IV. BIOLOGIC EFFECTS

A. TOXICITY

As this review pertains to the issue of carcinogenicity, only a brief summary of other aspects of toxicity will be presented. Aviado et al. (1975), Fuller (1976), and Waters et al. (1977) have presented recent reviews which support the assessment of toxic effects of TCE as presented in the NIOSH Criteria Document on TCE (NIOSH, 1973).

Toxic effects on the central nervous and cardiovascular systems, skin, liver, and kidney have been attributed to exposure to TCE. However, reports on the type and severity of effects occurring following industrial exposures were often sketchy and in some cases contradictory, especially as regards latent manifestations, including hepatic and renal toxicity.

Effects on the CNS, principally depression, have been well documented. Among the symptoms most often described are: headache, nausea, vomiting, dizziness, vertigo, fatigue, mental dullness, sleepiness, feeling of light-headedness, insomnia and burning eyes. Trigeminal palsies have been reported, as have several cases of visual deterioration. Considerable evidence has accumulated that indicates that dichloroacetylene is the likely neuropathy-causing agent in TCE exposures. Reichert et al. (1975), by exposing mice to dichloroacetylene, demonstrated degenerative lesions in the brain considered parallel to those responsible for neurotoxic symptoms observed in man. High, acute doses have also resulted in cardiovascular and respiratory effects with a number of deaths attributed to respiratory arrest, cardiac arrhythmias, including ventricular fibrillation, and primary cardiac standstill. Respiratory distress has been observed often, especially following intermittent inhalation exposures, with such symptoms as chest tightness and labored breathing.

Dermal effects have long been noted, principally as a localized reddening or flushing of the skin, although generalized dermatitis has also often occurred as a result of exposure to TCE.

Intolerance to alcohol is a well-characterized phenomenon among TCE-exposed workers. Not only do many TCE workers become inebriated with consumption of small quantities of alcoholic beverages but they also are subject to vasodilation of superficial skin vessels, resulting in skin blotches, a condition known as "degreasers flush." Stewart et al. (1974), in order to study this phenomenon, repeatedly exposed seven male volunteers to TCE and then gave them small oral doses of ethanol. A transient vasodilation in the skin reached its maximum within 30 minutes and completely subsided by 60 minutes. Flushing was most

common on the face, neck, shoulders, and back. From the studies of Stewart et al., "degreasers flush" appears to be a benign dermal phenomenon which occurs primarily following repetitive exposures to TCE of at least 3 weeks' duration. Further, it seems not to be a universal problem, as some individuals are more sensitive than others to the effect. Sensitivity to alcohol may also be a long-lasting effect in that some of the volunteers exhibited the effect for several weeks after cessation of the TCE exposure. One of the volunteers continued to flush upon consumption of a small quantity of alcohol 6 weeks after exposure to TCE for 5 days at 200 ppm. Small quantities of ethanol have been shown to increase the concentration of TCE in the body, of practical importance in assessing the hazard of TCE (Muller et al., 1975).

The effects of chronic exposure of humans to TCE have not been extensively studied and thus are not well characterized. Studies with laboratory animals and a few clinical studies, demonstrated latent effects of the liver, kidney and nervous system. The degree and dose relationships of these effects is unclear, at least at low levels (< 100 ppm). The few animal studies that have been conducted with inhalation exposures to TCE for periods of at least several months and at concentrations near the current Federal occupational exposure limit (100 ppm), have given conflicting results. Mosinger and Fiorentini (1955) observed both liver and kidney changes in cats exposed by inhalation to levels of 20 ppm for 1-1.5 hours per day for 4-6 months. Seifter (1944) also observed liver injury in dogs as soon as 3 weeks of exposure by inhalation to levels of 500-750 ppm, 4-8 hours per day, 5 days a week. Other reports (Adams 1957; von Oettingen 1955) indicated evidence for slight liver changes, and fatty degeneration, and weight increase, and degenerative effects on bone marrow, heart, and central nervous system following chronic exposures to animals.

Observations of liver disfunction in workers exposed to TCE have been observed only infrequently; however, relatively little surveillance of exposed workers has been conducted to ascertain any latent effects. Thus, it is unlikely that the potential magnitude of any liver or kidney abnormalities in humans exposed for long periods to TCE are fully known.

Evidence for latent toxicity and liver and kidney damage because of TCE is available from exposures other than those occurring in the workplace. Several cases of ingestion of liquid TCE have occurred and have resulted in severe liver and kidney damage. This is in apparent contrast to the usually slight hepatic and renal effects observed after inhalation exposures. Whether the same effects would have been encountered if ingestion of TCE were spread over a period of time similar to that of an inhalation exposure is difficult to predict. However, Baerg and Kimberg (1970) did document centrilobular hepatic necrosis and acute hepatic and renal damage in teenagers repeatedly sniffing a

cleaning fluid containing TCE and petroleum distillates. The toxic effects were attributed by the authors to TCE. As the vapor concentrations were likely high and were inhaled only for a short period, the effect cannot be directly extrapolated to inhalation of the same total dose over a longer period of time, as in typical occupational exposures.

In another chronic exposure study, El Ghawabi et al. (1973), 30 workers exposed to TCE for a period of 1-5 years at levels ranging from 41 to 163 ppm reported long-lasting symptoms, headache being the most common, followed in frequency of occurrence by dizziness, sleepiness, nausea and vomiting, conjunctivitis and lacrimation, diminished libido, fatigue, skin rashes and itching. Workers continued to suffer. Even when on leave for 2 weeks, their headaches worsened in some cases. Knee and ankle jerks were exaggerated in one exposed worker. A slight bradycardia was also observed in exposed workers. The blood analysis and liver function test results were comparable to those obtained from a group of 20 matched controls not exposed to TCE or to any other solvent. Based upon these latter results, El Ghawabi et al. concluded that no chronic toxicity was evident.

In a study of 50 workers chronically exposed to TCE, Grandjean et al. (1955), also were unable to diagnose a single case of liver or kidney trouble without difficulty; however, based upon modifications in biochemical tests, they were of the opinion that 10% of the workers had slightly impaired liver function. Of more concern to these investigators was the findings of chronic nervous disorders in workers exposed to levels between 20 and 80 ppm. Based upon those results, they concluded that the allowable concentration of TCE should be fixed below 40 ppm.

In assessing possible toxic effects associated with working with TCE, one must be concerned not only with the toxicity of TCE and its metabolites, but also with that of other chemicals formed by its environmental degradation or of products of reaction between TCE and components of normal biological systems. One of the most serious concerns relates to the possible formation of the highly hazardous chemicals, phosgene and dichloracetylene, under certain conditions of TCE use (as discussed in Section II). TCE vapor around open flames or even drawn through lighted cigarettes may degrade to phosgene and CO. Acute exposures to phosgene at 10-15 ppm may be fatal, with severe distress occurring at even lower concentrations. NIOSH has recommended a phosgene TWA workplace exposure standard of 0.1 ppm with a 0.2 ppm ceiling limit for no more than 15 minutes (NIOSH 1976a). Smoking, open flames, or very hot surfaces should be excluded in TCE operations to avoid inadvertent formation of phosgene.

B. PHARMACOKINETICS/METABOLISM

The NIOSH criteria document on TCE provided as thorough a discussion of the kinetics of its absorption, distribution, elimination, and metabolism as was known, at that time (1973). One omission, however, was the speculation about an epoxide intermediate metabolite, which has been proposed by several scientists who studied the metabolism of TCE. This issue is important to an assessment of carcinogenicity and will be discussed further in a subsequent section.

From the review in the NIOSH criteria document, as well as from a more recent one by Waters et al. (1977), a few general principles on the pharmacokinetics and metabolism of TCE may be stated: (1) trichloroethylene is readily absorbed after inhalation or ingestion and considerably less readily from the surface of the skin, (2) TCE is rapidly distributed via the blood stream throughout the body with accumulation mainly in fatty tissues, lungs, spleen, and liver, (3) the elimination of TCE following short-term exposures is primarily in the form of metabolites rather than as the parent TCE molecule and is largely via the urine, and (4) TCE is metabolized to, and excreted primarily as, two metabolites, trichloroacetic acid and trichloroethanol. The latter of these may be conjugated with glucuronic acid. A well-documented intermediate metabolite is chloral hydrate. Trichloroethanol and chloral hydrate are likely responsible for many of the toxic effects observed with TCE exposure. Other proposed metabolites are: chloroform, monochloroacetic acid, trichloroacetaldehyde, and TCE epoxide.

These general statements have been developed primarily on the basis of short-term animal experiments or brief exposures of human volunteers. Although very little is known about the effects of chronic exposure at various doses or concentrations on the pharmacokinetics of TCE, Leibman and McAllister (1967) demonstrated that repeated exposures to TCE modified the ability of rats to metabolize TCE. In those studies the conversion of TCE to chloral hydrate by rat liver microsomal preparations was shown to increase when the rats were repeatedly exposed to atmospheres containing TCE. The exposures were at 4000 ppm, 1/2 hour per day for 4 days. It is not possible to extrapolate from these results to effects of long-term exposures or to effects on other species, including humans.

