AN ACADEMIC REVIEW OF THE HAZARDS POSED BY TRICHLORETHYLENE

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I. Introduction and Executive Summary

The seemingly conflicting studies on the carcinogenic and other hazards of trichlorethylene (T.C.E.) were resolved recently at the Halocarbon Symposium held June 19-20, 1980 in Washington, D.C., a program of which is appended (Appendix A). This clarification was based on new in vivo work published by Van Duuren (1), and Henschler (2), as well as by the published proceedings of other recent academic symposia dealing with human and animal carcinogenesis (3,4,5).

The clarification begins with the other symposia. The major points from these are:

- the development of cancer in man is a multi-step process involving 1) gradual transformations in molecular cell structure and function, 2) immune competence, and 3) usually, repetitive, on-going factors that result in overcoming DNA repair systems and other defense mechanisms; these account for the 20-30 year period for neoplastic development in man

- the transformation of normal to malignant cells involves irreparable damage to the nuclear and perhaps mitochondrial DNA (6); injuries to genes and genetic expression are subsequently "promoted" by a variety of factors such as disordered nutrition (excess calories, fat-overloading as in the contemporary USA diet, obesity, deficiency of fiber and of trace nutrients such as antioxidants) (5) and toxic overloading with chemicals. "Promotion" generally means that cell division is increased, so that the irreparable DNA damage is replicated many times over and has an improved statistical chance of surviving and being ultimately expressed; the molecular-cellular events in promotion involve cell membrane pathology that is free radical in nature and results in increased levels of chemical substances (cGMP) that trigger division (7)

- the term "promotion" derives originally from the two-stage mouse skin carcinogenesis model wherein a subcarcinogenic dose of a potent initiator carcinogen is applied and this is followed by the frequent administration of a non-carcinogenic promoter such as phorbol ester (8); the concept of "promotion" for human carcinogenesis has been elaborated fully in recent publications (5)

- inducing the premature emergence of malignancies with chemical or physical means exclusively in certain strains and sexes of mice that have high spontaneous rates for the development of specific cancers is not properly termed carcinogenesis, but rather a form of "promotion"

- at the Cold Spring Harbor Symposium in the fall of 1979 (3), both Cesare Maltoni and Benjamin Van Duuren reported on the non-carcinogenicity of TCE in a variety of animal models, using multiple dosage regimens; the amounts administered were as high as could be tolerated without making the animals sick and were in the dose range of other carcinogenic halocarbons; these and other studies (5) have emphasized that very high doses that cause an animal to become ill (ruffled fur, hunched over appearance, skin ulcers, weight loss, tail infections) will also result

in altered liver metabolism such that carcinogenic metabolites are produced; Wattenberg and others (7,9) have shown that the endoplasmic reticulum hydroxylation system can metabolize chemicals in different ways, most often into non-toxic excretable substances; an ill-state in the animal which precludes sufficient nutrient (e.g. antioxidants) intake to produce non-toxic metabolites, and high drug doses are an artefact in carcinogenesis studies since metabolism is altered to foster the formation of toxic substances, which may include either chemicals endogenous to the animal (e.g., dysmetabolism of sterols) or the administered high dose agent.

In addition to the findings and discussion by Maltoni and Van Duuren at the Cold Spring Harbor meeting, the recent publications of Henschler (2) and Van Duuren (1), dealing with in vivo animal tests, offer unambiquous data on TCE. Their experiments are described as follows:

- Henschler (2) used pure TCE at high doses by inhalation (0, 100 and 500 ppm) over the greater part of the animal's life span (18 months) and employed both sexes of three animal species (mice, rats and hamsters); he found that TCE was non-carcinogenic despite these very high levels and a prolonged dose schedule of 6 hour/day, 5 days/week for 3/4 of the animal's life; the female mice of the particular strain (NMRI) employed have a high spontaneous rate of developing viral-related lymphomas and in the rigors of this course of TCE treatment showed an increased number of such neoplasms; Henschler concludes that the lymphomas which are peculiar to this type of sex of mouse are not indicative of any carcinogenic potential in TCE since there was no increase in tumor development in the other species, nor in the male NMRI mice, compared to controls.

- Van Duuren administered TCE to male and female Ha: ICR Swiss mice by intragastric tube (0.5 mg once every week for 622 days), subcutaneously (same regimen) and on the skin (using the sensitive two-stage mouse skin model of initiation and promotion, with TCE applied as the possible initiator); he found no increase in the number of tumors, compared to controls and concluded that TCE is not a carcinogen for mice, despite high doses and sensitive assays; a few other halocarbons, that are somewhat analogous to TCE did prove to be carcinogens in the dose-range used for TCE.

The recent animal work on TCE is conclusive. There is no evidence of carcinogenicity and the reasons for this include complex chemistry. Henschler has suggested that the TCE-epoxide (the form in which halocarbons damage DNA) which is formed undergoes immediate intra-molecular rearrangement to the non-reactive, non-carcinogenic chloral. This occurs while the TCE-epoxide is still within the endoplasmic reticulum (ER) mixed function oxidase; therefore, no TCE-epoxide is actually produced under these circumstances.

Since the ER is involved in metabolizing TCE, the many factors that can impact on the ER become important. If the animals are made ill by very high doses, such as those used in the original National Cancer Institute's (NCI) work that suggested TCE might have some carcinogenic potential, then an artefact will be produced, as discussed previously. Some TCE epoxide might develop and it is significant that the original NCI studies described the mice as having ruffled fur, a hunched over appearance, skin ulcers, weight loss and tail infections.

Other artefacts and uncontrolled factors in the early NCI TCE experiment included the presence of significant contamination by epichlorhydrin, a potent carcinogen and the use of a mouse strain that has a high spontaneous rate of developing liver neoplasms. The use of pure TCE, without epichlorhydrin, by other investigators has revealed no carcinogenic potential for TCE. Additionally, the acceleration, or "promotion" of spontaneously occuring liver neoplasms in the NCI TCE studies is not appropriately termed carcinogenesis under any circumstances.

The use of several in vitro assays has been helpful as a screening devise to help reduce the number of chemicals that eventually require testing by the very expensive in vivo animal systems. By themselves, however, in vitro assays have no significance for humans. Among the most reliable of these screening assays is the salmonella mutagenesis system devised by Bruce Ames. This has been in use for several years and has been verified by a multiplicity of investigators. By contrast, the more recently developed eukaryotic systems that use mammalian cells generally lack the solid record of the Ames test. Rosenkranz and others (10) have found that TCE is negative in the Ames test. The finding of mutagenicity with TCE in some selected eukaryotic systems has little meaning for regulatory purposes, especially in the light of the negative, definitive in vivo studies reported by Henschler, Van Duuren and Maltoni.

