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May 1994

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and Toxicology of Chemicals

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ECETOC Technical Report No. 60

TRICHLOROETHYLENE: ASSESSMENT OF HUMAN CARCINOGENIC HAZARD

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c032: errat

Erratum in ECETOC Technical Report No. 60.

Trichloroethylene: Assessment of Human Carcinogenic Hazard

On page 1, 3rd paragraph, the 4th sentence should read 'The second reported mortality as well as morbidity ...' (delete 'examined civilian aircraft maintenance workers and').

Brussels, 4 August 1994

SUMMARY AND CONCLUSION

Trichloroethylene (TCE) has been manufactured on an industrial scale since the beginning of this century and it is used for many different purposes.

The potential carcinogenic effect of occupational exposure to TCE has been the subject of a number of epidemiological studies conducted during 1978-1990, including 4 cohort studies, 2 cancer case-studies without control, one small cohort study without control and one colon cancer case-control study. Each of the above studies has some shortcomings, e.g. flawed study design or small study size. None of the studies demonstrate a link between exposure to TCE and an increase in cancer mortality or excess incidence of liver and colon cancer.

More recently 2 well-designed and well-conducted studies were published. The first, from the US National Cancer Institute studied mortality in a cohort of 6,929 workers with a follow-up of up to 30 years. This study included an extensive exposure assessment which indicated high exposures, in particular during the first part of the study period. The second examined civilian aircraft maintenance workers and reported mortality as well as morbidity in an extension of the initial cohort to 1,670 workers, with a follow-up period of 37 years. Both studies showed no association between exposure to TCE and cancer in general or any specific cancer.

Taken together, five cohort studies report on 18,183 workers with a follow-up period of more than 25 years for 4 out of five studies. None of these studies demonstrate a link between exposure to TCE and an increased risk for cancer in general or for any specific type of cancer in man.

These important findings contrast with the results from animal studies where evidence of carcinogenicity has been demonstrated. The principal tumour sites in the mouse are the liver and the lung (separate studies) and in the male rat, the kidney. The occurrence of an increased incidence of mouse liver tumours (hepatocellular carcinomas and adenomas) is the most frequently reported and significant observation in lifetime cancer bioassays of TCE following exposure by either inhalation or gavage. Increased incidences of lung tumours (adenocarcinomas) have been observed in female mice only.

Small increased incidences of kidney carcinomas were found in male Sprague-Dawley, F344 and Osborne-Mendel rats but not in female rats or in any of the other strains tested (ACI, August, Marshall and Wistar) although almost all rats exposed to high TCE levels had tubular cell

cytokariomegaly. The carcinogenicity results may have been confounded by the reduced survival due to the nephrotoxic effect of excessive doses of TCE.

A substantial number of biochemical studies have identified mechanisms for the development of the rodent tumours which do not require a direct interaction between TCE or its metabolites and DNA. In each case the mechanism is thought to be linked to species specific metabolism of TCE and to a range of biochemical responses which are either specific to rodents or are not seen at dose levels relevant to human exposure. The excess tumour incidences in the liver, lung or kidney in either mice or rats exposed to TCE are, therefore, considered to be of no real relevance to human carcinogenic hazard.

The mutagenic potential of TCE has been studied widely in *in vitro* and *in vivo* test systems. In many of the reported studies the purity of the test sample is not stated, although potentially mutagenic epoxide stabilisers were almost certainly present. Overall there is no convincing or conclusive evidence that pure TCE is genotoxic.

Taking all of this information into account, it is concluded that exposure to TCE does not present a carcinogenic hazard to man at levels of current occupational exposure standards.

SECTION 1. INTRODUCTION

Trichloroethylene (abbreviation TCE, CAS No. 79-01-6, IUPAC name: trichloroethene) has many synonyms, e.g. ethylene trichloride and tradenames, e.g. TRI. It is a colourless, volatile liquid used in metal degreasing and as a solvent in the textile and chemical industries. Commercial grade TCE is 99.9% pure, although up to 0.2% stabilisers such as 1,2-epoxybutane or diisopropylamine are added to commercial formulations. Occupational exposure to TCE vapours by inhalation or, to a lesser extent, to liquid TCE by skin contamination may occur in the workplace, especially in surface metal cleaning processes in non-closed systems.

The question of whether TCE presents a carcinogenic hazard to man has been the subject of much debate. TCE has been shown to be carcinogenic in animals, causing principally liver and lung tumours in mice and kidney tumours in male rats only. In contrast, epidemiological studies using large groups of exposed workers have shown no evidence of an association between exposure to TCE and the occurrence of cancer in man.

This report reviews TCE studies with respect to cancer epidemiology, animal carcinogenicity, metabolism and kinetics, mutagenicity and mechanisms of tumour formation. An assessment of the extent to which it represents a carcinogenic hazard for man is presented.

SECTION 2. CANCER EPIDEMIOLOGY

TCE has been manufactured and used extensively since the beginning of this century. Its use as an industrial solvent, metal cleaning agent, and historically as an inhalational anaesthetic and as an additive in drugs, food and consumer products, indicate that widespread human exposure has occurred (IARC, 1979; ACGIH, 1992). The potential effects from TCE on human health, in particular the risk for cancer, have been studied for many years among occupational groups and among other populations, with environmental exposure e.g. via drinking water.

There exist a multitude of studies of human health effects of TCE. The older studies in particular have significant limitations, but recently well-designed studies of occupationally exposed groups have become available. This overview concentrates on studies reporting on occupational groups but a few other studies with relevance for TCE will also be discussed briefly. Historically, the study of occupational groups has contributed most to our understanding of the potential risks associated with exposure to chemicals because workers typically had higher exposures for longer periods of time than other populations.

2.1 REVIEW OF COHORT STUDIES ON CANCER INCIDENCE AND MORTALITY

Axelson *et al* (1978) was the first to report an epidemiological study of workers exposed to TCE; their study was triggered by the carcinogenic findings in experimental animals. The authors identified exposed workers by means of a laboratory register of urine trichloroacetic acid (TCA) measurements, kept from 1950 by the sole Swedish manufacturer of TCE. The register is complete as from 1967. Participation in the monitoring program was voluntary and free of charge. The initial study concerned 582 workers exposed to TCE prior to 1970. The cohort was expanded and studied on 2 further occasions. At the first update Axelson (1986) reported on 1,424 male and 249 female workers exposed before 1975.

Subsequently the study was further updated by Axelson *et al* (1994) to encompasses 1,670 workers (1,421 males and 249 females) involved in the manufacture or use of TCE from 1950 through 1979. Follow-up was up to and including 1985. All had been monitored by analysis of TCA (a metabolite of TCE) in the urine. The mean urinary TCA level for each worker was used as an index of exposure. The majority of the subjects had mean urinary TCA levels below 50 mg/l, which the authors state roughly corresponds to an average exposure level of 20 ppm TCE in air (8-h time-weighted average). Mortality as well as morbidity was reported. Total male mortality was

significantly lower than expected (SMR, Standardised Mortality Ratio = 0.65: 95% CI, Confidence Interval: 0.47-0.89). Male mortality from disease of the circulatory system was slightly increased, reaching borderline statistical significance (SMR= 1.17: 95% CI: 1.00 - 1.37). Dose-response was studied using 3 subgroups based on overall mean TCA levels (<49, 50-99, >100mg/l) and by considering exposure time and 10 years of latency. The subcohort with the shortest exposure time had a slightly higher overall mortality than that with the longer exposure time. Morbidity analysis gave the same picture, with a borderline statistically significant increase in malignant skin tumours (SMR=2.36: 95% CI: 1.02 - 4.65) (Table 1). This excess as well as non-significant excesses of liver cancer, prostatic cancer and lymphomas occurred essentially in the low exposure group or after a short duration of exposure or both. However, these types of cancers were not increased in the medium or high exposure groups. The results from this study do not suggest an increased cancer risk from exposure to TCE at the exposure levels experienced by the cohort (Axelson *et al*, 1994).

This conclusion has a considerable weight as it is based on analysis of mortality as well as morbidity, while considering dose-effect, exposure time and latency. In addition, there are 2 arguments for assuming a higher level of TCE exposure than reported by the authors (20 ppm). First, assessing exposure from urinary levels of metabolites only gives a valid estimate when urine samples are collected at the appropriate time after exposure, which depends on the kinetics of excretion. The authors did not mention a prescribed sample collection procedure for the biomonitoring of TCE exposure. It is well accepted that random urine sampling will underestimate exposure (Lauwerys, 1983). Second, elevated exposures can be assumed to have existed during the first years of using TCE, when procedures and engineering controls to reduce exposure were not yet optimal. These high exposure levels are hidden by averaging all biomonitoring results obtained for a person during his entire working period, as has been done in this study.

A similar approach was used by Tola *et al* (1980), who identified Finnish workers with TCA exposure from a register of biological monitoring. Biological monitoring was part of the mandatory routine periodic medical examination. The cohort consisted of 1,148 men and 969 women (total 2,117) exposed to TCE between 1963 and 1976. 89 persons who had shown symptoms of acute toxicity due to TCE exposure during this period and 33 workers identified by employers as TCE exposed were included in the cohort in addition to the workers from the registry. Follow-up was up to and including 1976. TCE exposure was considered to have started at the time of the first urinary measurements for registered workers and follow-up commenced as of that date. The registered workers were grouped according to their highest recorded urinary TCA measurement. Ninety-one % of the registered workers had maximum urinary TCA measurements below 100 mg/l, which corresponds to 40 ppm TCE in air (Axelson *et al*, 1994). For the total cohort, observed deaths were

Table 1 Cause-specific SIR[a] and 95% CIs[b] Adjusted for Age and Calendar Period: White Men (<80 y) Exposed to TCE, Swedish Cohort (Axelson *et al*, 1994)

IDC 7[c]	Site	Observed	Expected	SIR	95% CI
140-209	All	107	111.9	0.96	0.80-1.16
151	Stomach	5	7.2	0.70	0.23-1.62
153	Colon	8	7.8	1.04	0.44-2.03
155	Liver	4	2.8	1.41	0.38-3.60
157	Pancreas	1	4.1	0.25	0.01-1.38
161	Larynx	2	1.4	1.39	0.17-5.00
162	Lung	9	13.2	0.69	0.31-1.30
177	Prostate	26	20.7	1.25	0.84-1.84
178	Testis	2	1.0	2.03	0.25-7.31
180	Kidney	6	5.2	1.16	0.42-2.52
181	Bladder	8	7.9	1.02	0.44-2.00
191	Skin	8	3.4	2.36	1.02-4.65
200	Non-Hodgkin lymphoma	5	3.2	1.56	0.51-3.64
201	Hodgkin's lymphoma	1	0.9	1.07	0.03-5.95
202	Other lymphoma	0	0.1	-	0.00-33.72
203	Multiple myeloma	1	1.8	0.57	0.01-3.17
	Other	21	31.2	0.67	0.43-1.03
	Person-years:	23,516			

a Standardised Incidence Ratio
b 95% Confidence Interval
c International Statistical Classification of Diseases and Related Health Problems

compared with expected deaths based on Finnish national mortality data. Overall mortality and cancer mortality were below expected (respectively 58 deaths observed vs. 84.3 expected; SMR = 69: 95% CI: 52-89, and 11 observed vs. 14.4 expected; SMR = 77: 95% CI: 38 -138). When allowing for a short latency period the results were essentially identical.