A crucial issue in this report is that of the relevance of animal carcinogenicity test results to the predictability of carcinogenicity in man. In such tests with TCE (to be discussed later), the test animals were exposed to high levels of TCE by gastric intubation for 18 months. As no pharmacokinetic measurements were made in these studies, it is not possible to assess with certainty the relevance of the results to hazard assessment. On the other hand, to disregard such evidence would

indeed run counter to all concepts of public health protection. There are several examples of animal test results obtained under similar conditions that accurately predicted potential hazard to man. Notable examples have been those obtained with vinyl chloride and benzidine, both considered at one time to be relatively innocuous.

An argument widely voiced is that high-dose levels utilized in such studies may saturate the usual metabolic pathways and lead to atypical metabolites and routes of metabolism. Gehring et al. (1976) conducted a series of studies with 1,4-dioxane and vinyl chloride to assess the dose-dependent fate of chemicals, especially as related to their metabolism, retention, and excretion. As might be expected, with high-dose levels, greater percentages of the chemicals were either excreted unchanged or retained in the body without undergoing metabolism. While Gehring et al. determined major differences in the kinetics of retention and elimination, they found no qualitative change in metabolites as the dose or exposure level was changed. They noted that the known pathways for vinyl chloride metabolism produce intermediate reactive metabolites that lead to the same end products.

A recent NIOSH review of vinyl chloride studies (NIOSH, 1977a) and the papers by Green and Hathway (1975) and by Reynolds et al. (1975) support the probability that two metabolic routes exist. These reports also propose that a dose-dependent depletion of liver glutathione may lead to decreased clearance of reactive electrophilic metabolites and to increased toxicity, including possible carcinogenicity. In studies conducted with rats, Watanabe et al. (1976) found that an exposure of at least 7 hours at 50 ppm vinyl chloride led to absorption and the metabolism of sufficient vinyl chloride to exceed the capacity of the liver to replenish the nonprotein sulfhydryl groups (glutathione and cysteine), so that presumably, free epoxidized vinyl chloride or some other reactive metabolite is available to react with nucleic acids.

Ample evidence exists that variations in exposure profiles, including different dose levels, periods, and durations of exposure, as well as concomitant exposure to other chemicals, modify the pharmacokinetics and metabolism of chemicals in the body. However, there is little evidence to support a generalization that a total shift to a more hazardous metabolite or pharmacokinetic pattern will result from exposure at high-dose levels. Results of pharmacokinetic studies with various doses of TCE, such as those described for vinyl chloride, have not been reported.

Related to this issue, however, are the studies of Ikeda et al. (1972), in which a decrease in the rate of urinary excretion of trichloroacetic acid was observed in workers with exposures above 50 ppm, essentially reaching a plateau at 100 ppm. In contrast, the levels of trichloroethanol excreted in urine

continued to rise at a constant rate with increased exposure-levels of TCE. Ikeda et al., suggested that a shift in the pattern of metabolism occurred at higher levels, with the formation of relatively more trichloroethanol, the more toxic of the two main metabolites. Based on these results, they expressed concern with 100 ppm as a permissible level of exposure.

As illustrated in recent reviews of carcinogenicity testing methods and applications of these results, extrapolation from high dose levels may in some cases underestimate the true effect that could result at low-dose levels (Kraybill, 1977; Page, 1977). This might occur where exposure levels exceed the capacity of the body to absorb, or where an "inert" chemical is metabolized to an active carcinogen by a system of finite capacity. In such cases sequential increases in exposure levels may not result in incremental increases in effect. As of this time, data are insufficient with which to assess with certainty the effect in either direction for exposures to TCE.

With respect to uptake, two recent studies have been conducted to study the influence of exercise on the pharmacokinetics of TCE following inhalation exposure. In one, Monster et al. (1976) observed that working or exercising men absorbed 40% more TCE than resting men during 4-hour exposures at 70 and 140 ppm, but there was no influence of exercise on either the distribution or the metabolism of TCE. In the other study, when 15 healthy men were exposed to TCE at about 100 and 200 ppm for four 30-minute periods similar results were obtained (Astrand, 1976). Some of the men were allowed to rest while others were exercised strenuously on a bicycle ergometer. The uptake of TCE was about 55% of that available in the inhaled air prior to beginning the exercise regimen. While the rate of uptake of TCE decreased to about 25% during the fourth period of exposure for those men exercising most vigorously, the total dose absorbed increased since the effect of exercise was to increase pulmonary ventilation (2.5 times that at rest). The concentration of TCE in alveolar air was about twice that at rest while that in arterial blood more than doubled. The decline in percentage uptake with increasing work intensity was attributed to a rate of diffusion to the blood from the alveoli slower than the rate of supply of TCE to the alveoli. Monster et al. suggested that the relative insolubility of TCE in blood contributed to the low-diffusion rate.

Perhaps even more important than the relative changes in the kinetics of absorption, retention, and excretion of a chemical is that of the metabolites that can be formed. Of particular importance is the potential for formation of a highly reactive metabolite that can be responsible for specific toxic effects.

It has been well established that some indirectly acting carcinogens may be metabolized to activated carcinogenic intermediates via cytochrome P-450-dependent mixed-function oxidases. Such metabolites may then interact covalently with

nucleophilic sites in nucleic acids and proteins, inducing carcinogenic lesions.

As long ago as 1945, Powell, and later, Daniel (1963), and Leibman (1965) speculated that TCE might be oxidized to an epoxide as an intermediate metabolite during its biotransformation. Renewed interest in this hypothesis resulted from the findings that vinyl chloride is carcinogenic in man and animals, and the observation that this in turn is likely due to the formation of an epoxide, chloroethylene oxide. The mechanism, proposed by Van Duuren (1975), gained support from findings of covalent binding of ^{14}C-vinyl chloride to tissue macromolecules, that it is catalyzed by rat liver microsomal preparations (Kappus et al., 1975), and by the formation of an alkylating metabolite, having an absorption spectrum identical with that of chloroethylene oxide, when vinyl chloride is passed through a mouse liver microsomal preparation (Barbin et al., 1975).

Since TCE is a structural homolog of vinyl chloride, a similar metabolic pathway and the production of TCE epoxide as an intermediate should be considered. This was proposed by Van Duuren (1975) and Corbett (1975). Such a postulation of an epoxide not only had been made as early as 1945, as indicated previously, but also was consistent with the earlier findings of McKinney (1955) on the autooxidation products of TCE.

Trichloroethylene epoxide has been synthesized by Kline and Van Duuren (1977) and by Derkosch (see: Bonse et al., 1975; Bonse and Henschler, 1976). Detection of, and thus proof of the existence of, such an epoxide in vivo has proved very difficult, primarily because of its instability, high reactivity and relatively short half-life. Presumptive evidence of its occurrence in vivo has been provided, however, by spectroscopic investigations (Uehleke et al., 1976). In Derkosch's studies, a synthesized TCE epoxide was found to have a half-life of 25 minutes in nonpolar solvents at 60°C. A much shorter half-life, 1.3 minutes, was measured by Kline and Van Duuren at pH 7.4. In-vitro, with heat, TCE epoxide thermally rearranges by migration of chlorine atoms to form dichloracetyl chloride. Opening of the oxirane-ring (epoxide) to form chloral hydrate does not occur in vitro in any considerable amount except in the presence of Lewis acids (Bonse et al., 1975). In-vivo, a somewhat different process occurs, as dichloroacetyl chloride apparently is not formed and chloral hydrate is the only observed primary intermediate, resulting from oxidation and intramolecular chlorine migration. According to Bonse et al. (1975), chloral hydrate may be evolved via formation of an oxirane (epoxide) through an interaction with the biological environment which prevents extra-molecular migration of chlorine and ring-opening to chloral, with further oxidation to trichloroacetic acid or reduction to trichloroethanol.

As discussed previously, evidence has accumulated that strongly supports the contention that TCE may be metabolized through a reactive alkylating epoxide, trichloroethylene oxide, as an intermediate metabolite. The toxicity of an epoxide depends on its chemical electrophilic properties, its rate of formation, stability against spontaneous rearrangement, rate and mode of degradation and the concentration of antioxidants in the specific system (Daly et al., 1972). All of these factors must be considered in assessing the impact of TCE epoxide on toxicity.

Additional studies, by Van Duuren and Banerjee (1976), Kline and Van Duuren (1977), Bolt et al. (1977), and Uehleke and Poplawski-Tabarelli (1977), lend considerable support to the hypothesis of epoxide formation followed by potential nucleophilic and microsomal interactions and binding. Using rat liver microsomal preparations incubated in vitro with ^{14}C-TCE, Van Duuren and his coworkers have adequately demonstrated that TCE binds covalently to microsomal protein and that trichloroethylene epoxide reacts rapidly with a variety of nucleophiles. In recent studies, Bolt et al. (1977) compared the uptake of highly-purified TCE vapor by rat liver microsomes and covalent binding to microsomal protein in vitro with that previously measured for gaseous vinyl chloride (Kappus et al., 1975).

The studies involved incubating rat liver microsomes in a closed system using atmospheres containing different concentrations of TCE vapor. Experiments were also conducted using albumin solutions and liposomal suspensions in order to study the affinity of TCE for protein and lipid structures. The results of the studies indicate that rat liver microsomes rapidly take up TCE vapor, as they do that of VC; however, certain quantitative differences were found. Relatively more TCE than VC was dissolved by the same preparation, indicating an apparently higher affinity of microsomes for TCE, with higher uptake of TCE at high TCE levels. Considerably more TCE than VC is also bound by albumin solutions and liposomal suspensions. Thus more TCE would be expected to bind to the lipid-containing membrane structures with resulting higher concentrations of TCE than of VC at the site of metabolism, especially in microsomal systems.