A final point in the evaluation of the possible hazards posed by TCE are the acute toxicities. TCE exposures must reach extraordinary levels before acute toxicities develop, and this is therefore a moot point. Another question that has been raised centers on synergism and the possibility that trace amounts of TCE might act synergistically with commonly used substances such as alcohol, and tranquilizers. There is no evidence to support such an idea. In general, synergistic possibilities become quite evident in medicine when a substance becomes widely used. For example, tranquilizers were found to synergize with alcohol to produce severe central nervous system depression and even coma. Chloroform has been found to act synergistically with ethanol to cause liver necrosis, based on the fact that both exert their effects through free radical pathologic mechanisms (11). In the case of TCE, this substance has been used widely, for many years in different countries, under a variety of environmental conditions. If there were any likelihood of synergism it would have been demonstrated by this time in the medical and forensic literature.

In conclusion, TCE is not a carcinogen, based on extensive high dose animal studies by several different research groups. In certain mouse strains that have high rates for the spontaneous development of liver tumors or viral related lymphomas, TCE can facilitate their appearance if it is used in very high doses; this type of enhancement in the appearance of autochthonous neoplasms is not carcinogenesis. Facilitation or "promotion" involve markedly different molecular pathologic mechanisms compared to true initiating carcinogens as summarized in tabular form below. Carcinogen regulations have been mandated by Congress in the USA, and represent feasible endeavors. However, the area of "promotion", tumor facilitation or enhancement are broad and impossible to regulate; further, Congress has not passed laws to regulate this vague area. TCE, at the very worst, might be in this ill-defined category.

TABLE I.

Initiating Carcinogens (e.g. benzo(a)pyrene)	"Promoters/Facilitators/Enhancers" (e.g. dietary fat)
• Parent compound or specific metabolites bind to key bases in DNA and cause non-reparable changes	• Require no metabolic activation
• Low, and/or infrequent doses are needed	• Do not bind to DNA
• Must be given before promotion	• Are not carcinogenic
• Mutagenic	• Affect cell membranes to cause changes in cyclic nucleotides
	• Must be given after the initiating agent
	• Trigger cell division
	• Action is reversible
	• Suppress immune system
	• Not mutagenic

2. Detailed Background

A. Multi-step process of carcinogenesis

When a group of normal cells are exposed to a carcinogen, for example benzo(a)pyrene, this does not immediately induce neoplasia. The earliest step is the metabolic activation to a dihydrodiolepoxide which links covalently via the 10 position to the 2-amino group of guanine in DNA (6). Many chemicals require such activation, while a few do not (see Table 2).

Table 2. Classification of Some Groups of Carcinogens

Indirect-acting (Metabolic Activation Required)	Direct-acting (Metabolic Activation Not Required)
Halogenated aliphatic and olefinic hydrocarbons*	Epoxides
Aromatic hydrocarbons	Lactones
Aromatic amines	Sulfur and nitrogen mustards
Nitrosamines	Halo ethers
Aflatoxins	Acylating agents
Pyrrolizidine alkaloids	

*Some of these are possibly direct-acting carcinogens.

Most often an epoxide is formed, and this occurs via the cell's endoplasmic reticulum hydroxylation system. However, not all epoxides are dangerous. As a matter of fact, many non-toxic chemicals are converted into epoxides. In fact, most of the molecules of even the potent carcinogens are converted into inactive epoxides. For the complex polycyclic aromatic hydrocarbons, the epoxide must be in the right position (the "K" region) to cause DNA damage. Figures 1 and 2 demonstrate comparisons of carcinogenic and non-carcinogenic epoxides.

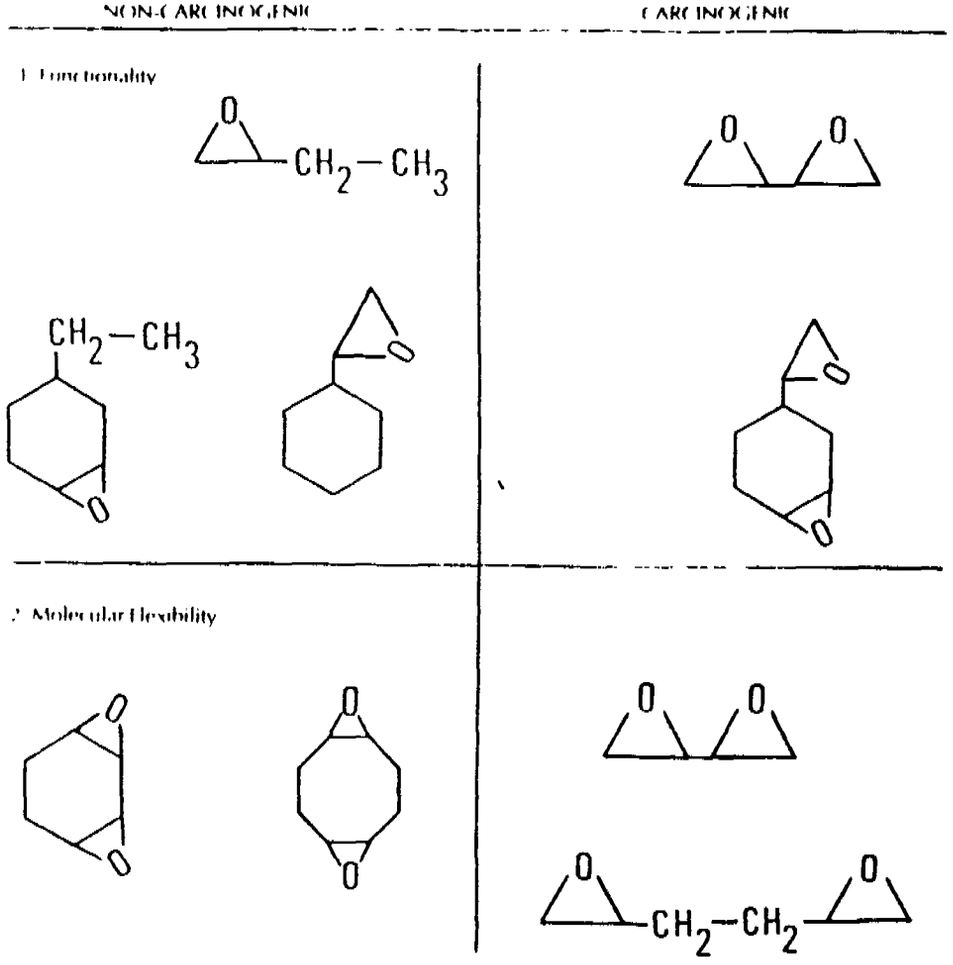

FIGURE 1. Comparison of carcinogenic and analogous non-carcinogenic epoxides using (1) functionality (mono- and bi-functional), and (2) molecular flexibility as indices