This study was not able to consider mortality outcome by a measure of cumulative exposure. It was also not possible to examine mortality for longer than 14 years after the presumed first exposure (although this is probably underestimated). Urine samples were not collected after potential exposure to TCE but during the routine periodic medical examination. Therefore, actual exposure levels were probably higher than those reported. In summary, the study did not demonstrate an increased cancer risk among workers including a group of very heavily exposed persons. However, this conclusion has limited weight due to the limitations of the study.

An American cohort mortality study of workers exposed to TCE was reported by Shindell and Ulrich (1985). They studied the workers in a manufacturing plant in which TCE was used for vapour degreasing and whose employees also drank water containing low levels of TCE. A total of 2,646

employees were identified who worked at least 3 months between 1957 and 1983. Expected mortality was determined using national rates. The data did not permit analysis by duration of exposure or cumulative exposure. Cancer mortality was less than expected for both sexes and races. Among white males there were 20 total cancer deaths and 32.2 expected (SMR=62: 95% CI: 38-95). The authors apparently included in this study everyone who had worked in the plant during the period of observation, including management, clerical and shipping personnel who were unlikely to have been exposed to TCE as part of their work. Moreover, there was no separate analysis for the groups of workers who were more likely to have been exposed. Because of these shortcomings, the conclusions fom this study are of limited value.

In 1985, the Hughes Aircraft Company commissioned a cohort mortality study of its workers in its Air Force plant No. 44. This investigation, conducted by Wong and Morgan (1990) of ENSR Health Sciences, has not been published. The study was initiated because of the detection of TCE in wells supplying water to the plant and to the surrounding community. It was believed that because the plant workers were exposed to TCE via degreasing operations inside the plant as well as via the water, their exposures must have been substantially higher than those of the community residents not employed in the plant. Therefore, any excess cancer risk would have been expected to be more readily detectable among the employee population. The investigators studied a total of 20,535 male and female employees of whom 4,733 were determined to have had occupational TCE exposure. Mortality follow-up was conducted from 1950 through 1985; approximately one-third of the cohort was observed for more than 25 years but another third had five or less years of follow-up. Both US and local county mortality rates were used to determine expected numbers of deaths for the study cohort. Duration of employment was used as an index of total TCE exposure. Also in this study a statistically significant deficit was observed for all cancers combined among the male and female workers. There were 125 cancer deaths observed compared with 159.7 expected (SMR=78: 95% CI: 65-93). For no specific anatomical site was the SMR for cancer mortality statistically elevated, although the SMR for liver cancer (including biliary passage cancer) was found to be elevated (SMR=184: 95% CI: 60-430). An SMR exceeding 200 was found for benign neoplasms (SMR=296: 95% CI: 109-646). Small deficits of mortality were observed for lung and kidney cancer. All-sites mortality appeared to increase somewhat by duration of employment, but in each category the SMR value was below 100. Three liver/biliary cancers were observed in the < 10 years employment category with none in the 10-20 years category and 2 in the longest duration category. The respective SMR's (178 and 434) were not statistically different from 100 (Wong and Morgan, 1990). The most important limitations of this study are the generally low TCE exposures reported for the employees and the small number of subjects with long duration of employment and

adequate follow-up time. This together with its relatively large size should be considered when evaluating this study.

The most recent and thorough evaluation of mortality among TCE-exposed workers is the US National Cancer Institute's study of civilian aircraft maintenance workers (Spirtas *et al*, 1991). The study was initiated because of concerns about lymphatic and haematopoietic cancer in one building at the aircraft base. Ultimately, the investigators studied the mortality experience of 14,457 workers from 1952 through 1982. The larger cohort contained a subgroup of 6,929 white male and female workers known to have been exposed to TCE, the specific agent thought to be responsible for the perceived cancer excess at the base. TCE exposure was assessed using air monitoring data from the later years in which TCE was used at the aircraft base, while estimates of earlier exposure were based on the description of historical changes in control of degreasing operations (Steward *et al*, 1991). For example, hygiene sampling data indicated that potential TCE exposures at the degreasing machines declined from a maximum of 400 ppm during the 60's to a maximum of 200 ppm in the 70's. Historical information suggested that peak exposures in earlier decades may have been as high as 600 ppm. A 3 way exposure classification, peak vs. low-level, frequent vs. infrequent and long duration vs. short duration (15 vs 5 min) was designated to all job-descriptions. A cumulative exposure score was calculated for each worker, which - according to the authors - should only be seen as relative score. The expected numbers of deaths were based on State rates. Mortality was examined by type of exposure, time since first exposure, highest exposure experienced, average exposure intensity and cumulative exposure. Deficits were observed for all cancers combined among both male and female workers (Table 2 and 3). For males, 248 cancer deaths were observed compared with 268.5 expected (SMR=92: 95% CI: 81-105). For females, 33 cancer deaths were observed compared with 49.4 expected (SMR=67: 95% CI: 46-94). For no anatomic site was the SMR for cancer mortality statistically elevated. No excesses were observed, for both sexes, at the anatomical sites of concern based on animal studies: liver, lung and kidney. The cumulative exposure analysis provided additional evidence for an absence of excess cancer risk in this cohort. The "all sites" cancer mortality rates were essentially equal across the 3 exposure categories. All 3 primary liver cancer deaths occurred in the lowest exposure category among males. A small excess of kidney cancer deaths was observed only in the lowest category among males. Lung cancer mortality was uniformly unremarkable across categories for males and females (Spirtas *et al*, 1991).

This study is the largest evaluation of a TCE-exposed workforce conducted to date. Nearly 7,000 workers with 45,000 person years of TCE exposure were included in this study, which covered a follow-up period of 30 years. This study had considerable power to uncover excess cancer risk. In

Table 2 Cause-specific SMRs[a] and 95% CIs[b] Adjusted for Age and Calendar Period: White Men Exposed to TCE, Hill Air Force Base, Utah, 1952-82 (Spirtas *et al*, 1993)

Cause of death	Observed	Expected	SMR[c]	95% CI[c]
All causes	1,508	1,647.8	92	87-96
All malignant neoplasms	248	268.5	92	81-105
Buccal cavity, pharynx	5	5.7	88	29-206
All digestive organs	74	74.7	99	78-124
Oesophagus	6	5.7	106	39-230
Stomach	14	16.0	88	48-147
Large intestine	27	23.3	112	73-164
Rectum	4	6.3	64	17-163
Biliary passages	6	2.5	238	87-519
Primary liver	2	1.6		
Pancreas	14	16.9	83	45-139
All respiratory organs	65	69.5	94	72-119
Larynx	1	2.9	34	1-191
Bronchus, Trachea, Lung	64	65.1	98	76-126
Prostate	22	27.6	80	50-121
Testes, genital organs	1	2.0		
Kidney	8	6.7	120	52-237
Bladder	10	7.3	137	65-251
Melanoma of skin	5	5.2	96	31-224
Central Nervous System	9	10.1	89	41-170
Thyroid, endocrine	1	1.5		
Bone	3	1.1	236	54-767
All lymphatic and haematopoietic tissues	30	34.6	87	59-124
Lymphosarcoma	9	8.0	112	51-213
Hodgkin's disease	4	4.3	93	25-237
Leukemia	9	13.1	69	31-130
Other lymphopoietic tissues	8	9.2	87	38-172
Multiple myeloma	5	4.5	111	36-259
Non-Hodgkin's lymphoma	10	9.8	103	49-189
All other cancers	15	21.2	71	40-117

a Standardised Mortality Ratio, Utah state deaths rates used as standard

b 95% Confidence Interval

c SMRs and 95% CIs were presented only if number of observed or expected deaths > 2

addition, the investigators completed a thorough and extensive exposure assessment which indicated high exposures, in particular during the first part of the study period. The only weakness of the study is the lack of information concerning potential confounding factors such as cigarette smoking and the possibility of exposure misclassification, inherent to all occupational studies without biological monitoring data.

Table 3 Cause-specific SMRs[a] and 95% CIs[b] Adjusted for Age and Calendar Period: White Women Exposed to TCE, Hill Air Force Base, Utah, 1952-82 (Spirtas *et al*, 1993)

Cause of death	Observed	Expected	SMR[c]	95% CI[c]
All causes	186	227.2	82	71-95
All malignant neoplasms	33	49.4	67	46-94
Buccal cavity, pharynx	0	0.6		
All digestive organs	7	13.0	54	22-111
Oesophagus	0	0.2		
Stomach	0	2.0		
Large intestine	2	5.5	37	4-132
Rectum	1	1.1		
Biliary passages	2	1.1		
Primary liver	0	0.2		
Pancreas	2	2.5	81	10-291
All respiratory organs	0	3.1	0	0-121
Larynx	0	0.1		
Bronchus, Trachea, Lung	0	2.8	0	0-131
Breast	9	11.5	79	36-149
Cervix uteri	4	1.8	224	61-574
Kidney	0	0.8		
Bladder	1	0.6		
Melanoma of skin	1	0.6		
Central Nervous System	0	1.4		
Thyroid, endocrine	0	0.4		
Bone	0	0.1		
All lymphatic and haematopoietic tissues	7	4.9	143	58-295
Lymphosarcoma	3	1.2	261	54-761
Hodgkin's disease	0	0.3		
Leukemia	2	1.9		
Other lymphopoietic tisssues	2	1.5		
Multiple myeloma	1	0.8		
Non-Hodgkin's lymphoma	4	1.4	286	78-731
All other cancers	0	4.2	0	0-86

a Utah state deaths rates used as standard
b 95% Confidence Interval
c SMRs and 95% CIs were presented only if number of observed or expected deaths > 2

2.2 STUDIES ON SPECIFIC CANCERS

A series of short reports of investigations of a possible association between TCE exposure and primary hepatic cancer appeared in the years around 1980. In a Czech cancer case-study, Novotna *et al* (1979) identified 56 cases of histologically confirmed primary carcinoma of the liver in 1972 and 1974 in Prague. Governmental records for obligatory registration of TCE exposed workers were studied but none of these liver cancer cases was related to occupational exposure to TCE. It should be noticed, however, that this was not a controlled study.

Another Czech study described the results of a study of 57 dry-cleaning workers in Prague (Málek *et al*, 1979). These were said to constitute 86% of all dry cleaners in Prague who had worked at least one year since the 1950's. Most workers were exposed for more than 5 years and had urinary levels TCA levels above 100 mg/l, suggesting high exposures. Half of the group had been exposed for more than 20 years prior to the study. The authors also indicated that exposures in this type of industry have been considerable as 32 cases of TCE intoxication were registered in Prague during the study period. No liver cancers were observed in this group of workers.