In the opinion of Bolt et al., covalent binding of both TCE and VC is of the same order of magnitude, which tends to support Van Duuren's theory (1975) that TCE and VC should exert similar carcinogenic effects. In this context, however, it should be noted that the primary target tissue in the liver for VC would appear to be the endothelium of the blood vessels rather than the hepatocyte. Another study, by Kelly and Brown (1974), confirmed binding of TCE to the cytochrome P-450 system. These investigators suggested that the binding was via an epoxide or other related electrophilic species.

A further assessment of the binding of ^{14}C-trichloroethylene both in vivo and in vitro has been made by Uehleke and Poplawski-Tabarelli (1977). Following intraperitoneal injection and oral gavage of TCE to male mice, radioactive-TCE became irreversibly bound to various portions of the hepatic protein, including (in order of degree of binding) that of the microsomes, mitochondria and cytosol. TCE also was bound to the hepatic lipids, both endoplasmic and mitochondrial. In the in vitro studies, the activity of rat liver microsomes was approximately 40% less than that of the mouse.

Banerjee and Van Duuren (1977) have demonstrated the covalent binding of a TCE-metabolite, most likely TCE epoxide, to DNA using in vitro methods and salmon sperm DNA. TCE was shown to covalently bind to microsomal proteins of not only the liver but also of the lung, stomach and kidney. Binding was also greater in mice than rats and in males over that of females. The significance of these observations is considered later in discussing the carcinogenicity results.

Henschler and his colleagues (Bonse et al., 1975; Greim et al., 1975; Henschler et al., 1975; Bonse and Henschler, 1976) have studied the chemical reactivity, metabolism, and mutagenicity of the chlorinated ethylene series, including vinyl chloride and TCE. They have demonstrated a rather remarkable correlation between biological activity and chemical structure in that those chlorinated ethylenes that are symmetrical (i.e., cis and trans -1,2 dichloroethylene and tetrachloroethylene) are relatively stable and not mutagenic. In contrast, the asymmetrical ethylenes, vinyl chloride, 1,1-dichloroethylene (vinylidene chloride) and TCE, are unstable and mutagenic. Although they acknowledged that oxiranes (epoxides) may be formed by all six chlorinated ethylenes, they concluded that the unsymmetrical oxiranes are far less stable than the symmetrical ones, are more highly electrophilic, and may react directly with nucleophilic constituents of cells, thereby exerting mutagenic or carcinogenic effects. The results of the mutagenic tests at this time correlate well with this structure/reactivity relationship.

Thus, there is strong presumptive evidence for the existence of TCE epoxide as an important intermediate step in TCE metabolism, and for the covalent binding of TCE metabolites to tissue macromolecules, including sulfhydryl nucleophiles. This is of great toxicological significance because of the possibility that alkylation of nuclear macromolecules may also occur. Further research is needed to confirm actual binding to nucleic acids under in vivo conditions. It should also be borne in mind that actual nuclear interactions may not necessarily be a prerequisite to cancer induction. Interaction between certain chlorinated hydrocarbons and the ergastoplasm, i.e., the endoplasmic reticulum holding nucleoprotein in the ribosomes attached to it, appear to result in nodule formation in the liver. Such nodules may develop into malignant tumors.

In order to produce a toxic effect, not only must a toxic chemical be present but it must also make contact with a vulnerable target tissue. Biotransformation processes may either enhance or decrease toxicity. The toxic effect, including carcinogenicity, may well depend upon the effectiveness of such detoxification mechanisms in inactivating reactive metabolites, including a possible epoxide. Indeed, studies by Moslen et al. (1977) suggest that hepatotoxic effects of TCE are related to glutathione loss and deactivation of cytochrome P-450 so that vulnerable cellular constituents are exposed to the activity of electrophilic TCE metabolites that might be formed. This suggestion, if true, would indicate a dose-dependent effect, low doses causing less effective depletion than higher ones. Such a relationship has been suggested for vinyl chloride as discussed earlier. As of this time this idea is no more than speculation as applied to TCE. Additional studies are necessary to verify that this can occur.

Along this same line, a reactive epoxide may be inactivated by a hepatic epoxide hydrase. Oesch et al. (1974), not only demonstrated such hydrase activity, but also compared the levels of humans with that of laboratory animals. Their results indicated that the hydrase activity in humans was 4 times that of mice, 2 times that of rats, about the same as that of guinea pigs, and markedly lower, about one-third, that of Rhesus monkeys. Styrene oxide was used as the substrate; however, Oesch and his colleagues have shown that the same purified epoxide hydrase can hydrate a large variety of other alkene and arene oxides.

Based upon this review the metabolic scheme presented in Figure 1 is proposed. It differs from that presented in the NIOSH criteria document only by describing events occurring in the conversion of TCE to chloral hydrate.

C. CARCINOGENIC POTENTIAL

On March 21, 1975, the National Cancer Institute reported preliminary results of a carcinogen bioassay which indicated no carcinogenic effects in rats but the induction of a highly significant number of hepatocellular carcinomas in both male and female mice (Saffiotti, 1975). These preliminary results were later confirmed and described in great detail (NCI, 1976).

An extensive literature review has not produced information on any other adequately conducted carcinogen test. Waters et al. (1977) listed four other toxicity studies, none of which revealed evidence for carcinogenicity (Adams et al., 1951; Mosinger and Fiorentini, 1955; Rudali, 1967; and Seifter, 1944). All, however, had major design weaknesses such as an inadequate period of exposure or observation, too few animals, and, in three studies, no deaths or data reported on any of the animals. None

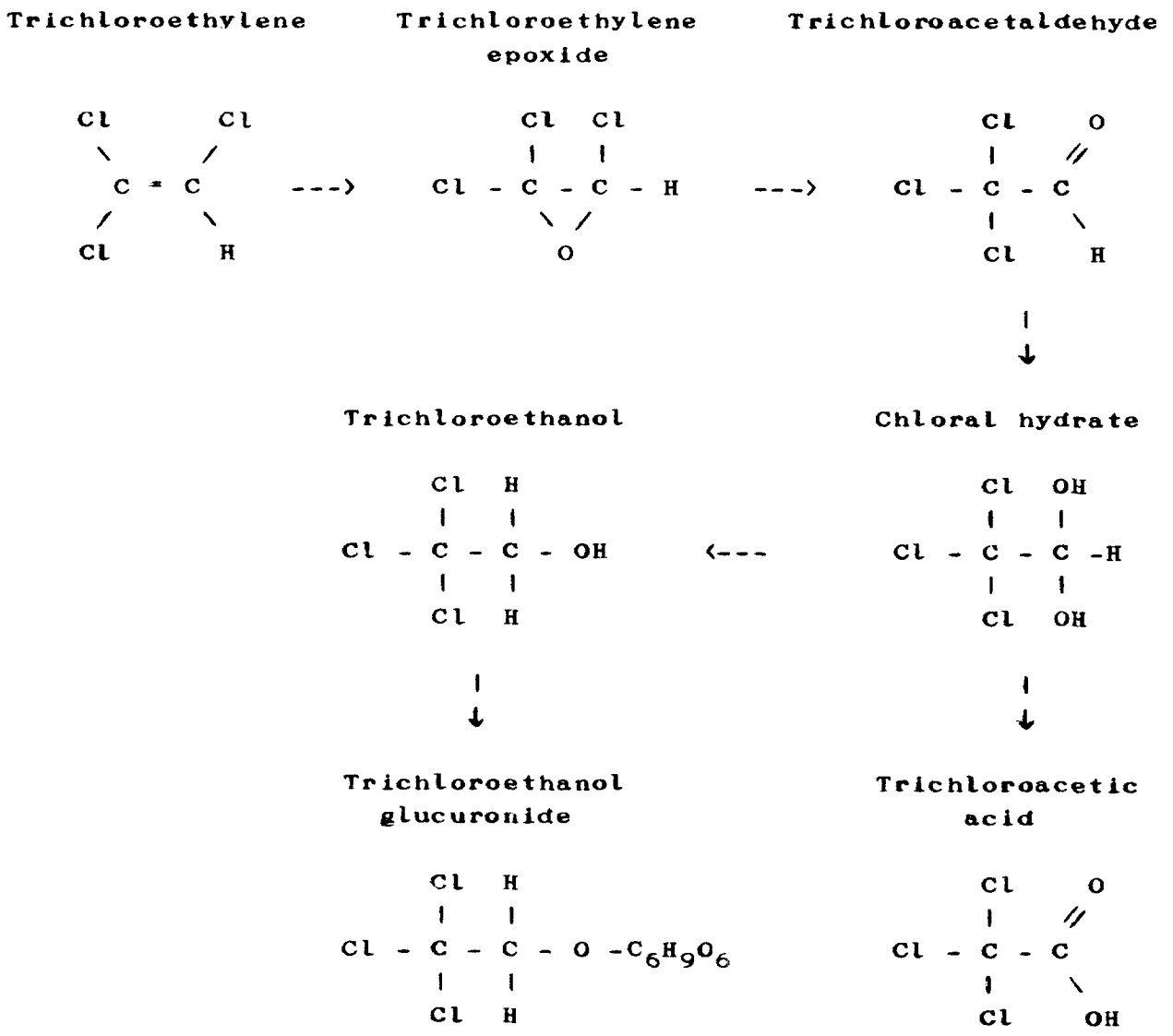

Figure 1. Proposed metabolism of trichloroethylene

can qualify as an adequate carcinogenicity test and will receive no further consideration in this review. They are, however, summarized in Table 4.