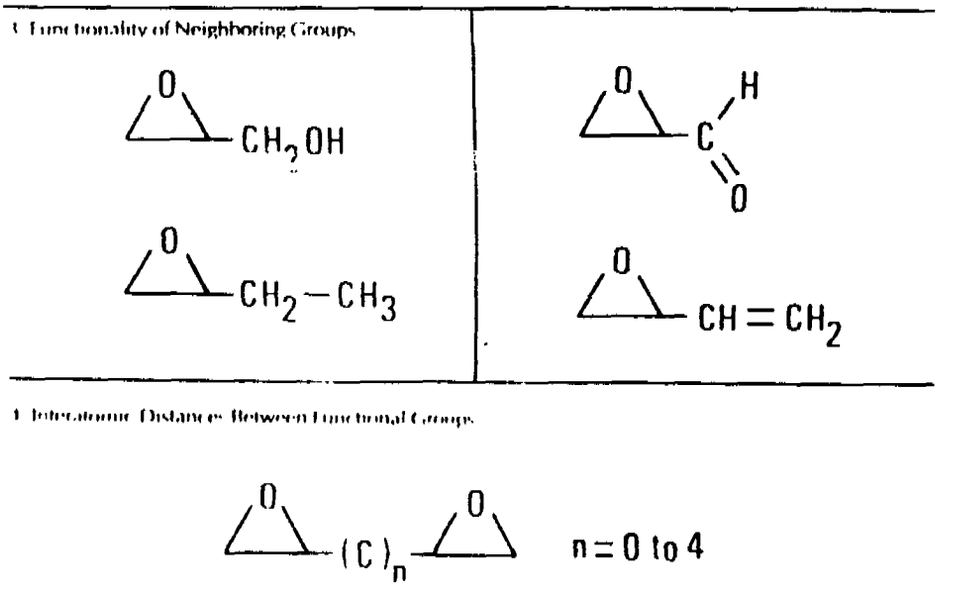

FIGURE 2. Comparison of carcinogenic and non-carcinogenic (3) mono-epoxides with small differences in neighboring groups, and (4) the effect of increasing chain length between functional groups in diepoxides

After appropriate metabolic activation, the epoxides must add to the DNA, on the right position of the proper base. See Figures 3 and 4 for examples of precise DNA-base damage.

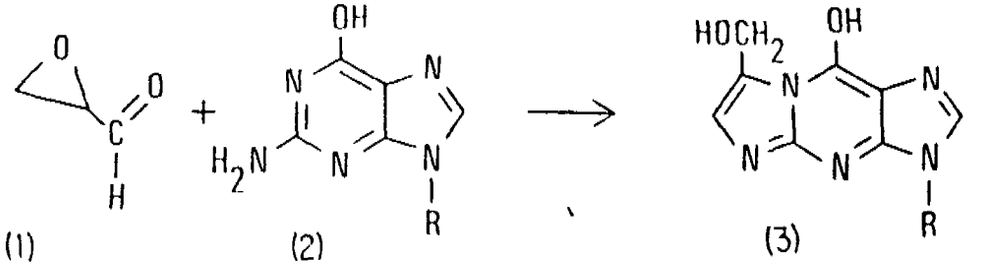

FIGURE 3. Reaction of carcinogen glycoaldehyde (1) with deoxyguanosine or guanosine (2). The reaction products are shown in structure (3). R refers to the sugar moieties.

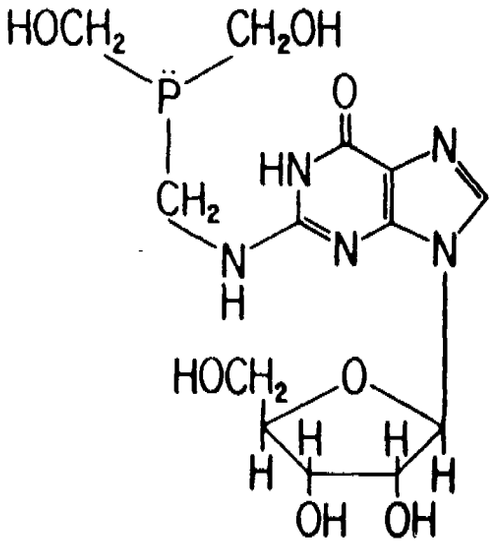

FIGURE 4. Reaction product of tetrakis(hydroxymethyl)phosphonium chloride (THPC) with guanosine.

Forming the epoxide is not the only requirement for forming a DNA-adduct. The epoxide must be stable for a long enough period of time to react with DNA. According to Henschler, trichlorethylene epoxide is unstable and undergoes immediate intramolecular rearrangement to chloral while the epoxide is still within the hydrophobic milieu of the mixed function oxygenase of the ER

hydroxylation system (13). TCE epoxide, when produced exogenously, is a reactive compound and decomposes at temperatures of 37°C and pH 7.4 by means of C-C and C-Cl fissions. However, metabolically, TCE is transformed in vivo exclusively to tri-chlorinated C_2 compounds, without any C-Cl fission. Hence, it is doubtful that epoxidation is a metabolic pathway for TCE, unless it occurs and undergoes immediate rearrangement, as suggested by Henschler. In either case, TCE epoxide either does not form in vivo, or it has such a short lifetime that it doesn't exist long enough to get out of the oxygenase system.

The DNA damage, if it does occur, is generally excised by the extensive DNA repair systems (12). Ultraviolet light, cosmic rays and strongly reactive chemicals have been parts of the natural environment for eons; it is therefore not surprising that living organisms have evolved many systems by which the action of the hazardous agents can be ameliorated. DNA repair systems consist of:

1. Some highly specific enzymes with very restricted substrate specificities (e.g., photoreactivating enzymes that act on cyclobutane pyrimidine dimers induced by DNA);

2. Enzymes that remove or relocate methyl and ethyl groups that are on incorrect sites of purines and pyrimidines (e.g., demethylases, glycosylases, transferases);

3. Enzymes with broad versatility that act on a wide variety of chemical carcinogen adducts to DNA.

The importance of repair systems is dramatically seen in various rare human diseases in which repair systems are lost because of hereditary defects. Three such conditions, xeroderma pigmentosum, ataxia telengectasia and Fanconi's anemia show elevated levels of carcinogenesis (12).