In a study by Paddle (1983), records of the local tumour registry were searched for new primary liver cancers in the period 1951 - 1977. The list of 95 cases was compared with the employee roster for the chemical company which had produced TCE in the area since 1909. None of the cases had ever worked at the chemical plant. The author noted that less than one primary liver cancer would have been expected among the plant's workforce during the registry period considered. By examining another record source, a single death due to secondary liver cancer was found, but no primary liver cancer deaths were observed.

Frederiksson *et al* (1989) reported the results of a case-control study of colon cancer, physical activity and occupation in Sweden. There were 312 living cases of adenocarcinoma of the large bowel diagnosed between 1980 and 1983 and 623 controls matched for age and sex. Exposure data were obtained by questionnaire. The authors did not observe an association with cancer for the job category "dry-cleaner" (which could be associated with TCE exposure) where the Odds Ratio (OR) was 2.0 (95% CI 0.5-7.1) or for the chemical agent category "trichloroethylene" (OR = 1.5, 95% CI 0.4-5.7). The arbitrary subcategory "trichloroethylene exposure among dry-cleaners" gave an OR of 7.4 (statistically significant, 95% CI 1.1-47.0). It should be noted that there were only 10 dry-cleaners in the study. The finding is based on small numbers and the quality of the exposure information is questionable as it was obtained by self-reporting. The category is arbitrary as there is no reason to expect TCE exposure will be much different for the category "dry-cleaner". Multiple comparisons, such as in this case-control study, will provide examples of high or low ORs for statistical reasons. Because of the inconsistency in the results of this study, the high OR for TCE exposed dry-cleaners is probably a statistical artefact. It is not supported by other studies and is therefore most probably without biological significance.

2.3 SUMMARY AND EVALUATION

TCE has been manufactured on an industrial scale and used for many different purposes since the beginning of this century. The first epidemiological study on cancer incidence in a cohort of workers

exposed to TCE was conducted by Axelson *et al* (1978). Subsequently 3 cohort studies (Tola *et al*, 1980; Shindell and Ulrich, 1985; Wong and Morgan, 1990), 2 uncontrolled liver cancer case-studies (Novotna *et al*, 1979; Paddle, 1983), one uncontrolled small cohort study (Málek, 1979) and one colon cancer case-control study (Frederiksson *et al*, 1989) were reported. None of the studies demonstrate a link between exposure to TCE and an increase in cancer mortality or excess incidence of liver and colon cancer.

Each of the above studies has some shortcomings, e.g. flawed study design or small study size.

More recently 2 well-designed and well-conducted studies were published. Spirtas *et al* (1991) studied mortality in a cohort of 6,929 workers with a follow-up of up to 30 years. This study included an extensive exposure assessment which indicates very high exposures, during the first part of the study period. Axelson *et al* (1994) reported mortality as well as morbidity in an extension of the initial cohort to 1,670 workers, with a follow-up period of 37 years. Both studies also showed no association between exposure to TCE and cancer in general or any specific cancer.

Taken together, five cohort studies (Table 4) report on 18,183 workers with a follow-up period of more than 25 years for 4 out of five studies. All these studies investigated mortality. The most recent study, by Axelson *et al* (1994) also reports on cancer incidence. Incidence is a much more sensitive indicator for types of cancer with high survival rates and this study also for this reason adds considerable weight to the overall negative findings. In these studies no link was demonstrated between exposure to TCE and an increased risk for cancer in general or for any specific type of cancer.

Table 4 Characteristics of Cohort Studies on Occupationally Exposed Workers

Cohort size	Period of potential exposure	Exposure level (ppm)		Follow-up period (y)	Reference
		TWA[a]	Maximum		
2,205	1963-1976	<40	>500[b]	14	Tola *et al*, 1980
2,646	1957-1983	Not specified	Not specified	27	Shindell and Ulrich, 1985
4,733	1950-1986	<50	>50	36	Wong and Morgan, 1990
6,929	1952-1979	Not specified	600[c]	30	Spirtas *et al*, 1991
1,670	1950-1979	<20	>40	Mortality: 37 Morbidity: 38	Axelson *et al*, 1994
Total: 18,183					

a TWA, Time-Weighted Average concentration (during an 8-h working day)
b Cohort also includes 89 persons who had "TRI poisoning"; no exposure level was reported for these subjects
c Peak exposure, reported as exposure index

SECTION 3. ANIMAL CARCINOGENICITY

3.1 INHALATION EXPOSURE (Table 5)

Groups of B6C3F$_1$ mice and Charles-River rats (100/sex/group) were exposed by inhalation (6 h/d, 5 d/wk) to atmospheres containing TCE (technical grade, purity >99%, 0.148 % epichlorhydrin) at concentrations of 0, 100, 300 and 600 ppm for 24 months. There was no carcinogenic effect in rats. An excess of hepatocellular adenomas and adenocarcinomas occurred in male and female mice but it was statistically significant only in the high concentration group. Due to several deficiencies (including wide variations in the TCE vapour concentrations in the chambers and animal substitutions in the groups) this study, conducted for the US Manufacturer's Chemical Association (US-MCA), was considered inadequate and remained unpublished (US-MCA, 1978).

Wistar rats, NMRI mice and Syrian hamsters (30/sex/group) were exposed by inhalation (6 h/d, 5 d/wk) to atmospheres containing 0, 100, or 500 ppm of pure TCE (stabilised only with an amine base) for 78 weeks. The animals were observed for their lifetime. The authors concluded that no significant increase in tumour formation was observed in any species or dosing group, except for malignant lymphomas, which were increased in female mice at the following incidence rates: control, 9/29; 100 ppm, 17/30; 500 ppm, 18/28. Due to the high spontaneous occurrence of lymphomas, which is peculiar to this strain of mice (NMRI) and very likely of viral etiology, it was concluded that no indication of a carcinogenic potential of pure TCE can be deduced (Henschler *et al*, 1980).

ICR mice (49 females/group) and Spraque-Dawley rats (51 females/group) were exposed by inhalation (7 h/d, 5 d/wk) to 0, 50, 150 or 450 ppm TCE (purity 99.8%, 0.02% epichlorohydrin) for 104 weeks. An increased incidence of lung adenocarcinomas was observed in female mice only. The effects were statistically significant at the 2 higher concentrations, the incidence rates being: control, 1/49; 50 ppm, 3/50; 150 ppm, 8/50; 450 ppm, 7/46. The incidence of total lung tumours (benign and malignant) was not significantly increased above control levels (Fukuda *et al*, 1983).

In a series of 7 inhalation studies, B6C3F$_1$ mice and Swiss mice, and Sprague-Dawley rats (90/sex/group) were exposed by inhalation (7 h/d, 5 d/wk) to 0, 100, 300 and 600 ppm TCE (99.9% epoxide-free) for 8 and 78 weeks (mice) or 8 and 104 weeks (rats) . All animals were observed for their lifetime (Maltoni and Maioli, 1977; Maltoni *et al*, 1986, 1988). Increases in hepatocellular adenomas and carcinomas were observed in both strains of mice which were statistically significant in the high exposure groups. The incidences, combined for male and female B6C3F$_1$ mice exposed

Table 5 Carcinogenicity Studies by Inhalation

Species	Strain, sex	Protocol	Conc. (ppm)	TCE purity, stabiliser	Observations	Reference
Rat	Wistar, ♂ and ♀	6 h/d, 5 d/wk for 78 wk, observation for 156 wk	100, 500	Highly purified, epoxide free	No increase in tumour incidence at any site	Henschler *et al*, 1980
Rat	Sprague-Dawley, ♂ and ♀	7 h/d, 5 d/wk for 104 wk, observation for lifetime	100, 300, 600	99.9%, amine, epoxide free	Increased incidence of kidney adenocarcinomas in ♂ of 600 ppm group: some Leydig cell tumours and immunoblastic lymphosarcomas	Maltoni and Maioli, 1977; Maltoni *et al*, 1986, 1988
Rat	Sprague-Dawley, ♀	6 h/d, 5 d/wk for 104 wk, observation for 3 wk	50, 150, 450	99.8%, trace of benzene and epichlorohydrin (0.02%)	No significant increase in tumour incidences	Fukuda *et al*, 1983
Mouse	NMRI, ♂ and ♀	6 h/d, 5 d/wk for 78 wk, observation for 130 wk	100, 500	Highly purified, epoxide free	Lymphomas in ♀ only (note: high spontaneous incidence of lymphomas in NMRI mice) Poor survival in all groups	Henschler *et al*, 1980
Mouse	ICR, ♀	6 h/d, 5 d/wk for104 wk, observation for 3 wk	50, 150, 450	99.8%, trace of benzene and epichlorohydrin (0.02%)	Only ♀ mice tested: increase in lung adenocarcinomas	Fukuda *et al*, 1983.
Mouse	Swiss, ♂ and ♀	7 h/d, 5 d/wk for 78 wk	100, 300, 600	99.9%, amine, epoxide free	Increased incidence of hepatocellular adenocarcinomas (♂+♀) and lung adenomas (♂)	Maltoni *et al*, 1988
Mouse	$B6C3F_1$, ♂ and ♀	7 h/d, 5 d/wk for 78 wk	100, 300, 600	99.9%, amine, epoxide free	Increased incidence of hepatocellular adenocarcinomas (♂+♀) and lung adenomas (♀)	Maltoni *et al*, 1988
Hamster	Syrian, ♂ and ♀	6 h/d, 5 d/wk for 78 wk	100, 500	Highly purified, epoxide free	No increase in specific tumours	Henschler *et al*, 1980

during 78 weeks, were: control, 2.2%; 100 ppm, 2.8%; 300 ppm, 3.9%; 600 ppm, 8.3%. The incidences combined for male and female Swiss mice exposed during 78 weeks, were: control, 2.2%; 100 ppm, 1.1%; 300 ppm, 4.4%; 600 ppm, 7.8%. A small increase in the incidence of lung tumours (essentially adenomas) was observed in female $B6C3F_1$ mice and in male Swiss mice with the following incidences: in female $B6C3F_1$ mice: control, 4.4%; 100 ppm 6.7%; 300 ppm, 7.8%; 600 ppm, 16.6%. In male Swiss mice: control, 11.1%; 100 ppm, 12.2%, 300 ppm, 25.5.%; 600 ppm, 30%. The effect was statistically significant only at the highest concentration in the $B6C3F_1$ mice and at the 2 higher concentrations in the Swiss mice. No increase in lung tumours was observed in male $B6C3F_1$ mice and in female Swiss mice in the same study using the same dosing regimen. Increases in the incidence of forestomach tumours in mice did not reach statistical significance. A small increase in the incidence of renal tubular adenocarcinomas has been observed only in male rats at 600 ppm. The increase (incidence control, 0/95; 600 ppm, 3/90) was described as borderline evidence by the authors.