A major carcinogenicity study is now underway, administered by the Manufacturing Chemists Association (Olson 1975a, 1975b; Clark 1977a). In these studies, male and female Charles River albino rats and $B6C3F_1$ mice are exposed to TCE (same commercial product tested by the NCI) by inhalation for a period of 24 months, beginning as young adults. Three dose levels, 100, 300, 600 ppm, are used with exposures for 6 hours per day, 5 days per week. Preliminary results, based upon rats and mice dying up to

21 months, and mice killed at 24 months, indicate an effect similar to that reported by the NCI, i.e., no evidence of tumorogenicity in rats with an apparent induction of liver cancer in mice (Clark, 1977b, 1977c).

Until the study supported by the MCA is completed, the only animal study that can be utilized in an evaluation of the carcinogenic potential of trichloroethylene is the one conducted for the NCI. The design can be summarized as follows: Rats and mice of both sexes were exposed once per day, 5 days/week to TCE, administered in corn oil by stomach tube, at two dose levels, beginning as weanlings and continuing for 78 weeks (18 months). At the end of 90 weeks (mice) or 110 weeks (rats), surviving animals were killed, necropsied, and submitted to an extensive gross and microscopic examination. Those dying or killed prior to the scheduled termination date were examined in the same manner. A total of 480 animals were employed in the chronic exposure study, consisting of groups of 50 TCE-exposed animals and of 20 controls (Table 5).

The observed incidences of hepatocellular carcinoma in mice were dose-related and highly significant ($p < .01$) at both dose levels in male mice and at the higher level in female mice (Table 6). The increase observed in low dose female mice was found to be significant at a p-value of 0.09.

A greater degree of confidence would be obtained if the control data for the entire colony were used, as opposed to the matched controls. The observed tumor incidences of 5% and 0% in the matched male and female control mice compares very favorably with incidences of 8.7% and 1.7% observed in over 2,000 colony controls of the same strain used in similar experiments by the NCI, (Page, 1977).

In reviewing these NCI test results, the response of male mice appeared to be greater than that of the female, not only in total incidence of hepatocellular carcinomas but also in shorter latency and in larger numbers of metastases to the lungs. These sex differences may have been more apparent than real, however, in as much as spontaneous hepatocellular carcinomas normally are not only of higher incidence in male controls but also appear earlier and metastasize with greater frequency.

As regards latency period, no hepatocellular carcinomas were found in female mice prior to the 90-week terminal killing. In comparison, 15 exposed males that died during the 90-week period had hepatocellular carcinomas. The first liver tumor was diagnosed at autopsy in a male mouse of the high-dose group that died after 27 weeks of exposure, with three others seen by the 36th week and six more by the end of the 78th week of exposure. By contrast, in the low-dose group, the first liver tumor was not observed until after 81 weeks in male mice. Thus, in male mice,

Table 4. Chronic toxicity studies of TCE reported in the literature other than the NCI studies

Species (Reference)	Route of Exposure	Exposure Regimen	Number Animals	Results	Comments
Mice (Rudali, 1967)	Gastric Intubation	0.1 ml in oil 2x/wk for unspecified period	28	No Tumors No Deaths	Insufficient data to judge adequacy - presumed to be inadequate
Rats	Inhalation	3000 ppm, 27 exposures	12	3 rats died No Tumors	Insufficient period of exposure/observation (too short for carcinogenicity study)
Guinea Pigs	"	100 ppm, 132 exposures	11	No data	
Monkeys	"	200 ppm, 148 exposures	2	No data	
Rabbits (Adams et al., 1951)	"	200 ppm, 178 exposures	4	No data	
Dogs (Seifter, 1944)	Inhalation	150-750 ppm in air; 20-48 hr/wk for 7-16 wks	16	No Tumors No Deaths	Insufficient period of exposure/observation
Cats (Mosinger and Fiorentini, 1955)	Inhalation	200 ppm for 75 min/day for 6 months	8	No Tumors No Deaths	Insufficient period of exposure/observation. Too few animals.

Table 5. Experimental design - NCI carcinogen bioassay of trichloroethylene

Experimental design		Experimental Groups	Dose levels (mg/kg/day)*
Mice ($B6C3F_1$)		Males:	
Route of exposure:	Intragastric Intubation	Controls	0
Treatment mixture:	10-24% TCE in Corn Oil	Low Dose	1169
Frequency of exposure:	Once Daily, 5x/Week	High Dose	2339
Duration of exposure:	78 Weeks	Females:	
Additional observation:	12 Weeks	Controls	0
Total period:	90 Weeks	Low Dose	869
Microscopic examination:	About 30 tissues/all animals	High Dose	1739
Rats (Osborne-Mendel)		Males:	
Route of exposure:	Intragastric Intubation	Controls	0
Treatment mixture:	60% TCE in Corn Oil	Low Dose	549
Frequency of exposure:	Once Daily, 5x/Week	High Dose	1097
Duration of exposure:	78 Weeks	Females:	
Additional observation:	32 Weeks	Controls	0
Total period:	110 Weeks	Low Dose	549
Microscopic examination:	About 30 tissues/all animals	High Dose	1097

Reference: NCI, 1976

* Time-weighted average doses. Actual doses listed below:

Mice (M) - 12 weeks at 1000/2000 mg/kg/day; 66 weeks at 1200/2400 mg/kg/day.
Mice (F) - 12 weeks at 700/1400 mg/kg/day; 66 weeks at 900/1800 mg/kg/day.
Rats (M/F) - 7 weeks at 650/1300 mg/kg/day; 9 weeks at 750/1500 mg/kg/day; 62 weeks at 500/1000 mg/kg/day.

Table 6. Incidence of hepatocellular carcinoma in $B6C3F_1$ mice exposed to trichloroethylene

Males		
Matched controls	1/20 (05%)	
Low dose	26/50 (52%)	(P = 0.004)
High dose	31/48 (64%)	(P < 0.001)
Females		
Matched controls	0/20 (0%)	
Low dose	4/50 (8%)	(P = 0.090)
High dose	11/47 (23%)	(P = 0.008)

Reference: NCI, 1976.

the latency period for the appearance of tumors was shorter in the group receiving the higher dose level.

The incidences of no other types of tumors in mice and of no tumors in rats were significantly increased (p=.05) above those observed (and expected) in the control groups, although there were increased incidences of malignant lymphoid tumors 17/195 versus 2/40) and pulmonary tumors (18/195 versus 1/40) in mice. No hepatocellular carcinomas, like those diagnosed in mice, were observed in any of the exposed rats.

The NCI report acknowledges that there were several design features that may have exerted a modifying or contributing role in the experiment. The NCI followed the recommendations of expert panels on carcinogenesis testing (Page, 1977) in employing high-dose levels so as to provide maximum sensitivity in the screening assay. Supposedly "maximum tolerated" doses were chosen after an 8-week range-finding study. Unfortunately, the doses were not as well tolerated as had been predicted and considerable toxicity was evident. In an attempt to achieve the "maximum tolerated dose," some subsequent minor downward adjustments were made in dose levels or frequency of administration.

Mice developed alopecia, skin sores, and a hunched appearance after a few weeks exposure. Abdominal distension, attributed to developing liver pathology, was noticed after 50 weeks. Although toxicity was clinically evident, the mortality rate by 90 weeks was not sufficient to reduce seriously the effective number of animals.

Survival of both TCE-exposed and control rats was poor at 110 weeks, 82% of male controls and 60% of the control females

having died by that time compared with 89 and 73% of the exposed male and female rats, respectively. In addition to an apparent exposure-related chronic nephropathy, the animals were afflicted with chronic respiratory disease. The renal pathology consisted essentially of degeneration of the epithelium of the proximal tubule, seen in the TCE-exposed animals only. No significant changes in the structure of the liver were observed. The NCI investigators concluded that the high mortality among the rats detracted from the usefulness of the experiment with that species in detecting carcinogenic potential.

Clinical signs of toxicity were observed in all exposed groups of rats, beginning in the first year and increasing in frequency progressively thereafter. Among the signs observed were rough haircoat, skin sores, reddish discharge from eyes, and a hunched appearance. While survival of all rats was poor, the exposed animals died earlier.

In addition to TCE-exposed animals, groups of animals to which other volatile chemicals had been administered were housed in the same room. Although the ventilation in the room conformed to the recommendations of the Institute of Laboratory Animal Resources (ILAR, 1976), it is possible that the TCE-exposed animals were also subjected to very low-level exposures to several other chemicals.