If repetitive DNA damage occurs such that it exceeds the capacity of the DNA repair systems, this still does lead to transformation. The genetic defect induced by the adduct must be viable and the damaged cell(s) must be induced to proliferate via some mechanism. Cells that do not divide cannot lead to cancer. This is where "promotion", in its broader sense becomes important. Promotion originally referred to the two-step process of mouse-skin carcinogenesis wherein an initiator like benz(a)pyrene is administered, usually in a subcarcinogenic dose. This is followed by repeated applications of a tumor promoter such as the phorbol ester, 12-O-tetradecanoyl-phorbol-13 acetate (TPA), which causes the appearance of a large number of skin tumors.

Promoters exert many effects on cells, particularly their cell membranes. They induce cell division and inhibit differentiation. See Tables 3 and 4.

TABLE 3. Effects of TPA on the Phenotype of Cell Cultures

Cell Surface and Membrane Changes
- Altered Na/K ATPase
- Altered morphology
- Increased phospholipid synthesis
- Altered hexose-glycopeptides
- Decreased EETS protein
- Increased uptake ^{32}P, ^{86}Rb, deoxyglucose
- Altered receptors
- Altered fluorescence polarization

Growth Properties
- Increased saturation density
- Altered cell-cell orientation
- Decreased serum requirement
- Decreased calcium requirement

Enzymatic
- Increased plasminogen activator synthesis
- Increased ornithine decarboxylase
- Increased prostaglandin synthesis

[a]Modified from Weinstein *et al.* 1979

TABLE 4. Examples of TPA Inhibition of Differentiation[a]

CELL SYSTEM	TYPE OF DIFFERENTIATION
Chicken Embryo Fibroblasts	Myogenesis
Chicken Embryo Chondroblasts	Chondrogenesis
Chicken Embryo Dorsal Root Ganglion	Neurite
Murine Erythroleukemia	Erythroid
Murine 3T3 Cell Line	Adipocytes
Murine Neuroblastoma	Neurite
Murine Melanoma	Melanogenesis
Hamster Epidermal Cultures	Keratinocytes
Mouse Epidermal Cultures	Keratinocytes
Sea Urchin	Embryogenesis

[a]See text and the following references: Slaga *et al.* 1978; Weinstein *et al.* 1979; Yamasaki, 1977.

Dr. Bernard Weinstein has recently discussed the idea that promoters can promote virus-related cancers (8) and this is relevant to Henschler's recent findings of the enhanced appearance of virus-related lymphomas in female NMRI mice following TCE inhalation at high doses (2). These lymphomas are caused by latent, inborn viruses. If promoters can accelerate virus-related cancers, as Weinstein believes (8), then this may explain some of the previous conflicts regarding the carcinogenicity of TCE. To date, TCE has been able to produce tumors only in Henschler's NMRI mice and in the B6C3F1 mice used in the original National Cancer Institute study (14).

In both instances, these mice have high rates of spontaneously developing lymphomas (NMRI) or liver tumors (B6C3F1), and the results may simply reflect some degree of promotion by TCE, and not carcinogenesis.

If a population of cells does undergo transformation and replicates to some extent, there are the additional stepwise changes of hyperplasia, metaplasia and benign tumor formation before malignant neoplasia occurs (8). The final hurdle that must be overcome is formidable and involves immunocompetence (15). Lewis Thomas originated the concept that cancer cells develop only if there is a failure on the part of the immune surveillance mechanism. This can occur either because of suppression of the immune system or by changes in the cell membranes of the newly emerged cancer cells such that they cannot be recognized as "foreign" (15). Henschler has offered the suggestion that the enhanced development of lymphomas in his experiments with TCE might be due to some change in the immune status of the mice. Among all the different kinds of tumors, the types that are most closely associated with failure of immunoregulation are the lymphomas (15). Lukes and others have classified Hodgkin's disease on the basis of immunoregulation (16); additionally, post-transplant recipients who receive immuno-suppressive therapy most commonly develop lymphomas. It is therefore reasonable to view Henschler's latest findings in his TCE treated mice as some alteration in the immune system, which allowed the naturally occuring lymphomas in this mouse strain to emerge more rapidly (2).

B. A Review of the In Vivo Studies Using TCE

Carcinogenicity - Van Duuren (1)

Because of the unique structural similarity of trichloroethylene (TCE) oxide to the -chloroethers, some of which are potent direct-acting alkylating carcinogens (17), it was suggested at a conference of the New York Academy of Sciences in 1974 that TCE may be a potential carcinogen (18).

This suggestion was based on the likelihood that TCE would be metabolized to its epoxide and that this epoxide would be the activated carcinogenic intermediate. A similar epoxide intermediate was suggested for vinyl chloride (VC) (18). These epoxide intermediates however were, at the time unknown compounds. VC was by then a well-known carcinogen, but other va. information concerning the carcinogenicity of TCE. The original suggestion that TCE might be carcinogenic was based on its structure-chemical reactivity relationship to the potent carcinogen, bis(chloromethyl) ether (20). TCE oxide, VC oxide as well as bis(chloromethyl) ether have a common structural feature, namely, one or two carbon atoms in each of the three compounds bear both an ether oxygen and a chlorine atom as shown in Figure 5. This implies that these epoxides are reactive alkylating agents, similar to bis(chloromethyl) ether. It should be noted, however, that they differ in hydrolysis mechanisms and products (13), and VC oxide rearranges spontaneously to chloroacetaldehyde (18). TCE oxide, according to recent work, does not form in vivo, or if it does, it is hypothetical since it undergoes immediate rearrangement to an inactive compound, as suggested by Henschler (13). The important point is that the