An increased incidence (statistically significant in the 2 higher concentration groups) of benign testicular Leydig cell tumours were observed in Sprague-Dawley rats when data were combined from 2 104-week studies (Maltoni *et al*, 1988). A slightly higher, not statistically significant and not dose-related incidence of leukemia (mainly immunoblastic lymphosarcomas) was observed in both female and male rats. However, this tumour type is known historically to have a variable incidence in groups of control Sprague-Dawley rats and the effect seen in this experiment is unlikely to be related to the TCE exposure.

3.2 ORAL ADMINISTRATION (Table 6)

$B6C3F_1$ mice (50/sex/exposed group, 20/sex/control group) and Osborne-Mendel rats (50/sex/group) were exposed by gavage (1x/d, 5d/wk) to TCE (dissolved in corn oil; purity of TCE 99%, 0.2% 1,2-epoxybutane and 0.1% epichlorohydrin) for 78 weeks. Rats were given time-weighed average (TWA) doses of either 1,097 mg TCE/kgbw or 549 mg/kgbw; male mice received TWA doses of 2,339 or 1,169 mg/kgbw and female mice 1739 mg/kgbw or 869 mg/kgbw. In mice, increased incidences of hepatocellular carcinomas were observed in both sexes. In addition, lung tumours were observed in male mice. No liver tumours were observed in rats; toxic nephropathy was found in both sexes (US-NCI, 1976). This study has been criticised because of the high content of stabilisers in the test material and, therefore, has been judged to be inadequate for the evaluation of the carcinogenicity of TCE (Henschler *et al*, 1977).

Table 6 Carcinogenicity Studies by the Oral (Gavage) Route

Species	Strain/sex	Protocol	Dose (mg/kgbw)	TCE purity, stabiliser	Observations	Reference
Rat	Osborne-Mendel, ♂ and ♀	5 d/wk for 78 wk	549, 1,098	99%, 0.2% 1,2-epoxybutane, 0.1% epichlorohydrin	No increase in specific tumours; toxic nephropathy	US-NCI, 1976
Rat	F344/N, ♂ and ♀	5 d/wk for 104 wk	500, 1,000	> 99.9%, epoxide free	Significant increase in renal adenocarcinomas in ♂; nephropathy in all treated groups: study judged inadequate by US-NTP	US-NTP, 1983
Rat	Sprague-Dawley, ♂ and ♀	5 d/wk for 52 wk, observation for lifetime	50, 250	99.9%, epoxide free	No significant increase in tumour incidence at any site; alteration of renal tubular cells in high dose ♂	Maltoni *et al*, 1986
Rat	August, ACI, Osborne Mendel, Marshalll	5 d/wk for 104 wk	500, 1,000	> 99.9%, epoxide free	Kidney carcinoma in male Osborne Mendel: study judged inadequate by US-NTP	US-NTP, 1988
Mouse	$B6C3F_1$, ♂ and ♀	5 d/wk for 78 wk	1,169, 2,339 for ♂; 869, 1,739 for ♀	99%, 0.2% 1,2-epoxybutane 0.1% epichlorohydrin	Increased incidence of hepatocellular carcinomas in both sexes	US-NCI, 1976
Mouse	$B6C3F_1$, ♂ and ♀	5 d/wk for 2 y	1,000	> 99.9%, epoxide free	Increased incidence of hepatocellular carcinomas in both sexes	US-NTP, 1983
Mouse	Ha:ICR, ♂ and ♀	5 d/wk for 78 wk	2,400 for ♂ (adjusted to TWA 1,900); 1800 for ♀ (TWA 1400)	Different samples, with and without stabilisers (see text)	Forestomach papillomas and carcinomas in groups treated with TCE stabilised with > 0.25% epichlorohydrin; no tumours with pure TCE	Henschler *et al*, 1984
Mouse	$B6C3F_1$, ♂	Drinking water for 61 wk	6 mg/kgbw/d	99%	No increase in liver tumours	Herren-Freund *et al*, 1987

B6C3F$_1$ mice and F344 rats (50/sex/group) were administered by gavage (1x/d, 5 d/wk) TCE (highly-purified, epoxide-free in corn oil) for 104 weeks. The dose levels were 1,000 mg/kgbw for mice and 500 or 1,000 mg/kgbw for rats. Increased incidences of hepatocellular adenomas and carcinomas were observed in male and female mice. A small (6%), but statistically significant increase in renal tubular adenocarcinomas (incidence: control, 0/48; test group, 3/49) was observed in male rats dosed at 1,000 mg/kgbw. Toxic nephropathy was observed in nearly all treated rats (US-NTP, 1983, 1990).

Four different strains of rats (ACI, August, Osborne-Mendel and Marshal; 50/sex/group/strain) received TCE (highly-purified, epoxide-free in corn oil, dose levels 500 and 1,000 mg/kgbw) by gavage (1x/d, 5 d/wk) for 104 weeks with an observation period of 3 to 6 months following treatment. A small, but statistically non-significant increase in renal cell adenocarcinomas, was observed in male Osborne-Mendel rats only. However, TCE caused tubular cell cytomegaly in 82-100% of all dose animals and may be judged as nephrotoxic (US-NTP, 1988).

The US-NTP judged both gavage studies above to be inadequate because of insufficient survival, significant non-tumour renal pathology and deficiencies in the conduct of the studies (US-NTP, 1983, 1988).

HA:ICR Swiss mice (50/sex/group) received high doses of TCE (highly-purified, stabilised with 0.0015% triethanolamine; in corn oil) and TCE (99.4% pure, stabilised with epichlorohydrin 0.8%, 1,2-epoxybutane 0.8%, or 1,2-epoxybutane 0.25% and epichlorohydrin 0.25%; in corn oil) by gavage (1x/d, 5d/wk; initial dose levels 2,400 mg/kgbw for males and 1,800 mg/kgbw for females) up to 78 weeks. Due to high toxicity, gavage was interrupted for several weeks, and all dose levels were reduced by a factor of 2 from week 40. Consequent TWA doses were calculated to be 1,900 mg/kgbw for males and 1,400 mg/kgbw for females. The animals were observed up to 104 weeks. No increase in liver or lung tumours was observed in this study. A statistically significant increase in forestomach tumours was observed only in the animals treated with TCE containing epichlorohydrin or with TCE containing 1,2-epoxybutane and epichlorohydrin. The latter is a genotoxic carcinogen known to cause tumours at the site of application. In the same study, a sample of TCE stabilised with 1,2-epoxybutane (0.25%) and a sample which contained 0.0015% triethanolamine did not cause an increase in forestomach tumours (Henschler *et al*, 1984).

Maltoni *et al* (1986) reported a chronic study in Spraque-Dawley rats, where TCE (purity 99.9%; in olive oil; dose levels 50 or 250 mg/kgbw) was administered by gavage (1x/d, 4-5 d/wk) for 52 weeks. The rats were observed for their lifetime. Alterations of renal tubular cells were found in

high dose males but there was no increased incidence of renal tumours. The only finding was an increase in immunoblastic lymphosarcomas in male rats (incidence: controls, 3.3% ; 50 mg/kgbw, 6.7% ; 250 mg/kgbw, 10.0%). This finding was considered by the authors as a "limited evidence" in view of the large variations in historical controls.

Herren-Freund *et al* (1987) exposed male B6C3F$_1$ mice to TCE (purity 99%) via drinking water for 61 weeks at a level (40 mg/l) that provided a dose of 6 mg/kgbw/d. No significant increase in liver tumours were observed. In the same study, TCE did not show tumour promoter activity in mice which were pretreated with ethylnitrosourea.

3.3 DERMAL ADMINISTRATION (Table 7)

The skin of Ha:ICR Swiss mice was treated with purified TCE (3x1 mg/wk) or TCE and phorbol ester (3x1+2.5 mg/wk), and TCE was injected s.c. (1x0.5 mg/wk) for 89 weeks (Van Duuren *et al*, 1979). No skin tumours were observed in these studies.

3.4 CARCINOGENICITY OF METABOLITES (Table 8)

In a drinking-water carcinogenicity study, Herren-Freund *et al* (1987) exposed B6C3F$_1$ mice (22-32 males/group) to trichloroacetic acid (TCA), a major TCE metabolite and dichloroacetic acid (DCA), a minor metabolite (Section 4.1), both at a dose level of 1 g/kgbw/d (5 g/l drinking water) for 61 weeks. Both TCA and DCA induced significantly increased incidences of hepatocellular adenomas and carcinomas.

Bull *et al* (1990) administered TCA and DCA (both 1 or 2 g/l) to B6C3F$_1$ mice (males and females) via the drinking water for up to 52 weeks. Both compounds induced increased incidences of hepatocellular tumours in male mice. Female mice were not responsive.

De Angelo *et al* (1991) conducted a drinking-water bioassay with DCA (0.05, 0.5, 3.5 or 5 g/l) in B6C3F$_1$ mice (50 males/group) for up to 75 weeks. Significantly increased incidences of hepatocellular adenomas and adenocarcinomas were found in the 2 highest concentration groups.

In a bioassay on by-products from the chlorination of drinking water, Daniel *et al* (1992) exposed male B6C3F$_1$ mice via their drinking water to DCA (0.5 g/l), to chloral hydrate (1 g/l) and 2,2-chloroacetaldehyde (0.1 g/l) for 104 weeks. All 3 compounds induced significant increase of hepatocellular adenomas and carcinomas.