It should be pointed out that the NCI test had serious limitations as the ultimate test for the carcinogenic potential of TCE. As the exposure began when the animals were young adults, no assessment for transplacental carcinogenesis can be made. Tissues of the fetus or newborn animal are generally regarded as more sensitive to chemical carcinogenesis than those of older offspring. The tests were terminated at 90 and 110 weeks for mice and rats, respectively. It is possible that late developing tumors might have been observed had the animals been allowed to survive until they died or the study terminated after a longer period of observation. Over 80% of exposed and control mice and 70% of control rats were alive at time of termination. It should be noted that several expert committees have recommended that exposure begin prior to conception, continue during pregnancy, with exposure to offspring for life, especially for those chemicals to which the fetus may be exposed (FDA, 1971; Tomatis, 1974; Canada, 1975). The FDA further recommended that studies not be terminated until cumulative mortality has reached 75% in a group showing negative results. They also consider that sample sizes greater than 40-50 per group are required for testing weaker carcinogens. The NCI and MCA studies both suffer from failure to follow these guidelines.

Another design weakness of the NCI test, detracting from its sensitivity, was that the exposure levels were too high in the rat study, such that less than 15% of male rats and 25% of female rats survived after 110 weeks.

While these deficiencies in basic design and performance detract somewhat from the confidence that one might attach to the NCI study, the differences observed in liver cancer rates between exposed and control mice are real. At this point, one must conclude that a carcinogenic potential in the mouse has been demonstrated for TCE under the test conditions. It then becomes necessary to examine several scientific issues to assess the probability that TCE would represent a cancer risk in the workplace under normal conditions of exposure.

Species Differences:

Differences in species responses to chemical carcinogens can often be attributed to differing metabolic pathways and metabolites, and to an inability of some species to effectively convert the test chemical to an active carcinogen. The high sensitivity of the $B6C3F_1$ mouse and the low sensitivity of the NCI strain of Osborne-Mendel rat (Table 7) not only to TCE but to the positive control, carbon tetrachloride (NCI 1976), and to tetrachloroethylene (NCI 1977b) indicate innate species differences in sensitivity to chlorinated aliphatic compounds.

Henschler (1977) is of the opinion that the difference in response of rats and mice might be attributed to the comparatively low activity of epoxide hydrase in mice, i.e., the decreased ability of the mouse to detoxify an epoxide, whether the product of metabolism or its presence in the administered TCE. Another obvious possibility is that of a difference in the degree of epoxide formation between the two species. Indeed, Banerjee and Van Duuren (1977) recently demonstrated, using in vitro methods, differences in the metabolism of TCE by the $B6C3F_1$ mouse and the Osborne-Mendel rat used in the NCI study. These differences agree well with the NCI carcinogenicity test results. The binding of TCE to liver microsomal protein was 46% higher in male $B6C3F_1$ mice than in the male Osborne-Mendel rat. Binding of TCE to liver microsomal proteins was 37% higher for males than that for female mice. Covalent binding of TCE to DNA was also much higher for microsomal proteins from male mice than from females. These differences correlate with the carcinogenic response in mice but not rats and a greater response in male mice over that of female mice in the NCI test. In an ancillary study, Banerjee and Van Duuren (1977) also substantiated the role of epoxide hydrase in the interaction of TCE and DNA. Enhanced covalent binding of TCE to DNA resulted from the addition of a known inhibitor of epoxide hydrase.

Based on the above observations, the greater metabolism of TCE, the larger covalent bindings of TCE to microsomal protein and to DNA, and the lower level of epoxide hydrase in mice are all factors which may have contributed to the greater carcinogenic response in the $B6C3F_1$ mouse, than in the Osborne-Mendel rat.

Table 7. Incidence of hepatocellular carcinoma in rats exposed to trichloroethylene (TCE), tetrachloroethylene (PCE), chloroform ($CHCl_4$), and carbon tetrachloride (CCl_4).

	Male rats			Female rats		
	Controls	Low dose	High dose	Controls	Low dose	High dose
TCE	1/99	0/50	0/50	0/98	0/48	0/50
PCE	1/99	0/49	0/49	0/98	0/50	0/50
MCh	1/99	0/50	0/50	0/98	0/50	0/50
CHCL3	1/99	0/50	1/50	0/98	0/49	0/48
CCL4	1/99	2/50	2/50	0/98	4/49	1/49

While it is possible that the epoxide hydrase level in humans is greater than that of rats and mice (Oesch et al., 1974), additional research is needed to compare the rate of formation, rate of detoxification and ultimate reactivity or alkylating potential of a carcinogenic chemical that might be formed in the metabolism of TCE by humans.

Significance of Mouse Liver Cancer as an Indicator of Carcinogenic Potential to Man:

One of the most controversial issues in cancer research in the past decade has been that of the relevance of liver cancer induction in mice as a predictor of carcinogenic potential in man. Indeed, in an effort to resolve the many uncertainties, two major working conferences have been held; in 1969 (IARC, 1971), and 1975 (Butler and Newberne, 1975). Neither has been able to provide clear guidance in the application of such data to risk assessment.

Challenges to the biological significance of mouse liver cancer and even to the use of the mouse in carcinogen testing have been primarily from the English pathologists Butler, Grasso, and Roe (Butler, 1971; Grasso and Crampton, 1972; Grasso and Hardy, 1975; Roe and Tucker, 1974). Recent support for their position has been expressed by Newberne (1974) of the United States.

In considering this issue, the World Health Organization in 1973 (WHO, 1974) agreed that "there is a serious lack of knowledge regarding the processes involved in the development of liver tumors by mice and that it would be unwise to classify a substance as a carcinogen solely on the basis of evidence of an increased incidence of tumors of a kind that may occur spontaneously with such a high frequency". In reviewing the basis for this conclusion, it appears that the concern is for use of animals in which the spontaneous incidence of liver tumors is very high. In the present case it does not appear that the 1.7 and 8.7% spontaneous incidences in female and male mice used by the NCI would engender this concern. In addition, the discussion cited largely pertains to hepatic nodules, considered benign liver tumors, rather than to hepatocellular carcinomas.

The issue of terminology may be more one of semantics than of substance, as the typical hepatocellular carcinoma diagnosed in the NCI study was of nodular appearance, and although it rarely invaded surrounding tissues, it frequently metastasized to the lungs. On the basis of both morphology and metastasis, they are truly malignant.

In the NCI study, approximately 12% (7 of 57) of the hepatocellular carcinomas in male mice were observed to have metastasized to the lungs. Of the 15 carcinomas in exposed female mice or the single hepatocellular carcinoma in control animals, none was found to have metastasized. The extent of

metastasis, however, was judged on the basis of single sections through each lung. There can be little doubt that a more thorough examination would be required to detect rare or early lesions, including metastatic foci of liver tumors in the lung. Kyriazis et al. (1974) illustrated the inadequacy of a cursory examination to detect such small lesions. A simple examination of randomly selected lung lobes revealed only 4% and 0% metastatic foci of diethylnitrosamine-induced hepatocellular carcinomas in male and female $B6C3F_1$ mice, respectively. Sections of the whole lung at two different levels detected foci in over 20% of animals with liver cancer. Additional sections may well have revealed an even higher rate of metastasis.

Pathologists have found it difficult to reach a consensus in the classification of liver tumors, and Terracini et al. (1973) stated that strict criteria for distinguishing between benign and malignant liver cell tumors were not available. Grasso and Hardy (1975) stated that the meaning of the term hepatoma in experimental cancer research is equivocal so that the reader is never certain whether investigators are using it to indicate benign tumors or hepatic cell carcinomas.

Andervont and Dunn (1952) transplanted hepatomas in mice and attempted to correlate the histologic structure of primary hepatomas with their ability to propagate in new hosts. Microscopic examination failed to reveal any consistent morphologic differences between tumors which did and did not grow.

In a dose response study of Dieldrin, Walker et al. (1973) distinguished two types of liver tumors. Benign hepatomas, or type A tumors, consisted of solid cords of closely packed parenchymal cells differing little in morphologic and staining characteristics from the rest of the parenchyma. The mitotic index was low and the lesion appeared to be growing by expansion with compression of normal adjacent liver parenchyma. Malignant, or type B, hepatomas were distinquished from type A by the presence of areas of papilliform or adenoid formations of liver cells with wide and irregular vascular channels. Nuclei showed a variable increase in size and enlarged nucleoli. The mitotic index was frequently increased and multinucleated forms were seen. Cytoplasmic alterations included hydropic and fatty changes and hyaline-droplet formation. Areas of necrosis were also present. Metastases were found in association with type B lesions only. In most other published series the incidence of metastases also was low.

Walker et al. (1973) did not distinguish between a hyperplastic nodule and a benign hepatoma. Lemon (1967) indicated that some weight should be given to the presence of portal tracts within the nodule in making the distinction between spontaneous nodular hyperplasia (small hepatoma) and an induced tumor.

Farber (1976) and Miller and Miller (1976), have reviewed the pathogenesis of liver cancer. From their reviews, it appears that the majority of experimental studies on the pathogenesis of chemically induced liver cancer have implicated proliferative stages, such as hyperplastic nodules, as probable precursor lesions.