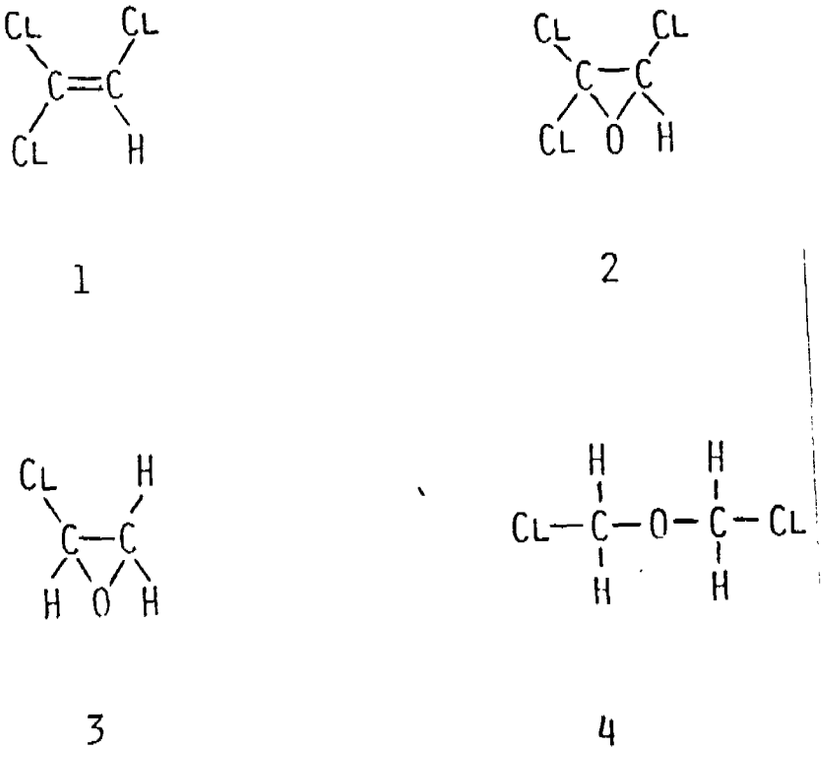

Figure 5. Structural similarity between the epoxides of TCE and VC and the carcinogen bis(chloromethyl) ether. (1) TCE; (2) TCE oxide; (3) VC oxide; and (4) bis(chloromethyl ether.

reaction products of TCE, in vivo, now show that TCE oxide is not formed; however, this was not known until the work of Henschler and others in the late 1970's (13).

Before any metabolic studies were done to determine whether significant, stable amounts of TCE oxide were formed, and before any screening in vitro tests were performed, the National Cancer Institute launched a high-dose in vivo study of TCE (21). In their bioassay, TCE was fed at high doses to B6C3F1 hybrid mice and Osborne-Mendel rats of both sexes. Hepatocellular carcinoma was observed in both sexes of mice in significant incidences ($P<0.01$). In the Osborne-Mendel rats, the tumor incidences observed, when compared to no-treatment controls, could not be ascribed to TCE, that is, it was not carcinogenic in this strain of rat. This report (21) (Public Health Service 1976a) was widely publicized and criticized (22) for a variety of reasons. The reasons most frequently cited were the high dosages used, the use of a liver tumor-susceptible strain of mice, and the lack of significant liver tumor incidences in Osborne-Mendel rats.

In the mid-1970's, studies were begun on the metabolism, carcinogenicity and mode of action of a series of halogenated hydrocarbons of which TCE was one.

Four bioassays were used to test TCE for carcinogenicity in ICR/Ha Swiss female mice. This is a random-bred strain of mice with a relatively low incidence of spontaneous tumors. The tests used were: 1) two-stage carcinogenesis on mouse skin, that is, a single application of TCE followed by repeated applications of the tumor promoter, phorbol myristate acetate (23); 2) repeated mouse skin application; 3) once weekly subcutaneous injection; and 4) once weekly intragastric intubation. The duration of the four tests ranged from 342 to 622 days and the medial survival times in all tests were good, except in those tests where tumors developed. The complete details of these tests together with all or some of the same tests using other halogenated hydrocarbons were recently published (1). TCE had negative results in all four tests. The two-stage carcinogenesis and subcutaneous injection are, in our experience particularly sensitive.

TCE oxide, a possible carcinogenic intermediate, was tested by us as an initiating agent in two-stage carcinogenesis. This test was negative also (1). TCE epoxide is thought to be the active substance, if TCE is carcinogenic, hence its administration should be an additional definitive test, especially when used in a sensitive assay such as two-stage carcinogenesis in mouse skin with TCE epoxide used as the possible initiator and phorbol myristate acetate (PMA) as the promoter. Despite using the supposed ultimate carcinogenic metabolite in a sensitive assay system, TCE epoxide was found to be a non-carcinogen (1).

Since the publication of this work (1), Van Duuren has expanded the TCE epoxide testing to include two additional assays of: 1) repetitive skin application of 2.5 mg TCE epoxide in 0.1 ml acetone, three times weekly for a median of 526 days and 2) subcutaneous injection of 500 µg of TCE epoxide in 0.05 ml tricaprylin once a week for a median of 547 days. No tumors were produced, compared to controls. See Tables 5 and 6 which represent pre-publication data from Dr. Van Duuren's laboratory.

The cumulative evidence from Dr. Van Duurens published and most recent pre-publication data regarding TCE epoxide shows that TCE is not a carcinogen. As indicated earlier, the mere fact that a substance forms an epoxide does not mean that it will have carcinogenic or other properties; definitive animal studies, using the epoxide must be done, as in Van Duuren's work.

Carcinogenicity - Henschler (2)

Henschler bega. his most recent in vivo studies because of the deficiencies in the original NCI experiments (14). He questioned the use of massive gavage doses used by NCI as "maximum tolerated doses" and noted that the results merely represented "...an acceleration of the occurence of a spontaneous tumor in an extremely sensitive strain of mice...". Additional defects were the presence of significant contamination of NCI's TCE with the potent carcinogen, epichlorhydrin, and the lack of tumorigenicity in rats also subjected to massive TCE doses (2).

Henschler (2) used pure TCE stabilized by an amine base and administered it by inhalation at 0, 100 and 500 ppm for 6hr/day, 5 days/week for 18 months to mice, rats and hamsters of both sexes. The 18 months represents the greater part of the life span of these rodents. TCE was found to be a non-carcinogen in all the species tested. The dose levels and regimens used by Henschler were high considering the fact that Guberan (25) has calculated that the amount of TCE

Table 5

Skin Painting of Halo-Epoxides

30 ♀ ICR/Ha Swiss mice per group were painted on the dorsal skin 3 times weekly with halo-epoxides at the doses indicated in 0.1 ml acetone by micropipet, except where noted. Duration of test was 432 to 580 days.