Table 7 Carcinogenicity Studies by the Dermal Route

Species	Strain, sex	Protocol	Dose/concentration	TCE Purity	Observations	Reference
Mouse	Ha:ICR, ♀	3 x/wk for 83 wk, or same dose plus 2.5 mg phorbol ester, 3 x/wk	1 mg/kgbw	Undefined	No skin papillomas	Van Duuren *et al*, 1979
Mouse	Ha:ICR, ♀	Subcutaneous injection	0.5 mg 1 x/wk for 89 wk	Undefined	No local sarcomas	Van Duuren *et al*, 1979

Table 8 TCE Metabolites: Carcinogenicity Studies by the Oral Route

Compound	Species, strains	Sex	Dose/concentration	Observations	Reference
Trichloroacetic acid	Rat, F344	♂	0.05, 0.5 and 5 g/l in drinking water for 100-104 wk	No carcinogenic effect although hepatic toxicity evidence was present	DeAngelo *et al*, 1992
	Mouse, B6C3F$_1$	♂	5 g/l in drinking water for 61 wk	Increased incidence of hepatocellular adenomas and carcinomas	Herren-Freund *et al*, 1987
	Mouse, B6C3F$_1$	♂, ♀	1 and 2 g/l in drinking water for 52 wk	♀: no carcinogenic effect ♂: increased incidence of hepatocellular adenomas and carcinomas	Bull *et al*, 1990
Dichloroacetic acid	Mouse, B6C3F$_1$	♂	In 5 g/l in drinking water for 61 wk	Increased incidence of hepatocellular adenomas and carcinomas	Herren-Freund *et al*, 1987
	Mouse, B6C3F$_1$	♂	0.05, 0.5 and 5 g/l in drinking water for up to 75 wk	Increased incidence of hepatocellular adenomas and carcinomas at 0.5 and 5 g/l	DeAngelo *et al*, 1991
	Mouse, B6C3F$_1$	♂	0.5 g/l in drinking water for 104 wk	Increased incidence of hepatocellular adenomas and carcinomas	Daniel *et al*, 1992
Chloral hydrate	Mouse, B6C3F$_1$	♂	1 g/l in drinking water for 104 wk	Increased incidence of hepatocellular adenomas and carcinomas	Daniel *et al*, 1992
S-1,2-dichloro-vinylcysteine	Mouse, Swiss Webster	♂	0.01 to 0.1 mg/ml in drinking water for up to 37 wk	No carcinogenic effects; severe kidney damage at termination of study at 37 wk	Jaffe *et al*, 1984
	Rat, Wistar-Porton	♂, ♀	Dose levels up to 10 mg/kgbw/d for up to 46 wk	No carcinogenic effects at termination of study at 87 wk	Terracini and Parker, 1985

S-1,2-dichlorovinyl cysteine, a cystein conjugate of TCE, was studied by Terracini and Parker (1965) for chronic toxicity/carcinogenicity in Wistar rats. In a pilot experiment, the animals (13 males, 5 females) were dosed via their drinking water at a dose level equivalent to 10 mg/kgbw/d for up to 46 weeks. No carcinogenic activity was found at the termination of the study at 87 weeks, although there was severe kidney damage.

Jaffe *et al* (1984) exposed male Swiss Webster mice (24 in the test groups, 36 in control) to S-1,2-dichlorovinylcysteine dissolved in drinking water at 0.01, 0.05 and 0.1 mg/ml. At termination of this study at 37 weeks severe renal injury was observed in all treatment groups.

In a brief abstract, DeAngelo *et al* (1992) report a carcinogenicity sytudy in male F344 rats exposed to TCA and DCA at 0.05, 0.5 and 5 g/l via their drinking water for 100 to 104 weeks. TCA induced hepatic toxicity without neoplasia. DCA induced increases in altered foci and hyperplastic nodules in the liver at 0.5 g/l. High dose DCA treatment resulted in mortality.

3.5 SUMMARY

3.5.1 Trichloroethylene

TCE has been shown to increase the incidence of certain tumours in the mouse and, to a lesser extent, in the rat. The principal tumour sites in the mouse are the liver and the lung (observed in separate studies) and for the male rat, the kidney.

The occurrence of an increased incidence of mouse liver tumours (hepatocellular carcinomas and adenomas) is the most frequently reported and significant observation in lifetime cancer bioassays of TCE. These tumours have been observed in both male and female Swiss and $B6C3F_1$ mice following exposure by either inhalation or gavage. Increased incidences of liver tumours have not been reported in other strains of mice (e.g. NMRI or ICR) exposed to TCE, nor have they been reported in other species, for example, rat and hamster.

Increased incidences of lung tumours (adenocarcinomas) have been observed in the female ICR mouse and the female $B6C3F_1$ mouse. The effect was not observed in the Swiss mouse nor in males of any of the mouse strains tested. The effect also appears to be species specific.

Small increased incidences of kidney tubular cell carcinomas were found in Sprague-Dawley, F344 and Osborne-Mendel rats. These findings were confined to males and no increased incidences were found in any of the other tested rat strains (ACI, August, Marshall and Wistar) although almost

all rats exposed to high TCE levels had tubular cell cytokariomegaly. The carcinogenicity results may have been confounded by the reduced survival due to the nephrotoxic effect of excessive doses of TCE.

Isolated findings of increased tumour incidences were: Leydig cell tumours in male Sprague-Dawley rats following inhalation, leukemia (mainly immunoblastic lymphosarcomas) in 1 oral gavage and 1 inhalation study conducted in Spraque-Dawley rats, and forestomach tumours in a gavage study with HA:ICR mice.

Leukemia is known historically to have a variable incidence in groups of control Sprague-Dawley rats. The effect was unlikely to be related to exposure to TCE because it was not dose related and the absolute incidence was small. This type of tumour was not observed in any of the other rat studies or in the mouse studies conducted with TCE.

An increased incidence of forestomach tumours was found in HA:ICR mice receiving by gavage high doses of TCE stabilised with epichlorohydrin (> 0.25%). In the same study, a sample of TCE stabilised with 1,2-epoxybutane and a non-stabilised sample did not cause an increase in forestomach tumours. There were no statistically significant changes in the incidence of forestomach tumours in mice receiving TCE by inhalation, using non-stabilised solvent.

The significance of the Leydig cell tumours will be discussed in Section 6.4.

3.5.2 Metabolites

The major TCE metabolite, trichloroacetic acid, as well as 2 other, minor metabolites, dichloroacetic acid and chloral hydrate, were found to induce hepatocellular carcinomas in several long term studies conducted in B6C3F$_1$ mice. The TCE cysteine conjugate, S-1,2-dichlorovinyl cysteine, did not have carcinogenic activity in rats. In the only study conducted in rats, trichloroacetic acid did not show carcinogenic activity.

SECTION 4. METABOLISM AND KINETICS

A detailed review of the absorption, distribution, metabolism and elimination of TCE in animals and man has been published by Davidson and Beliles (1991).

Predictions of the uptake, distribution, metabolism and elimination of TCE at dose levels outside the range of experimental studies have been derived from a number of physiologically based pharmacokinetic models constructed from the existing experimental data (Bogen, 1988; Fisher *et al*, 1989, 1990, 1991; Fisher and Allen, 1993; Allen and Fischer, 1993).

4.1 ANIMAL STUDIES

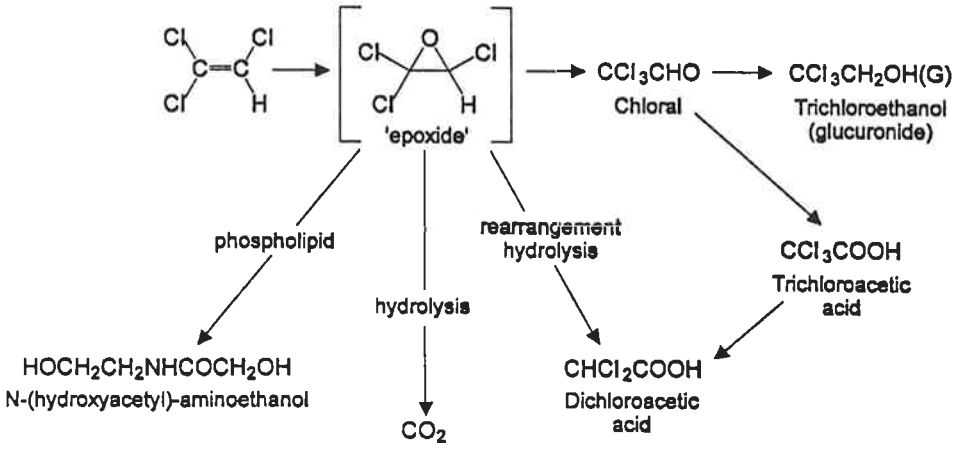
TCE is rapidly and extensively absorbed through the lungs and from the gastrointestinal tract. Skin absorption from TCE vapour is negligible although dermal contact with liquid TCE may result in significant absorption. Following absorption it is widely distributed throughout the body via the systemic circulation. Highest concentrations are found in adipose tissues due to its high lipid solubility. Unmetabolised TCE is eliminated by exhalation.

TCE is rapidly metabolised by cytochrome P450, possibly P4502E$_1$, to a postulated epoxide intermediate which spontaneously rearranges to chloral (hydrate), which is in turn further metabolised to trichloroethanol, trichloroethanol glucuronide and trichloroacetic acid (TCA). TCA is also reported to occur as glucuronide and coenzyme A conjugates. Minor metabolites include carbon dioxide, dichloroacetic (DCA), oxalic acid and N-(hydroxyacetyl)-amino-ethanol (Green and Prout, 1985; Dekant *et al*, 1986a; Bruckner *et al*, 1989) (Figure 1). It has been proposed that these metabolites are formed by hydrolysis of the TCE epoxide although this has not been established experimentally. The existence of the epoxide has not been confirmed *in vivo* and a number of authors have questioned the role of an epoxide in the metabolism of TCE (Bruckner *et al*, 1989; Miller and Guengerich, 1982). The latter authors proposed a rearrangement of the initial oxidation product within the enzyme site which precluded the formation of an epoxide.

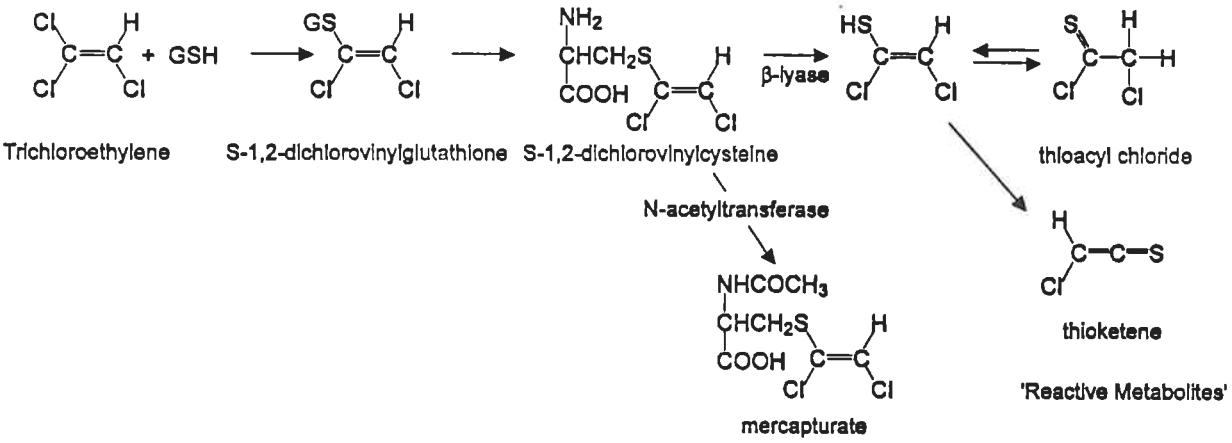
Qualitatively, the metabolism of TCE is similar between species and sexes, the major metabolites in all species being trichloroethanol (as its glucuronide) and TCA. There is, however, evidence of metabolic differences between strains of mice. In Swiss mice and the B6C3F$_1$ strain, TCA accounts for 7-12% of the dose (Green and Prout, 1985) whereas in NMRI mice this metabolite was only 0.1% of the dose (Dekant *et al*, 1984). Quantitatively, there are differences between species with respect to the rate of oxidation of TCE and the relative yields and kinetic behaviour of its

Figure 1 Metabolic Pathways of Trichloroethylene

Cytochrome P-450 pathway



Glutathione-S-transferase pathway



metabolites. In the mouse, the kinetics of the cytochrome P450 metabolism of TCE have been shown to be linear up to dose levels of 2 g/kgbw (Buben and O'Flaherty, 1985; Prout *et al*, 1985) but in the rat this pathway is saturable at dose levels between 500 and 1,000 mg/kgbw (Stott *et al*, 1982; Prout *et al*, 1985). As a result of these differences the levels of trichloroethanol and trichloroacetic acid in blood were 4 to 7 fold higher in mice than rats, and peak concentrations were reached within 2 h in mice, compared with up to 10 h in rats. In the study by Stott *et al* (1982) a comparison of the amount of TCE metabolised on a weight basis indicated that the mouse metabolises 1.2 times more than the rat at 10 ppm and 2.6 times at 600 ppm.