Grasso and Crampton (1972) were of the opinion that induction of liver tumors in mice did not indicate chemical carcinogenicity, no matter what the tumor class. Reasons advanced were: possible presence of oncogenic viruses, unpredictable background or high spontaneous incidence of such tumors in untreated controls, and the ease of induction by carcinogens and other agents. Their concern seems to have been more related to the interpretation of benign lesions, often called hepatic nodules or hepatomas, rather than to that of such hepatocellular carcinomas as were observed in the NCI experiments.

Grasso and Hardy (1975) proposed that tissue injury and repair may be important in the development of hepatic nodular lesions. As examples they cited the efficacy of CCl_4 and chloroform in producing extensive necrosis as well as apparently increased incidences of liver tumors. Other factors, e.g., sex hormones and diet, have been suggested as possible modifiers of the carcinogenic activities of primary carcinogens and perhaps even of the natural occurrence of spontaneous liver tumors.

To evaluate this problem, Tomatis et al. (1973) surveyed the available literature and compiled a list of chemicals reported to induce liver tumors in mice. They then searched for test results in the rat and hamster with this list of chemicals in order to assess the correlation of induction of liver tumors in mice with the capability of the chemical to also induce tumors in the other two species. From this survey of 58 chemicals, a strong positive correlation existed when the chemical induced tumors of the liver in the mouse and also induced tumors in the liver or at another site in at least one of the other two species. Among the 58 chemicals considered, 7 were recognized or suspected to be human carcinogens. All seven were hepatocarcinogenic in the mouse and six were carcinogenic in the liver and/or other organs in the rat. Of the 58, 51 had been tested in rats and 29 in hamsters, of which 78% and 79% were positive, respectively in these species. Negative results were obtained in both rats and hamsters for only one chemical. Tomatis et al. concluded that the induction of liver tumors in the mouse should be considered to be as valid as the evidence obtained in the rat and/or the hamster at any site. These reviewers also demonstrated that target organs may differ in the rat and/or hamster (and perhaps man) from that or those in the mouse. Saffiotti (1977) undertook a critical evaluation of the tests used by Tomatis et al., and concluded that the correlation was even stronger than stated by the latter authors. In Saffiotti's opinion, there is no

scientific basis to support the suggestion that the carcinogenicity of a chemical in mice is not representative of its activity in other species, including man.

As indicated in the preceeding discussion, many eminent scientists have disagreed about the validity of results obtained in the mouse for predicting carcinogenic activity in man. The opponents claim: (1) that factors unconnected with the administration of the test compound may have a considerable influence on the incidence of hepatomas in mice and (2) that a mechanism may be responsible for the effect which is unlikely to occur in humans exposed to the chemical at lower levels. In their opinion, additional evidence is required if claims of carcinogenicity based upon the induction of this type of tumor are to be seriously considered.

In contrast, proponents of the use of the mouse can point to the many results first obtained in the mouse which were later confirmed with other species, including humans. The mouse was the first animal used to demonstrate the carcinogenicity of coal tars, polynuclear aromatic hydrocarbons, azo dyes, aromatic amines, estrogenic hormones, and carbon tetrachloride. The mouse has been accepted by carcinogenesis researchers for years as the species of choice. As pointed out by Saffiotti (1977), serious questioning has arisen only after findings of carcinogenicity of organochlorine pesticides. The mouse, like the rat and other laboratory species, has strengths and limitations as an experimental model. Resolution of this controversy is beyond the scope of this review. However, we have chosen to adopt the approach of gathering all additional data that might either support or refute the animal bioassay results.

It should be noted, that while this discussion has focused on the induction of mouse liver tumors, a similarity exists in the interpretation of induced lung tumors of the mouse, mammary tumors in the rat and a number of other spontaneously occurring tumors in laboratory animals and humans. It should not be expected that an index for carcinogenicity is the induction of a tumor-type never observed as a spontaneously occurring one as the cell populations transformed are the same and since many so-called spontaneous tumors are possibly related to exposures to low-levels of chemicals in the animals' environment.

Route of Exposure:

In the NCI study, TCE was administered by gastric intubation whereas occupational exposures are predominantly by the inhalation and dermal routes. While the NCI did not measure gastrointestinal absorption, it is likely that a high concentration of TCE reached the liver via the portal circulation for a comparatively short time each day following intubation, as TCE is readily absorbed from the intestinal tract (Fabre and Truhaut, 1952; Barrett and Johnston, 1939). Because tumors

appeared outside the intestinal tract, absorption of TCE and then systemic exposure of all tissues to TCE can be assumed. However, the relative distributions to other organs were not measured and would be difficult to estimate. It is worthy of note, however, that absorption from the gastrointestinal tract is the route that is the most likely to ensure that metabolites of TCE, rather than TCE itself, impinge upon not only the liver, but also other organs and tissues of the body. Here, the balance between activation and detoxication becomes of great importance also.

The kinetic relationships of absorption and distribution of TCE following inhalation and oral exposures are likely to be of a similar qualitative nature, with the liver the main organ responsible for biotransformation and detoxication in both situations. In the case of a chemical absorbed readily from either the lungs or the intestinal tract, and with an effect of a systemic nature, or at a site different than the point of initial contact or absorption, the results obtained with one route may be applicable generally to the other. NIOSH considers that the NCI test is appropriate for assessing the carcinogenic potential of TCE via inhalation. The inhalation studies underway, administered by the MCA, may provide useful data in assessing the route of exposure as a modifier of potential carcinogenicity of TCE. Preliminary results, recently released by the MCA, indicate a carcinogenic effect in mice similar to that found by the NCI after gavage.

Selection of Dose Levels:

Dose levels selected were to be the highest consistent with long-term survival. Such high doses have been referred to as "Maximum Tolerated Doses" in accordance with the methods proposed by Weisburger and Weisburger (1967). While such high levels may increase the probability of a tumorigenic response by the test system, objections to such levels are that they may introduce toxic conditions that interfere with survival and the carcinogenic process, or that they may introduce atypical metabolites by saturation of the usual metabolic pathways.

As indicated earlier, the doses used in the NCI study were too high, so that survival of the rats was poor and, consequently, the ability of the test to detect carcinogenicity was comparatively low. As regards the second objection to the use of high-dose levels, dose modifying effects on the pharmacokinetics of retention, metabolism and elimination have been demonstrated for vinyl chloride and dioxane (Gehring et al., 1976); however, an insufficient number of studies have been performed along this line to permit generalizations in this regard. As previously discussed in the metabolism section, Gehring et al. did not demonstrate qualitative or quantitative changes in metabolites as the exposure was changed. Unless a pathway is demonstrated to be the only one responsible for the

carcinogenic effect, and unless that pathway is demonstrated to be completely lacking at low doses, then the modification of metabolism can only relate to some relative quantitative differences (of questionable value in extrapolation) and not an "all-or-none" effect. Data relevant to the NCI bioassay studies are not yet available.

Role of Exposure to Chemicals Other Than TCE:

TCE-exposed animals may have been exposed also to low levels of known carcinogens by way of additives or contaminants in the commercial grade TCE, the air, water, or the feed.

Trichloroethylene used in the NCI test was of a commercial grade, 99% pure, with a number of minor contaminants or additives. Those identified were 1,2-epoxybutane (0.19%), ethyl acetate (0.04%), epichlorohydrin (0.09%), N-methylpyrrole (0.02%) and diisobutylene (0.03%). Epichlorohydrin has carcinogenic and mutagenic potential (NIOSH 1977c), as does 1,2-epoxybutane (Walpole, 1958; Rosenkranz and Speck, 1975; McCann and Ames, 1976). Henschler et al. (1977) analyzed a sample of the TCE used by the NCI and identified contaminants amounting to 0.65%, including 0.22% epichlorohydrin and 0.20% epoxybutane.

Henschler et al. (1977) are of the opinion that the carcinogenic effect observed in the NCI study was predominantly, if not exclusively, due to the epoxides present in the TCE sample used in the test. While this possibility cannot be discounted, it should be noted that the TCE used in several positive mutation and cell transformation tests was highly-purified and without the epoxide stabilizers. An additional argument against implicating the epoxide stabilizers is that other chlorinated chemicals, e.g., tetrachloroethylene, have induced the same tumor response in mice and did not contain the epoxide stabilizers. While we are of the opinion that the carcinogenic effect is primarily related to exposure to TCE itself, the possible additive or modifying effects of other chemicals is recognized. As the TCE utilized in the MCA studies was the same grade as that used in the NCI test, possible effects of minor contaminants is also an issue.

In addition to TCE, 17 other chemicals were on test in the same room housing the TCE-exposed mice. Included in the group were five other chemicals which have carcinogenic potential: carbon tetrachloride, chloroform, tetrachlorethylene, 1,2-dibromo-3-chloropropane and 1,2-dibromoethane. The first three have been known to induce liver cancers similar to those observed in the TCE-exposed mice. The mice were maintained in filtered cages, the air in the room was changed 10-15 times per hour, and the chemicals were administered under a hood in a separate room. Even under these conditions it is possible that certain amounts of chemicals other than TCE were exhaled or excreted, so that contamination of the air within the room by low

concentrations of other toxic substances may well have existed throughout most or all of the exposure to TCE. Sansone et al. (1977) have documented the spread of chemicals, mixed in feed, within and between animal rooms even when cages with filters and high-level containment practices were used. The escape and movement of volatile chemicals might be even greater than particulate matter. Thus, the TCE-exposed mice may well have been exposed also to low levels of other carcinogenic chemicals under test in the same room, especially to those of a relatively volatile nature. As no environmental measurements were made, these levels can not be estimated.