Compound, dose	Days to first tumor	No. animals with papillomas[a] / Total papillomas		Median survival time (days)	P
cis-1-Chloropropene oxide, 10 mg	225	14/20[b]	(10)	432	<0.0005
trans-1-Chloropropene oxide, 10 mg	345	15/20	(10)[c]	513	<0.0005
cis-1,3-Dichloropropene oxide, 10 mg	371	16/19	(11)	522	<0.0005
trans-1,3-Dichloropropene oxide, 10 mg	255	20/27[d]	(17)[e]	511	<0.0005
Trichloroethylene epoxide, 2.5 mg	-	0		526	-
Tetrachloroethylene epoxide, 7.5 mg[f]	268	3/3[g]	(1)	>459	0.014
m-Dichlorobenzene, 3.5 mg	-	0		520	-
Dichloroacetyl chloride, 250 µg	-	0		>576	-
d,l-Diepoxybutane, 3.3 mg, positive control	147	26/72	(21)[h]	462	<0.0005
Acetone, 0.1 ml	-	0		>576	-
No treatment, 100 animals	-	0		551	-

[a]Number of mice with squamous cell carcinoma is indicated in parentheses.
[b]Three of these were keratoacanthomas.
[c]One animal developed both a sarcoma and a squamous cell carcinoma.
[d]One of these was a histiocytoma and one was a keratoacanthoma.
[e]One of these was a sarcoma at the treatment area.
[f]Tetrachloroethylene epoxide was applied at 5 µl (7.5 mg) by Eppendorf pipet followed immediately by 0.1 ml acetone by micropipet.
[g]One of these was a keratoacanthoma.

Table 6

Subcutaneous Injection of Halo-Epoxides

30 ♀ ICR/Ha Swiss mice per group were injected in the left flank once weekly with halo-epoxides at the doses indicated in 0.05 ml tricaprylin. Duration of test was 551 to 580 days.

Compound, dose	Days to first tumor[a]	No. animals with local tumors	Median survival time (days)	p
cis-1-Chloropropene oxide, 1 mg	355	1 sarcoma 1 sq. cell carcinoma 1 plasmacytic lymphoma	482	0.052
trans-1-Chloropropene oxide, 1 mg	67	4 sarcomas 1 carcinoma	418	0.003
cis-1,3-Dichloropropene oxide, 500 µg	77	4 sarcomas 1 sq. carcinoma and scirrhous adenocarcinoma	500	0.003
trans-1,3-Dichloropropene oxide, 500 µg	189	5 sarcomas	498	0.003
Trichloroethylene epoxide, 500 µg	529	1 sarcoma	547	N.S.[b]
Tetrachloroethylene epoxide, 500 µg	245	1 sarcoma 1 lymphocytic lymphoma	491	N.S.[b]
m-Dichlorobenzene, 165 µg	-	0	525	-
Dichloroacetyl chloride, 50 µg	503	1 sarcoma	>560	N.S.[b]
d,l-Diepoxybutane, 330 µg, positive control	35	9 sarcomas 2 sq. cell carcinomas	475	<0.000
Tricaprylin, 0.05 ml	-	0	>524	-
No treatment, 100 animals	484	1 sarcoma	551	-

[a]Day to first tumor is the day the tumor was first palpated as a solid subcutaneous mass.

[b]p >0.10.

absorbed during an 8-hour occupational exposure to 100 ppm TCE is approximately equivalent to 1.4 grams of chloral hydrate; the chloral is formed from TCE and is then hydrated. Henschler went as high as 500 ppm TCE, 6 h/day, 5 days/week for 3/4's (18 months) of the animals life time and still did not see carcinogenesis.

The only statistically significant increase in tumor formation was in virus-related lymphomas that already occur spontaneously with high incidence in female mice of the strain employed (NMRI). This high prevalence of lymphomas in female NMRI mice as first reported by Cole and Furth (26). Many other investigators subsequently confirmed the autochthonus nature of the lymphomas in the NMRI females (27, 28). The viral nature was known as early as 1951 and was defined by Gross (29).

The enhancement of naturally occuring neoplasms that are highly specific for sex and species is not carcinogenesis, but rather a form of the broad type of "promotion" (8). Weinstein has helped to pioneer the concept that virus-related animal tumors can be "promoted", in a manner analogous to the promotion of subcarcinogenic doses of initiator carcinogens (8). Some loss of immuno-regulation occurs in the multistep process of carcinogenesis, as summarized by Filipovich (15). The strongest evidence regarding immunosuppresion and cancer development is found in the human lymphomas, as summarized by Filipovich (15) Lukes (16) and others. It is therefore very likely that Henschler's own discussion of his lymphoma results is accurate, i.e., the high doses of TCE simply facilitated, or "promoted" the appearance of lymphomas in mice that already have a high rate of developing them, and this occurred through a mechanism of immunosuppresion. Henschler's conclusion was that this did not represent carcinogenesis (2).

Carcinogenicity - Maltoni (24)

Cesare Maltoni reported gavage administration of TCE, delivered by stomach tube, in olive oil, once daily, 4-5 days/week, for 52 weeks at two dose levels: 250 mg/kg and 50 mg/kg; the control group was given olive oil alone. Each group had 60 rats, 30 males and 30 females that were 13 weeks old at the start of the experiments. The study lasted 140 weeks and all animals were observed until spontaneous death. Maltoni found no evidence of carcinogenicity in these studies (24). It should be noted that the doses employed were of the same order of magnitude as employed in the original NCI study (14).

At the Banbury Center conference (3) at Cold Spring Harbor the Comments by the participants were recorded, transcribed and published as part of the symposium (3). Maltoni's comment following Van Duuren's presentation is particularly relevant:

> Maltoni: "Just a comment to Dr. Van Duuren. National Cancer Institute (NCI 1976) data showed that in one single strain of mice, the usual one used for the NCI tests, extremely massive doses of TCE were shown to produce hepatomas in the mice. Thereafter, there were, I think three kinds of experiments - those by Dr. Van Duuren, one sponsored by

industry which was not finished, and our experiments, which had results in line with the results of Dr. Van Duuren. We treated for 52 weeks, five times weekly with 0.25g/kg body weight. We didn't get any type of carcinogenic effect.

It was found that probably the TCE used by NCI was not pure TCE. And so industry promoted an experiment of mutagenesis with highly pure TCE with negative results on an O-series carcinogenicity test.

We are now conducting what we do believe are the largest studies ever done on EDC-it's the largest one performed in our laboratory at once on rats. We are using Sprague-Dawley rats and our own Swiss mice. A large group of 800 animals were kindly provided to us by NCI. We are testing by inhalation starting by very heavy dose - 600 ppm down to 300 ppm on 3500 animals altogether. This study was started in February 1979 and we are confident in 1 year we will have enough time to finalize the results.