The rate of elimination of the major metabolites differs markedly. Chloral and trichloroethanol are cleared from blood with a half-life of 1-2 h whereas the concentrations of TCA in rat and mouse

blood are sustained for up to 30 h and are thereafter cleared within 48 h of dosing (Prout *et al*, 1985).

Low levels of S-1,2-dichlorovinylglutathione have been detected in rat bile (Dekant *et al*, 1990) and S-1,2-dichlorovinyl-N-acetylcysteine (<0.1% of the dose) in mouse and rat urine after dosing with TCE, demonstrating a second metabolic pathway involving conjugation with glutathione and metabolism through the mercapturic acid pathway (Dekant *et al*, 1986a, 1990; Green *et al*, 1990; Birner *et al*, 1993) (Figure 1). The S-2,2-dichlorovinyl-N-acetylcysteine isomer was also detected in rat urine (Commandeur and Vermeulen, 1990).

4.2 STUDIES IN MAN

The kinetics and metabolism of TCE in man have been reported from a large number of occupational and volunteer studies (see Davidson and Beliles, 1991 for review). Pulmonary uptake is initially rapid, but at least 8 h are required for complete tissue equilibrium to be achieved. Uptake is increased with exercise and approximately doubles from rest to a work rate of 50W. There is little direct information about tissue distribution. Tissue concentrations are expected to be proportional to exposure duration and concentration and to follow the distribution pattern seen in animals. TCE could still be detected in exhaled air 18 h after exposure due to the relatively long half-life of elimination from adipose tissues (3.5-5.0 h) compared to other tissues (Fernandez *et al*, 1977; Monster, 1979). The principal metabolites excreted in urine are those known in animals, trichloroacetic acid and trichloroethanol, the latter being conjugated with glucuronic acid. Trichloroethanol and its glucuronide are rapidly eliminated in urine with a half-life of approximately 10 h. In contrast to this short half-life, that of trichloroacetic acid is approximately 52 h (range 35-70 h). Saturation of metabolism has not been established in man within the range of experimental studies (up to 380 ppm).

The second minor pathway of TCE metabolism involving glutathione conjugation has also been described in man. S-(dichlorovinyl)-N-acetylcysteine, was detected in the urine of workers exposed to TCE of undefined purity during an 8-h work shift when cleaning metal parts in a TCE bath (Birner *et al*, 1993). Both the 1,2- and 2,2- isomers of S-dichlorovinyl-N-acetylcysteine were detected in urine. Details of the exposure concentrations were not given. Comparisons of the levels of this conjugate with the levels of trichloroacetic acid in the same urine samples led the authors to conclude that glutathione conjugation was a more significant pathway in man than in rodents. However, urine was only collected for 16 h post-exposure in this study. The long half-life of trichloroacetic acid in man, only 6% of the total body burden of TCA was excreted in 36 h in a human volunteer study (Müller *et al*, 1974), precludes any such comparisons of the relative extents of the 2 pathways.

SECTION 5. MUTAGENICITY AND RELATED END-POINTS

Mutagenicity studies have been conducted on a routine basis for the last 15 years. Validated methods which have been recognised and accepted by health authorities or relevant expert committees have been only available for the last 7 to 9 years, in accordance with OECD guidelines 471-485 from 1984 (OECD, 1993) and Commission directives 84/449/EEC and 87/302/EEC (EC, 1984, 1988). Hence a considerable number of unreliable and not confirmed test results are found in the published literature. Another important aspect of the internationally accepted methods is the inclusion of negative and positive controls, the repetition of *in vitro* experiments, and a knowledge on the purity of the test substance. Frequently, published studies are confounded by the use of test material of unknown purity or by the use of an industrial grade of the test material (Leuschner and Leuschner, 1991).

With TCE, for example, samples containing low concentrations of mutagenic oxirane stabilisers (epichlorohydrin and/or 1,2-epoxybutane) have been used in many studies.

In this report the original interpretation of test results is based on current standards for mutagenicity testing, in accordance with OECD guidelines 471-485 from 1984 (OECD, 1993) and Commission directives 84/449/EEC and 87/302/EEC (EC, 1984, 1988), which means that a positive effect is indicated by a reproducible, dose-related response, and e.g. in the Ames test by at least a doubling of the spontaneous revertants rate. The original interpretation of the authors of the studies described below may, therefore, be different from that in this text. Studies which do not meet these criteria or which do not contain enough information about the purity of the test substance or in which oxirane-stabilised TCE has been used, have been shortly described but have been disregarded in the final evaluation.

5.1 *IN VITRO*

Details concerning the various test systems, including experimental protocols, strains, mode of exposure, doses and metabolic activation systems are summarised in Tables 9 to 11. The bacterial mutagenicity studies using vapour phase exposure (the most relevant method for volatile substances) have been separated from those using liquid phase exposure. In addition, studies conducted with pure or non-oxirane stabilised TCE have been separated from those conducted with TCE of undefined purity (no information provided in the original publication) or oxirane-stabilised TCE.

5.1.1 Bacteria (Table 9)

Vapour Phase Exposure

Tests with Pure or Non-oxirane Stabilised TCE

A borderline increase (less than 2 times over the control values) in his^+ revertants was detected in *Salmonella typhimurium* TA100, only in the presence of S9 from Aroclor-induced rat or mouse liver, with an epoxide-free TCE sample (Simmon *et al*, 1977). Similar results were reported by Baden *et al* (1979), Bartsch *et al* (1979) and Crebelli *et al* (1982). In the latter study no mutagenic activity was detected when liver S9 from uninduced rats, mice and hamsters was used for activation (Crebelli *et al*, 1982).

A non-oxirane stabilised TCE sample did not show mutagenic activity in *S. typhimurium* TA1535, TA 1537, TA 98 or TA100, with and without liver S9. (Shimada *et al*, 1985; Koorn, 1988; McGregor *et al*, 1989).

Tests with TCE of Undefined Purity or with Oxirane-stabilised TCE

With a TCE sample of undefined purity no mutagenic activity was detected in *S. typhimurium* TA 98 and TA 100 when liver S9 from uninduced rats (Voogd and van der Stel, 1979) or when purified rat liver microsomes was used for activation (Waskell, 1978).

Positive effects were found in *S. typhimurium* TA 100 and TA 1535, with and without metabolic activation, with TCE of low purity (97-99%) (Milman *et al*, 1988). The same results were reported in poorly described studies by Riccio *et al* (1983) and Warner *et al* (1988).

A technical-grade TCE sample containing 1,900 ppm of 1,2-epoxybutane and 900 ppm of epichlorohydrin was mutagenic in *S. typhimurium* TA100, with and without metabolic activation (Crebelli *et al*, 1982). Similar results with an oxirane-stabilised TCE sample were reported in *S. typhimurium* TA 1535 and/or TA 100 by other authors (Shimada *et al*, 1985; Koorn, 1988; McGregor *et al*, 1989).

Vapour-phase tests with 1,2-epoxybutane showed that an atmospheric concentration of 0.009% could induce 12-fold and 3-fold increases, respectively in strains TA1535 and TA100. These increases would account for the mutagenic activity of the oxirane-stabilised TCE sample (McGregor *et al*, 1989).

Table 9 Mutagenicity Assays in Bacteria

Test species	Strain, type	Exposure time, temperature	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
Vapour phase exposure with pure or non-oxirane stabilised TCE								
S. typhimurium	TA1535, TA100	7 h, 37°C	RA, MA	Epoxide free	1-2.5%	(-)	In TA100, +S9	Simmon *et al*, 1977
S. typhimurium	TA1535, TA100	7 h, 37°C	RA	99%, epoxide free	1-3%	(-)	In TA100, +S9	Baden *et al*,1979
S. typhimurium	TA100	2-16 h, 37°C	MP	99.5%, epoxide free	5-10%	(-)	In TA100, +S9	Bartsch *et al*, 1979
S. typhimurium	TA100	7 h, 37°C	RA	99.9%, epoxide free	0.33-1.33%	(-)	In TA100, +S9	Crebelli *et al*, 1982
S. typhimurium	TA100	7 h, 37°C	R, M, H	99.9%, epoxide free	0.33-1.33%	-ve		Crebelli *et al*, 1982
S. typhimurium	TA1535, TA100, TA98, TA1537, TA1538	18 h, 37°C	RA	99.98%, epoxide free	1-5%	-ve		Shimada *et al*, 1985
S. typhimurium	TA1535, TA1537, TA98, TA100	48 h, 37°C	RA	99.9%, 7 ppm thymol	Up to 2%	-ve		Koorn, 1988
S. typhimurium	TA1535, TA98, TA100	Undefined	RA, SA	> 99.96%, 8 ppm diiso-propylamine	Up to 20%	-ve		McGregor *et al*, 1989

Table 9 Mutagenicity Assays in Bacteria (cont.)

Test species	Strain, type	Exposure time, temperature	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
Vapour phase exposure with TCE of undefined purity or oxirane-stabilised TCE								
S. typhimurium	TA98, TA100, TA1535	48 h, 37°C	Mi	Undefined	0.5-10%	-ve		Waskell, 1978
S. typhimurium	TA98, TA100	Undefined	R	> 99.5%	0.0001-0.05 mol/l	-ve		Voogd and Van der Stel, 1979
S. typhimurium	TA98, TA100, TA1535, TA1557	Undefined	RA, MA	97-99%	No data	+ve	TA100, TA1535, ±S9	Milman *et al*, 1988
S. typhimurium	TA98, TA100, TA1535, TA1537	8 h, 37°C	RA, MA	Undefined	No data	+ve	±S9, insufficient details	Riccio *et al*, 1983
S. typhimurium	TA100	Undefined	H	Undefined	No data	+ve	No details	Warner *et al*, 1988
S. typhimurium	TA100	7 h, 37°C	RA	Technical, 900 ppm 1,2-epoxy-butane, 900 ppm epichlorohydrin	0.33-1.33%	+ve	TA100, ±S9	Crebelli *et al*, 1982
S. typhimurium	TA1535, TA100, TA98, 1537, 1538	18 h, 37°C	RA	99.5%, 1,2-epoxybutane	1-5%	+ve	TA1535, TA100, ±S9	Shimada *et al*, 1985
S. typhimurium	TA1535, TA1537, TA98, TA100	48 h, 37°C	RA	99.9%, 0.1% oxirane	Up to 2%	+ve	TA1535 ±S9	Koom, 1988
S. typhimurium	TA1535, TA98, TA100	Undefined	RA	TCE containing 0.5-0.6% 1,2-epoxybutane	0.32-5%	+ve	TA1535, TA100, ±S9	Mc Gregor *et al*, 1989

Table 9 Mutagenicity Assays in Bacteria (cont.)