Artesian well water was provided to the mice ad libitum. This contained low levels of nitrate, nitrite, chloride, chromium and many other chemicals commonly present in water supplies. As the water was not chlorinated, the concentrations of chloroform or other chlorinated carcinogens were probably quite low. Very low levels of DDT, DDE, Aroclor 1254, and Dieldrin were present in some samples of the commercial feed used in the study.

The NCI concluded that there was no evidence that either the housing or the presence of other chemicals in the same room modified the results of the experiment. This conclusion was based on the lack of an unusual incidence of hepatocellular carcinomas in the controls kept in the same room, a dose-response relationship in the TCE-exposed groups, and the thousand-fold increase in dose levels achieved by gastric intubation versus that possible by any inadvertent inhalation of contaminants.

The possible role that exposure to these very low levels of other chemical carcinogens may have played in the NCI study is impossible to assess. As the controls were also exposed to the same air, diet, water, and room environment and since some tests conducted along with that of TCE did not reveal carcinogenic activity (NCI 1977a), a major effect of such minor exposures may be viewed as highly unlikely. However a cocarcinogenic effect or possible stimulatory activity on liver enzymes cannot be discounted. Other chemicals which affect the liver, such as phenobarbital and DDT, have been reported to be able to induce a significant enhancing effect with another liver carcinogen, 2-AAF (Peraino et al., 1975). The concentrations used in those studies were far in excess of those of the contaminant or incidental chemicals in the NCI tests.

Assessment of NCI Carcinogenicity Test:

The criteria for carcinogenicity developed by the National Cancer Advisory Board (NCAB, 1977) have been met by the NCI bioassay test in that: (1) the studies were adequately designed and conducted, (2) the increases in incidences of neoplasms were statistically significant, and (3) the only major experimental variable between control and experimental groups was the absence or presence of the test agent. In addition, positive results

were observed in more than one group and the occurrence of neoplasms followed a dose-dependent relationship. In NIOSH's opinion, the use of high-dose levels, the gavage route of exposure, and the low-level contamination of air, food, and water with other chemicals do not negate the validity and usefulness of the test results as an indicator of carcinogenic potential. The NCI studies were designed in accordance with recommendations of expert international and academic committees and were conducted according to laboratory standards existing at the time.

Comparison of TCE Results With Studies of Other Organochlorine Chemicals:

Trichloroethylene was placed on carcinogenic bioassay as one of a series of short-chain organochlorine compounds. As of this time, results with four other structurally related chlorinated chemicals have been reported. These are carbon tetrachloride, chloroform, tetrachloroethylene and methyl chloroform. Of these, the first three induced hepatocellular carcinomas much like those induced by TCE. In addition, chloroform induced kidney cancers also. Methyl chloroform was tested at levels higher than used for the other chemicals. However, the mortality was excessive, which detracted from the confidence in that study. Table 8 presents information on this series of studies. While it is difficult to compare the results of these studies because of possible differences in absorption and mortality, it would appear from Figures 2 and 3 that the relative potency of TCE, as compared with those of the other chemicals capable of inducing liver cancer, may be somewhat less. This is based on the higher incidences of liver tumors at lower dose levels for the other three chemicals, and with the exception of the high-dose males in the TCE study, a shorter latency period.

D. MUTAGENICITY

Following the announcement of positive carcinogenic results by the NCI, considerable research was initiated to assess the mutagenic potential of TCE using in vitro systems. Initial tests proved negative. However, with the use of more sensitive test systems, to which metabolic activation systems were added, mutagenicity was demonstrated in four test systems: two bacteria, Salmonella typhimurium and E. coli, in yeast and in Tradescantia. In none of these systems, however, was a particularly strong response observed.

Simmon et al. (1977) found TCE to be weakly mutagenic in the S. typhimurium (Ames) bacterial system, using the TA100 tester strain coupled with liver homogenate. While positive results were obtained with Sprague-Dawley liver S-9 mix, the response was greater using liver homogenate obtained from male $B6C3F_1$ hybrid mice. The assay plates were placed in 9-liter

Table 8. A comparison of NCI carcinogenesis bioassay tests of trichloroethylene (TCE). tetrachloroethylene (PCE), methyl chloroform (MCh), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4).

Chemical/expt'l group		Dose levels[1] (mg/kg)		Percent alive at 78 weeks		Hepatocellular carcinomas in mice	
		Rats	Mice	Rats	Mice	Percent incidence[2]	Time to 1st tumor (wks)
TCE	Males						
	Low dose	549	1169	60	80	52	81
	High dose	1097	2339	24	48	65	27
	Females						
	Low dose	549	869	40	82	8	90
	High dose	1097	1739	46	45	23	91
PCE	Males						
	Low dose	471	536	43	53	65	27
	High dose	941	1072	14	25	56	40
	Females						
	Low dose	474	386	50	25	40	41
	High dose	949	772	42	17	40	50
$CHCl_3$	Males						
	Low dose	90	138	78	86	36	80
	High dose	180	277	54	81	98	54
	Females						
	Low dose	100	238	56	86	80	66
	High dose	200	477	50	72	95	67
MCh	Males						
	Low dose	750	2807	2	42	0	-
	High dose	1500	5615	4	28	2	50
	Females						
	Low dose	750	2807	18	56	0	-
	High dose	1500	5615	24	28	0	-
CCl_4	Males						
	Low dose	47	1250	68	22	100	48
	High dose	94	2500	68	4	98	26
	Females						
	Low dose	80	1250	76	25	100	16
	High dose	159	2500	42	9	96	19

[1] Chemicals were administered by stomach intubation at predicted maximum tolerated dose levels for 78 weeks and observed for an additional 12 weeks (mice) or 32 weeks (rats).

[2] Incidence in all animals at end of experiment, i.e., 90 weeks for mice and 110 weeks for rats. Colony control incidence (n=2208) of hepatocellular carcinoma in B6C3F1 mice: Males = 8.7%; Females = 1.7% (Page 1977).

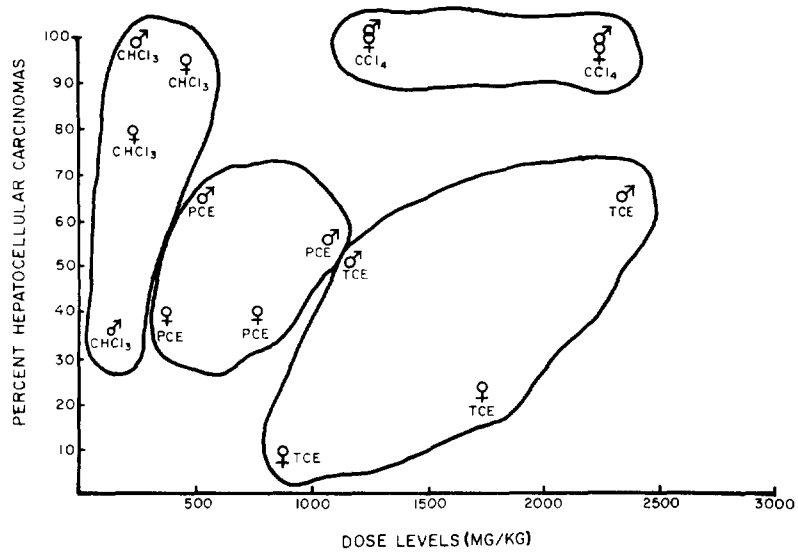

Figure 2. Relationship of hepatocellular carcinoma incidence with dose levels for trichloroethylene (TCE), tetrachloroethylene (PCE), chloroform ($ChCl_3$), and carbon tetrachloride (CCl_4).

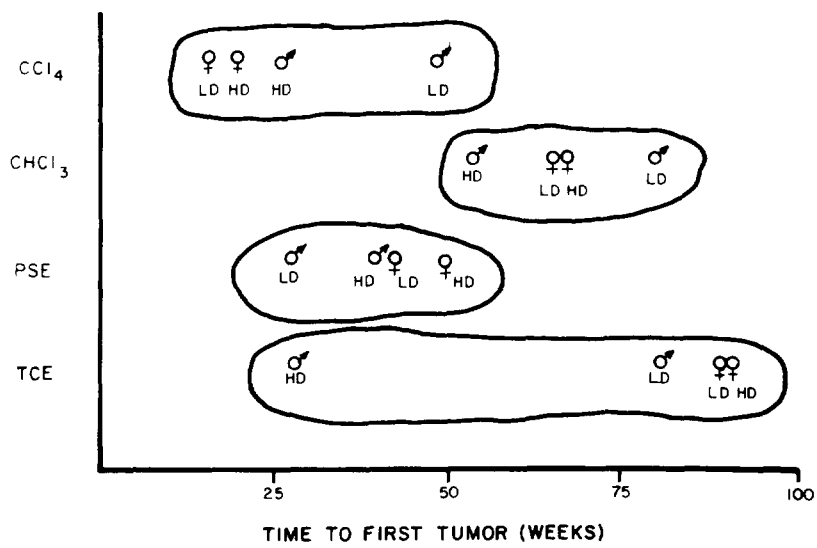

Figure 3. Initial tumor appearance with TCE, PCE, $ChCl_3$, and CCl_4.

desiccators and subjected to concentrations of up to 2,000 ppm of TCE for about 7 hours. A dose-related increase in revertants was observed up to about 1,500 ppm of TCE (1.5 ml). Higher dose levels resulted in a decrease in revertants because of toxicity and cell death. The increase in mutants was relatively small, but proved to be reproducible. The mutations detected in this system are histidine-requiring reverse gene mutations. High-purity, reagent grade TCE was used in the study. Chemical analysis did not detect either epichlorohydrin or 1,2-epoxybutane. Baden et al. (1976) had earlier alluded to these findings. Mutagenic activity was not found unless the liver homogenate was included in the system, indicating that TCE must be metabolized to an active agent for mutagenesis. In companion studies, both methyl chloroform and methylene chloride were found to be more active than TCE even without activation.