On the basis of our present evidence, we agree entirely with what Dr. Van Duuren has said this morning. We didn't have any carcinogenic effect up to this point".

Maltoni's data are partially summarized in Tables 7, 8 and 9. No significant differences were found, compared to controls. The Tables shown here are not as extensive as in Maltoni's presentation. He studied the animals completely and looked for all types of tumors.

The Cold Spring Harbor Symposium (3) ended the discussion of TCE with the agreement of that Maltoni's inhalation studies using TCE at doses of 300-600 ppm, in mice and rats would be important (3). However, since that symposium, Henschler's studies (2) were published wherein TCE was used at 100-500 ppm in mice, rats and hamsters for the greater part of the animals life span (18 months); Henschler's inhalation work showed that TCE was not a carcinogen.

Carcinogenicity - Original NCI study (14)

Industrial grade (>99% pure) trichloroethylene was tested using 50 animals per group at 2 doses and with both sexes of Osborne-Mendel rats and B6C3F1 mice. Twenty of each sex and species were maintained as matched controls, in addition to colony and positive carcinogen controls. Animals were exposed to the compound by oral gavage 5 times per week for 78 weeks. At the end of treatment, animals were observed until terminal sacrifice at 110 weeks for rats and 90 weeks for mice. A complete necropsy and microscopic evaluation of all animals (except 7 of the original 480) was conducted.

TABLE 7

Experiment BT301: Exposure by ingestion (stomach tube) to Trichloroethylene in olive oil at 250 and 50 mg/Kg body weight, once daily, 4-5 days weekly, for 52 weeks. Results after 140 weeks (end of experiment).

DISTRIBUTION OF THE DIFFERENT TYPES OF TUMOURS

GROUPS NO.	CONCEN-TRATION	ANIMALS (Sprague-Dawley rats, 13 weeks old at start)			ANIMALS WITH TUMOURS								
					Mammary tumours			Zymdal gland carcinomas			Leukaemias		
		Sex	No.at start	Corrected number	Total No.	%	Average latency time (weeks)	Total No.	%	Average latency time (weeks)	Total No.	%	Average latency time (weeks)
I	250 mg/kg	♂	30	30	1	3.3	79.0	0	-	-	3	10.0	67.3
		♀	30	29	16	55.2	83.3	0	-	-	0	-	-
		♂ & ♀	60	59	17	28.8	83.0	0	-	-	3	5.1	67.3
II	50 mg/kg	♂	30	29	4	13.8	87.0	1	3.4	119.0	2	6.9	70.0
		♀	30	30	22	73.3	81.2	0	-	-	0	-	-
		♂ & ♀	60	59	26	44.1	82.1	1	1.7	119.0	2	3.4	70.0
III	Olive Oil Control	♂	30	28	1	3.5	111.0	0	-	-	0	-	-
		♀	30	30	16	53.3	93.0	0	-	-	1	3.3	80.0
		♂ & ♀	60	58	17	29.3	94.0	0	-	-	1	1.7	80.0
TOTAL			180	176									

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TABLE 7 continued

Experiment BT30i: Exposure by ingestion (stomach tube) to Trichloroethylene in olive oil at 250 and 50 mg/Kg body weight, once daily, 4-5 days weekly for 52 weeks. Results after 140 weeks (end of experiment).

DISTRIBUTION OF THE DIFFERENT TYPES OF TUMOURS

GROUPS No.	CONCENTRATION	ANIMALS WITH TUMOURS													
		Forestomach epithelial tumours			Subcutaneous sarcomas			Encephalic tumours: Neuroblastomas			Encephalic tumours: Other types		Others		
		Total No.	%	Average latency time (weeks)	Total No.	%	Average latency time (weeks)	Total No.	%	Average latency time (weeks)	No.	%	Total No.	Benign No.	Malignant No.
I	250 mg/Kg	0	-	-	0	-	-	0	-	-	0	-	3	2	1
		0	-	-	0	-	-	0	-	-	0	-	3	1	2
		0	-	-	0	-	-	0	-	-	0	-	6	3	3
II	50 mg/Kg	0	-	-	1	3.4	119.0	0	-	-	1	3.4	0	0	0
		1	3.3	95.0	0	-	-	0	-	-	1	3.4	3	2	1
		1	1.7	95.0	1	3.4	119.0	0	-	-	2	3.4	3	2	1
III	Olive Oil (control)	0	-	-	0	-	-	0	-	-	1	3.6	5	4	1
		0	-	-	0	-	-	0	-	-	0	-	8	6	2
		0	-	-	0	-	-	0	-	-	1	1.7	13	10	3

TABLE 8

Experiment BT301 : Exposure by ingestion (stomach tube) to Trichloroethylene in olive oil at 250 and 50 mg/Kg body weight, once daily, 4-5 days weekly, for 52 weeks. Results after 140 weeks (end of experiment).

DISTRIBUTION OF THE DIFFERENT TYPES OF MISCELLANEOUS ("OTHER") TUMOURS

GROUPS NO.	SEX	ANIMALS BEARING OTHER TUMOURS				TOTAL
		Benign		Malignant		
		No.	Distribution of histotypes	No.	Distribution of histotypes	
I	M	2	1 skin acanthoma 1 Leydig cells tumour	1	1 sarcoma of the lung	3
	F	1	1 pheochromocytoma	2	1 adenocarcinoma of the uterus 1 pericytosarcoma of the uterus	3
II	M	0	--	0	--	0
	F	2	1 adrenal gland cortical adenoma 1 leiomyoma of the uterus	1	1 adenocarcinoma of the uterus	3
III	M	4	2 dermatofibromas 1 pheochromocytoma 1 bladder papilloma	1	1 retroperitoneal liposarcoma	5
	F	6	1 adrenal gland cortical adenoma 1 pheochromocytoma 1 ileo-caecal fibroma 1 polypus of the uterus 1 bladder papilloma 1 neurilemoma	2	2 adenocarcinomas of the uterus	8

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TABLE 9

Experiment BT36 Exposure by ingestion (stomach tube) to Trichloroethylene in olive oil at 250 and 50 mg/Kg body weight, once daily, 4-5 days weekly, for 52 weeks. Results after 140 weeks (end of experiment).