Test species	Strain, type	Protocol	Exposure time, temperature	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
Liquid phase exposure with pure or non-oxirane stabilised TCE									
S. typhimurium	TA100	Plate incorporation	Undefined	RA	Epoxide free	0.1-10 µl	-ve		Henschler *et al*, 1977
S. typhimurium	TA1535, TA1537, TA97,	Preincubation	20 min, 37°C	RA, SA	99.9%, 8 ppm diisopropylamine	10-1,000 µg/ plate	-ve		Mortelmans *et al*, 1986; US-NTP, 1988
S. typhimurium	TA1535, TA100	Incubation	2 h, 37°C	RA	99.9%, epoxide free	Leading to 0.1-10% in atmosphere	-ve		Baden *et al*, 1979
Liquid phase exposure with TCE of undefined purity or oxirane-stabilised TCE									
E. Coli	K_{12} 343/113	Incubation	2 h, 37°C	M	Undefined	3.3 mM	+ve	+S9	Greim *et al*, 1975
S. typhimurium	TA98, TA100, TA1535, TA1538, TA1950, TA1951, TA1952	Preincubation	20 min, 37°C	No	Undefined	0.5-50µl/ plate	+ve	In TA1535, TA1538	Černa and Kypěnová, 1977
S. typhimurium	No data	Preincubation	10 min, 37°C	No data	Undefined	No data	+ve		Hughes *et al*, 1987
S. typhimurium	TA98, TA100	Plate incorporation		R	> 99.5%	0.0001-0.1% in top agar	-ve		Voogd and v.d. Stel, 1979
S. typhimurium	TA97a, TA98, TA100, TA102	Preincubation	20 min, 37°C	No	Undefined	No data	-ve	No details	Calandra *et al*, 1987
S. typhimurium	TA1535, TA98, TA100	Preincubation	20 min, 37°C	RA	96.8%, 0.5-0.6% 1,2-epoxybutane	Up to 10,000 µg/plate	-ve		McGregor *et al*, 1989

a MP, phenobarbital-induced mouse liver S9; RA, Aroclor-induced rat liver S9; SA, Aroclor-induced Syrian hamster liver S9; MA, Aroclor-induced mouse liver S9; R, M, H, uninduced rat, mouse and Syrian hamster liver S9; Mi, Aroclor- or phenobarbital-induced rat liver purified microsomes

b +ve, positive (reproducible and dose-related increase in number of revertants); -ve, negative; (-) negative: approximately 2 x background level, but reported +ve by the authors

Liquid Phase Exposure

Tests with Pure or Non-oxirane Stabilised TCE

Highly purified, epoxide-free TCE samples were not mutagenic in *S. typhimurium* TA100 with and without metabolic activation using the plate incorporation assay (Henschler *et al*, 1977), and in strains TA1535, TA1537, TA97, TA98 and TA100 when assayed following the preincubation protocol (Mortelmans *et al*, 1986; US-NTP, 1988) or liquid incubation test (Baden *et al*, 1979).

Tests with TCE of Undefined Purity or Oxirane-stabilised TCE

In a study reported without sufficient detail, an analytical-grade TCE sample (only one dose was tested) showed a borderline effect in *Escherichia coli* K12 strain 343/113, in the presence of metabolic activation (Greim *et al*, 1975).

With a TCE sample of undefined purity, positive effects were reported in *S. typhimurium* TA1535 and TA1538, in a preincubation test (Černa M and Kypěnová H, 1977; Hughes *et al*, 1987).

Negative results were obtained with TCE of undefined purity in plate incorporation assays with *S. typhimurium* TA98, TA100 and TA1535, with and without metabolic activation (Voogd and van der Stel, 1979), and in a preincubation assay with TA97a, TA98, TA100 and TA102 (Calandra *et al*, 1987).

No mutagenic response was observed with oxirane-stabilised TCE in a preincubation assay, both with and without S9 (McGregor *et al*, 1989).

5.1.2 Fungi and Yeasts (Table 10)

A highly purified TCE sample gave negative results in a forward mutation assay with *Schizosaccharomyces pombe* strain P1, with and without metabolic activation (Rossi *et al*, 1983).

A highly purified, epoxide-free sample of TCE was tested in the mould *Aspergillus nidulans* for induction of forward mutations and mitotic segregation. The assay with the haploid strain 35 revealed a weak mutagenic effect in cultures grown in the presence of TCE vapours. Furthermore, a dose-related increase in the frequency of mitotic segregants (non-disjunctional diploids and haploids) was detected in colonies of the diploid 35x17 strain exposed to very high concentrations of TCE. No effect was found when the stationary phase strain 35 was used (Crebelli *et al*, 1985).

Table 10 Mutagenicity Assays in Fungi or Yeasts

Test species	Endpoint	Protocol	Exposure time, temp.	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
With pure or non-oxirane stablised TCE									
S. pombe P1	Forward mutation	Liquid incubation	1-4 h, 32°C	RPN, MPN	99.98%, epoxide free	5-25 mM	-ve	Stationary and growing phase tested	Rossi *et al*, 1983
A. nidulans 35	Forward mutation, mitotic segregation	Vapour exposure	5 d, 37°C	E	Epoxide free	2,500-5,000 ppm	+ve	In growing phase only	Crebelli *et al*, 1985
A. nidulans 35x17	Mitotic segregation	Vapour exposure	24 h, 37°C	E	Epoxide free	7,500 - 17,500 ppm	+ve	In growing phase only	Crebelli *et al*, 1985
With TCE of undefined purity or oxirane-stabilised TCE									
S. cerevisiae XV185-14C	Back mutation at *b* loci	Liquid incubation	1-4 h, 30°C	M	Technical	0.1-2.0 µl/ml	+ve	+S9, at low survival rate (1%)	Shahin and Von Borstel, 1977
S. cerevisiae D7	Reversion gene conversion recombination	Liquid incubation	4 h, 37°C	M	Undefined	10-40 mM	+ve	+S9, at survival rate ≥ 50%	Bronzetti *et al*, 1978
S. cerevisiae D7	Mitotic recombination, gene conversion	Liquid incubation	1 h, 37°C	E[c]	Undefined	15-22 mM	+ve	At survival of 67%	Callen *et al*, 1980
S. cerevisiae D7	Gene conversion reversion	Liquid incubation	2 h, 30°C	MA	Analytical grade	11.1-22.2 mM	-ve	Poor survival with S9	Koch *et al*, 1988
S. cerevisiae D61.M	Aneuploidy	Liquid incubation	2.30 h, 30°C	MA	Analytical grade	5.5-16.6 mM	+ve	-S9, at poor survival (45%)	Koch *et al*, 1988
S. pombe	Forward mutation	Liquid incubation	1-4 h, 32°C	RPN, MPN	Technical, 1,900 ppm 1,2-epoxy- butane, 900 ppm epichlorohydrin	5-25 mM	-ve	Stationary and growing phase tested	Rossi *et al*, 1983

a E, endogenous metabolic activation; M, uninduced mouse liver; RPN, liver S9 from rats pretreated with phenobarbital and β-naphthoflavone; MPN, liver S9 from mice pretreated with phenobarbital and β-naphthoflavone; MA, liver of mice pretreated with Aroclor

b +ve, positive (reproducible and dose-related increase in mutants); -ve, negative

c High cytochrome P-450 content

In contrast an oxirane-containing technical TCE sample gave negative results in a forward mutation assay with *S. pombe* strain P1, with and without metabolic activation (Rossi *et al*, 1983).

Shahin and Von Borstel (1977) tested a technical-grade TCE sample in *Saccharomyces cerevisiae* XV185-14C. In this study back mutations were scored at 3 loci. Dose-related increases in revertant rates were reported at all loci studied but only in assays with metabolic activation and at very low survival rates.

With a TCE sample of unspecified purity, slight increases in revertant and convertant frequencies were observed at high survival levels in *S. cerevisiae* strain D7 (point mutation and gene conversion), only in assays with exogenous metabolic activation (Bronzetti *et al*, 1978).

In a study by Callen *et al* (1980) exposure of *S. cerevisiae* strain D7 to a TCE sample of unspecified purity produced, at high survival levels, significant increases of gene convertants and mitotic cross-overs and a doubling in the spontaneous frequency of revertants. In their conclusion, the authors suggested that the compound needs metabolic activation for its genetic activity to be expressed and that metabolism is more rapid in strain D7.

A slight positive effect was found in *S. cerevisiae* strain D61.M, but not in D7 with a analytical grade sample of trichloroethylene, with and without metabolic activation by S9 derived from liver of Aroclor-pretreated mouse (Koch *et al*, 1988).

5.1.3 Mammalian Cells (Table 11)

Gene Mutations

With pure TCE, a borderline increase in mutants at the thymidine kinase locus was reported in mouse L5178Y lymphoma cells, only with metabolic activation (US-NTP, 1988). A similar response in mouse lymphoma cells with a TCE sample of undefined purity was reported by Rudd *et al* (1985), Caspary *et al* (1988) and Myhr and Caspary (1991). In the latter study the mutagenic activity was found only at concentrations near or above the solubility limit of TCE. A negative response of TCE was observed in human lymphoblasts (Caspary *et al*, 1988).

Chromosomal Effects

In a study by Galloway *et al* (1987) no significant induction of structural chromosome aberrations was found in CHO cells exposed to TCE of undefined purity at concentrations up to 14,900 mg/ml.