In a similar series of experiments also using the TA100 S. typhimurium strain, Bartsch et al. (1976) did not observe mutagenic activity even with the addition of human or mouse liver microsomes. In that series, vinyl chloride was the least active mutagen, less so than 1,1-dichloroethylene, vinyl bromide, 1- or 2- chloro-1,3 butadiene or 3,4-dichlorobutene-1. While epoxide formation by mouse liver microsomes was detected for vinyl chloride and vinyl bromide, such was not the case for TCE. Neither was an alkylating intermediate detected.

Greim et al. (1975) reported the induction of reverse mutations by analytical grade TCE using K12 E. coli bacteria coupled with a mouse liver microsomal enzyme activation system. The responding systems employed back mutation gal+ and arg+ mutants. Vinyl chloride and vinylidene chloride (1,1-dichloroethylene) were also positive, whereas tetrachloroethylene, cis 1,2-dichloroethylene and trans-1,2-dichloroethylene were not mutagenic. Because of toxicity (cell death), the concentration of TCE that could be used was only one-third that of vinyl chloride. Nevertheless, it was concluded that the mutagenicity of TCE was less than that of vinyl chloride but greater than that of vinylidene chloride. Without the liver microsomal enzyme system, no mutagenic activity of any of the chlorinated ethylenes was detected.

TCE has also been shown to induce both frameshift and base substitution mutations in the yeast, Saccharomyces cerevisiae, strain XV185-14C (Shanin and Von Borstel, 1977). In those studies, TCE was not mutagenic without activation but in the presence of mouse liver homogenate, it was considered to be a powerful mutagen. TCE was also very toxic in that system. Also using Saccharomyces cerevisiae (strains D4 and D7), Bronzetti et al. (1977) detected point mutations and mitotic gene conversions following both in vitro and in vivo exposures to certified, ACS grade TCE. In the in vitro study, no mutagenic activity was detected without activation; however, with added mouse liver homogenate, concentration dependent increases in mutations

occurred. TCE concentrations of 10-40 mM were used. The in vivo, intrasanguinous or host-mediated assay, was used to assess mutagenicity from a single dose as well as repeated treatments of TCE. In the single-dose study, 400 mg/kg was administered by gavage to CD-1 mice, immediately after retroorbital sinus instillation with Saccharomyces cerevisiae. Four hours later the organisms harvested from the kidney and liver had high levels of mutation while those obtained from the lung revealed only few mutations.

In the multiple-dose study, 22 doses of TCE, each of 150 mg/kg, were administered over a 5-day period, followed by a dose of 400 mg/kg on the day of assay, total dose = 3,700 mg/kg. While the increase in the induced frequency of mutations of yeast recovered from the liver was greater (about 2X) than that found in the single-dose study, the increase in the kidneys was considerably greater (about 5X). A low response, like that in the single dose experiment, was found in the lung. The authors suggested that the results following multiple-doses may be indicative of either the retention of an active metabolite in the kidney or selective induction of kidney enzymes responsible for the metabolism of TCE to a mutagen. They further speculated that both the kidney and liver might be primary target organs for carcinogenicity.

In tests using Tradescantia (clone 4430), Sparrow (1976) observed TCE to be mutagenic following 6-hour exposures at levels as high as 30 ppm. The maximum response, however, was observed at 0.5 ppm, inasmuch as higher levels produced an inhibiting or interfering toxic effect.

PPG Industries, Inc. (Bell, 1977), recently reported a dominant lethal mutation study in which 15 male rats were exposed to inhalation of TCE at a level of 300 ppm, 6 hours per day, 5 days per week for 9 months. Following that exposure, the males were then each mated weekly to two unexposed virgin females for 8 consecutive weeks. As reproductive performances by the TCE-exposed and control rats were comparable, it was concluded that no dominant lethal mutagenic effect had resulted.

E. CELL TRANSFORMATION

Using a highly sensitive in vitro cell system, Price et al. (1977) reported the transformation of Fischer rat embryo cells (F1706) to tumor producing cells upon exposure to TCE. The cells were phenotypically transformed, characterized by the appearance of progressively growing foci of cells lacking in contact inhibition and in orientation with growth of macroscopic foci in semisolid agar. Upon subcutaneous inoculation of the morphologically-changed cells into newborn Fischer rats (1×10^6 cells), undifferentiated fibrosarcomas developed within 55 days at the sites of inoculation in all animals. No spontaneous transformation was observed in media or acetone controls. The

TCE tested was of reagent grade (99.9% purity). Price et al. concluded that TCE has a carcinogenic potential on the basis of these results. The cell cultures were incubated for 48 hours with concentrations of 1.1×10^3 and 1.1×10^4 M of TCE. Three other chlorinated chemicals, tetrachloroethylene, methyl chloroform, and methylene chloride were also tested in this system and also induced transformation equal to or perhaps even more efficiently than TCE. In this system TCE was considerably less toxic than the other three halocarbons. The positive control, methylcholanthrene, was considerably more effective in transformation-frequency than any of the 4 halocarbons.

No other reports of cell transformation studies are known.

F. TERATOGENICITY/REPRODUCTIVE EFFECTS

Only two teratogenicity studies have been found in a literature review. In the first, Fink (1968), in a very limited study, exposed fertile chicken eggs to 1% TCE and observed a significant increase in anomalies in chick embryos (total 96) harvested on the 3d day of embryo development. The significance of results observed after only 3 days incubation is open to question. In the other, Schwetz et al. (1975), exposed 18 pregnant Sprague-Dawley rats and 12 pregnant Swiss Webster mice by inhalation to 300 ppm of commercial grade TCE for 7 hours per day on days 6-15 of gestation. Caesarian sections were performed on days 18 and 21, respectively, on the mice and rats. While all fetuses and dams were examined grossly for visible abnormalities, one-third of each litter was randomly selected for visceral exam and two-thirds from each litter for skeletal exam. All these were fixed in toto, sectioned, stained, and examined microscopically. The authors reported that exposure to TCE caused little or no maternal, embryonal or fetal toxicity as indicated by standard, visible abnormality criteria. An examination of the tables of data does, however, suggest three possible fetal effects among the mouse litters: hemorrhage into cerebral ventricles (17 in 2 litters versus 4 in 1 litter in controls), undescended testicles (17 in 2 litters versus none in controls), and split sternebrae (17 in 2 litters versus none in controls). The authors did not indicate whether the listed effects were observed in the same two litters. While the incidence of abnormalities was increased above that found in controls, these were not found to be significant by the investigators at $P = 0.05$. While these studies would have detected major teratogenic effects, they were not sufficiently sensitive or adequately designed to detect weak teratogens, especially using noninbred animals. Neither would teratogenic neurological effects have been detected.

In a study recently reported by the PPG Industries, Inc. (Bell, 1977), no teratogenic effects were observed in offspring from female Charles River albino rats exposed by inhalation to

300 ppm TCE, 6 hours each day, from the 6th to the 15th days of gestation. In this study, 17 pregnant females bore 138 offspring, of which 95 were examined for fetal skeletal development and 43 for fetal internal development. As in the previously discussed study, only gross skeletal or organ effects would have been observed. More subtle behavioral or latent teratogenic effects would not have been detected.

A few reports allege possible TCE-induced teratogenic effects in humans. In one, sacral agenesis was observed in five infants whose mothers were exposed during pregnancy to organic solvents (Kucera, 1968). As only one or two cases involved trichloroethylene, the causal relationship is not strong.

The other report pertained to the medical use of gaseous anesthetics (Corbett et al., 1973; NIOSH, 1977b). A higher rate of miscarriages was observed among operating room nurses than would be expected among women in the general population. Exposure to TCE may have been a contributing factor, although it was impossible to implicate any single chemical because of the wide variety of anesthetics in use in typical operating rooms over a long period.

In a somewhat related study, TCE was demonstrated to readily cross the placenta into the fetuses of sheep and goats, reaching a higher blood level than in the dam after only 16 minutes (Helliwell, 1950). As such a differential exposure to the fetus might also occur in humans, the possibility of reproductive or teratogenic effects warrants consideration.

Thus, while a teratogenic effect of TCE in humans has not been established, there is sufficient reason, based upon weak evidence from both animal and human studies, the ability of TCE to cross the placenta, and possibly expose the developing fetus, to be concerned about a teratogenic potential of this chemical. Further research is needed, especially to assess the occurrence of subtle latent effects, including neurological/behavioral effects and transplacental carcinogenesis.