DISTRIBUTION OF THE DIFFERENT TYPES OF MAMMARY TUMORS

GROUPS NO.	CONCENTRATION	ANIMALS (Sprague-Dawley rats, 13 weeks old at start) Sex	No. at start	Corrected number	MAMMARY TUMORS Total No.	%	Average latency time (weeks)	No. of tumors/ tumors bearing animals	Histologically examined Total No.	%	Histotype: Fibromas and fibroadenoma No.	%	Average latency time (weeks)	Carcinomas No.	%	Average latency time (weeks)
I	250 mg/Kg	♂	30	30	1	3.3	79.0	1.0	1	100.0	1	100.0	79.0	0	-	-
		♀	30	29	16	55.2	83.3	1.6	14	87.5	12	85.7	85.7	3	21.4	62.3
		♂ & ♀	60	59	17	28.8	83.0	1.5	15	88.2	13	86.7	85.2	3	20.0	62.3
II	50 mg/Kg	♂	30	29	4	13.8	87.0	1.0	3	75.0	3	100.0	88.3	0	-	-
		♀	30	30	22	73.3	81.2	1.3	20	99.0	17	85.0	87.4	3	10.0	63.0
		♂ & ♀	60	59	26	44.1	82.1	1.2	23	88.5	20	86.9	87.5	3	13.0	63.0
III	Olive Oil (control)	♂	30	28	1	3.5	111.0	1.0	0	-	0	-	-	0	-	-
		♀	30	30	16	53.3	93.0	1.7	16	100.0	16	100.0	93.0	3	18.7	110.0
		♂ & ♀	60	58	17	29.3	94.0	1.6	16	94.1	16	100.0	93.0	3	18.7	110.0
TOTAL			180	176												

Two doses were used with animals started on test at approximately 6 weeks of age. The initial doses used in this test were the estimated maximum tolerated dose (MTD) and ½ MTD, as predicted from data obtained in a 6-week toxicity study. For rats, the initial doses were 1300 and 650 mg/kg body weight. These were changed, based upon survival and body weight data, so that the "time-weighted average" doses were 549 and 1097 mg/kg for both male and female rats. For mice, the initial doses were 1000 and 2000 mg/kg for males and 700 and 1400 mg/kg for females. The doses were increased so that the "time-weighted average" doses were 1169 and 2339 mg/kg for male mice and 869 and 1739 mg/kg for female mice.

With both male and female mice, primary malignant tumors of the liver, i.e., hepatocellular carcinoma, were observed in high numbers. For males, 26/50 low dose and 31/48 high dose animals had hepatocellular carcinomas as compared with 1/20 matched controls and 5/77 colony controls. The differences between treated and matched control males at both doses were highly significant ($P < 0.001$). For females, hepatocellular carcinomas were observed in 4/50 low dose and 11/47 high dose animals as compared with 0/20 matched controls and 1/80 colony controls. While the difference between the high dose female mice and matched controls was also highly significant ($P < 0.01$), the difference at the low dose was less ($P = 0.09$). For both male and female mice, age-adjusted tests for linear trend (dose response) were highly significant for hepatocellular carcinoma ($P < 0.001$ for males and $P = 0.002$ for females).

Carbon tetrachloride (CCl_4) was used as a positive control for the series of chlorinated chemicals which included trichloroethylene. While virtually all male and female mice developed hepatocellular carcinomas following carbon tetrachloride treatment, the response in the Osborne-Mendel rat was considerably less. Only about 5% developed hepatocellular carcinomas. Thus, there appears to be a marked difference in sensitivity to induction of carcinomas by chlorinated compounds between the B6C3F1 mouse and the Osborne-Mendel rat.

The original NCI test has been reviewed and found to have serious flaws including: 1) extremely high doses; Maltoni had described them as "...extremely massive doses of TCE..." (3); Van Duuren believes quite strongly that disordered metabolism is induced by such doses and that such artefacts preclude an evaluation of carcinogenicity (3); 2) use of mice that spontaneously develop liver tumors; this is facilitation or a type of "promotion"; it is not carcinogenesis; 3) lack of tumor production in a different species; most chemical carcinogenesis workers will not consider a substance as carcinogenic as long as only one species is affected. The NCI studies have just revealed a peculiarity of the mouse strain employed. Other workers like Van Duuren (1), Henschler (2) and Maltoni (24) have studied rats, hamsters and other mouse strains, by varied routes and have found no evidence of carcinogenicity; and 4) use of TCE that was heavily contaminated with significant quantities of the potent carcinogen, epichlorhydrin; this latter point disqualifies the entire NCI study.

C. A Review of the In Vitro Studies Using Pure TCE

The use of TCE preparations that contained potent carcinogenic contaminants, such as the epichlorhydrin in the original NCI in vivo study (14) are invalid as are studies wherein the purity of the TCE was not specified in vitro (33,35,37).

A further point is that the in vitro assays are for screening the miriads of chemicals, in order to select the ones that should be tested by the costly, and prolonged in vivo animal tests. Van Duuren (30) has defined a multi-tier system for studying carcinogens and this is shown in Table 10. In view of the recent extensive in vivo data by Van Duuren (1), Henschler (2) and Maltoni (3), it is reasonable to ask, "Why in vitro assays?". These are only screens which test for mutagenicity and by themselves have no significance for humans, or for animals.

Since Ames first developed his Salmonella mutant lines, a number of other short-term in vitro assays have been developed (31), and include whole organisms such as flies (34). The Ames test requires a liver homogenate to mediate the metabolic activation of the chemical in question. There has been an extraordinarily good correlation in a series of more than 300 compounds between the number of back-mutations to histidine auxotrophy in various tester strains of Salmonella, and the carcinogenicity of the compounds; there are approximately 10% false positives and 10% false negatives (32). The newer tests using eukaryotes (yeast and mammalian cells such as Heidelberger's C3H/10T ½ cells) do not have the excellent "track record" of the Ames test in that they have not been correlated with over 300 compounds, and the percent of false positives and false negatives are higher.

A review of the available literature (32-41) reveals mixed data, equally negative and positive, even with pure TCE. There are several reports that are negative and the few that are positive are weakly so. In the weakly positive results with Salmonella, only one tester strain, TA100, was sensitive. The lack of consistently positive data, particularly in several Salmonella tester strains seriously undermines any premise of significant mutagenicity for pure TCE. Further, it is well to keep in mind the 10% false positive and 10% false negative aspects of the most reliable in vitro system, the Ames Salmonella test. To place any reliance on the in vitro data, wherein the positives are quite weak and only in one tester strain, is to push the reliability of the in vitro systems beyond credibility.

TABLE 10

Tier System for Assessment of Potential Human Carcinogens

1. Structure Activity Studies
2. Short-term Bioassays
3. Chronic Testing in Animals
4. Epidemiology

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