Table 11 Genotoxicity Assays in Mammalian Cells *In Vitro*

Cell type	Protocol	Exposure time	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
Forward mutation								
L5178Y mouse lymphoma cells	Liquid incubation	4 h	RA.	> 99.9%, 8 ppm diiso-propylamine	25.0-200.0 nl/ml	(-)	+ S9	US-NTP, 1988
L5178Y mouse lymphoma cells	Not specified	Not specified	Yes	Undefined	Not specified	+ve	+ S9	Rudd *et al*, 1983
L5178Y mouse lymphoma cells	Liquid incubation	4 h	RA	Undefined	Up to 400 µg/ml	(-)	+ S9	Caspary *et al*, 1988
Human lymphoblast TK6 cells	Liquid incubation	4 h	RA	Undefined	Up to 600 µg/ml	-ve		Caspary *et al*, 1988
L5178Y mouse lymphoma cells	Liquid incubation	4 h	RA	Undefined	25-300 nl/ml, -S9; 15.6-500 nl/ml, +S9	(-)	+ S9	Myhr and Caspary *et al*, 1991
SCE								
CHO	Vapour exposure	1 h	RA	Undefined	0.17%	-ve		White *et al*, 1979
CHO	Liquid incubation	25 h	None	Undefined	17.9-700 µg/ml	(-)		Galloway *et al*, 1987
CHO	Liquid incubation	2 h	RA	Undefined	49.7-14,900 µg/ml	(-)		Galloway *et al*, 1987
Chromosomal aberration								
CHO	Liquid incubation	8-12 h	None	Undefined	745-14,900 µg/ml	-ve		Galloway *et al*, 1987
CHO	Liquid incubation	2 h	RA	Undefined	499-14,900 µg/ml	-ve		Galloway *et al*, 1987

Table 11 Genotoxicity Assays in Mammalian Cells *In Vitro* (cont.)

Cell type	Protocol	Exposure time	Metabolic activation[a]	TCE purity, stabiliser	Dose (range)	Result[b]	Comment	Reference
UDS								
Rat hepatocytes	Vapour exposure	3-18 h	E	99.98%, epoxide free	0.1-2.5%	-ve	Toxicity ≥ 50 %	Shimada *et al*, 1985
Rat hepatocytes	Vapour exposure	3-18 h	E	99.5% + 1,2-epoxy-butane	0.1-2.5%	-ve	Toxicity ≥ 50 %	Shimada *et al*, 1985
Human NI-38 cells	Liquid incubation	1.5 h	RA	99.9%	0.1-5.0 µg/ml	+ve	± S9	Beliles, 1980
Human lymphocytes	Liquid incubation	1 h	RP	Undefined	2.5-10 µg/ml	+ve	+ S9 only	Perocco and Prodi, 1981
Phenobarbital-induced rat hepatocytes	Liquid incubation	1 h	E	Undefined	2.8 mM	+ve		Costa and Ivanetic, 1984
Transformation								
Fischer rat embryo cells	Liquid incubation	48 h	E	99.9%	1.1-11 mM	+ve		Price *et al*, 1978
Syrian hamster embryo cells	Liquid incubation	7 d	E	Undefined	5-25 µg/ml	(-)		Amacher and Zelljadt, 1983
BALB/c-3T3	Vapour exposure	24 h	E	Undefined 97-99%	4-250 µg/ml	+ve		Tu *et al*, 1985

a RA, Aroclor-induced rat liver; RP, phenobarbital-induced rat liver S9; E, endogenous

b +ve, positive (reproducible and dose-related increase in number of revertants); -ve, negative; (-) negative: approximately 2 x background level, but reported +ve by the authors

Both negative and weakly positive results were reported in studies carried out to evaluate the possible induction of sister-chromatid exchanges (SCE) in Chinese hamster ovary cells (CHO). Negative results in were reported by White *et al* (1979). A TCE sample of undefined purity produced weak but dose-related increases in SCE, both with and without metabolic activation (Galloway *et al*, 1987).

DNA Damage

The induction of unscheduled DNA synthesis (UDS) in different cell systems gave contrasting results. Two TCE samples with and without oxirane stabilisers were tested in the vapour phase in primary rat hepatocytes by Shimada *et al* (1985). Both samples did not induce DNA repair, even at doses producing extensive cell killing. Perocco and Prodi (1981) tested a TCE sample of unspecified purity in human lymphocytes and found positive results only in assays with metabolic activation. Significant incorporation of radioactive DNA precursors in hepatocyte suspensions isolated from phenobarbital-treated rats was detected after TCE exposure of unspecified purity (Costa and Ivanetich, 1984).

DNA Binding In Vitro

Covalent binding of TCE or its metabolites to microsomal protein, RNA or DNA has been demonstrated *in vitro* (Bolt and Filser, 1977; Banerjee and Van Duuren, 1978).

Mazullo *et al* (1992) reported *in vitro* binding of TCE to calf thymus DNA mediated by phenobarbital-induced liver microsomes of Wistar rats and Balb/c mice. The level of binding with rat liver microsomes was double that seen with mouse microsomes and is therefore not consistent with either the species differences in hepatocarcinogenicity, or the significantly higher rates of TCE metabolism found in mice *in vivo*. The higher rates of binding seen with rat liver microsomes were not reproduced in an *in vivo* study described in the same report.

Cell Transformation

Morphological transformation was studied in 3 cellular systems with weakly positive results reported in all cases. TCE exposure produced transformed foci in Fischer rat embryo cells, which developed into malignant tumours when inoculated subcutaneously into newborn rats (Price *et al*, 1978). Weakly positive results were also obtained with TCE samples of undefined purity in Syrian hamster embryo cells (Amacher and Zelljadt, 1983) and in the BALB/c-3T3 cell transformation assay where

a weak but dose-related increase in the number of transformed type-III foci was observed (Tu *et al*, 1985).

5.1.4 Summary of *In Vitro* Studies

Many mutagenicity studies on TCE have been carried out the past with oxirane-stabilised test material (epichlorohydrin, 1,2-epoxybutane). As *in vitro* bacterial mutagenicity assays are particularly sensitive to these type of substances, positive results observed in studies with TCE, known to contain epoxides, or with TCE of undefined purity have been disregarded in this summary.

TCE was non-mutagenic in *in vitro* bacterial mutagenicity assays when the pure compound was tested.

Tests in fungi with pure TCE or with TCE of undefined purity did show evidence of weak mutagenic activity (aneuploidy) in specific strains. Most of the positive results were found at high dose levels and with added metabolic activation or high endogenous metabolic activation. It is known that TCA, a major metabolite of TCE, causes protein denaturation due to reduction of pH, which could be responsible for the effects seen in fungal assays.

The majority of studies on gene mutations in mammalian cells were negative. A borderline increase in gene mutations was reported in one study in mammalian cells *in vitro*. In most studies no information is provided about the purity of the TCE used. Moreover, positive effects reported were observed at high dose levels, at the limit of the solubility of TCE.

In vitro chromosomal aberration assays showed that TCE is not clastogenic; all available tests were conducted with TCE of undefined purity.

Contradictory results were obtained in assays for DNA damage. Both positive and negative results were reported in UDS studies in which TCE of undefined purity or technical TCE was used. Weakly positive results were reported in cell transformation studies with TCE of undefined purity or with pure TCE.

In conclusion, pure TCE is non-mutagenic in the majority of *in vitro* mutagenicity test systems. In a few reports it was weakly positive. Many reports do not state the purity of TCE and are, therefore, inadequate to draw a final conclusion with respect to the mutagenicity of pure TCE because of the presence of mutagenic stabilisers. The weakly positive findings were observed only at high dose levels with no evidence of reproducibility in or between laboratories.

5.2 IN VIVO

Details concerning species, type of assay, route of exposure, doses and TCE purity are summarised in Tables 12 (animal studies) and 13 (studies in man).

5.2.1 Host Mediated Assays

No mutagenic activity was found in an i.p. host-mediated assay using mice exposed to pure TCE by inhalation repeatedly for 5 d, with *S. typhimurium* TA 98 as inoculum (Beliles *et al*, 1980).

Negative results were obtained in an intraperitoneal and intrasanguineous host-mediated assays with male mice, inoculated with yeast cells *(S. pombe* strain P1) and treated by gavage (2 g/kgbw, 4 and 16 h before sacrifice) with a highly purified TCE sample as well as a technical sample containing 1,2-epoxybutane (0.19%) and epichlorohydrin (0.09%) as stabilisers (Rossi *et al*, 1983).

S. cerevisiae strains D4 and D7 were used in intrasanguineous host-mediated assays with male mice receiving TCE by gavage as an acute dose (400 mg/kgbw) or by repeated administration (total dose 3,700 mg/kgbw). In both cases significant increases in the frequency of revertants (strain D7) and convertants (strains D4 and D7) were detected in yeast cells recovered from liver, lungs and kidneys (Bronzetti *et al*, 1978).

5.2.2 Gene Mutations

In a mouse spot test with 99.5% pure TCE (stabilisers not specified), 2 hair spots were observed in 145 surviving offspring in the low-treatment group (140 mg/kgbw) and 2 in 51 survivors in the high-dose group (350 mg/kgbw). The author suggested some weak genotoxic activity of TCE *in vivo* (Fahrig, 1977). The relevance of this finding is questionable because of the i.p. route of exposure and the near-lethal doses used.

5.2.3 Chromosomal Effects

Animal Studies (Table 12)

Positive effects have been reported in a micronucleus assay in the CD-1 mouse after oral administration of an analytical-grade TCE sample (Duprat and Gradiski, 1980). The authors stated that they analysed for micronuclei "including microbodies appearing to be of nuclear origin" and that

Table 12 *In Vivo* Mutagenicity Assays with TCE

Assay	Animal species	Strain	TCE purity, stabiliser	Protocol[a]	Dose (range) or concentration	Result[b]	LEC[c], comment	Reference
Host mediated								
S. typhimurium TA 98	Mouse	CD1	Undefined	I.p. after inhalation for 5 d	100-500 ppm	-ve		Beliles, 1980
S. pombe P1	Mouse	C57BLx CD1	99.98%, epoxide free	I.p. and i.s. after acute oral dose	2,000 mg/kgbw	-ve		Rossi *et al*, 1983
S. pombe P1	Mouse	C57BLx CD1	Technical, 1,900 ppm 1,2 epoxy-butane + 900 ppm epichloro-hydrin	I.p. and i.s. after acute oral dose	2,000 mg/kgbw	-ve		Rossi *et al*, 1983
S. cerevisiae D7	Mouse	CD1	Undefined, epoxide free[f]	I.s. after acute dose	400 mg/kgbw	+ve	Liver, kidney, lung	Bronzetti *et al*, 1978
S. cerevisiae D4	Mouse	CD1	Undefined, epoxide free[f]	I.s. after acute dose	400 mg/kgbw	+ve	Liver, kidney	Bronzetti *et al*, 1978
S. cerevisiae D4	Mouse	CD1	Undefined, epoxide free[f]	I.s. after repeated adm.	3,700 mg/kgbw (total dose)[e]	+ve	Liver, kidney	Bronzetti *et al*, 1978
Spot test	Mouse	C57BLxT	99.5%	I.p., acute	140-350 mg/kgbw	(-)	140 mg/kgbw, toxic concentration	Fahrig, 1977
Micronucleus	Mouse	CD1	99.5%	Gavage, acute	375-3,000 mg/kgbw	+ve	375 mg/kgbw, bone marrow cells	Duprat and Gradiski, 1980

Table 12 *In Vivo* Mutagenicity Assays with TCE (cont.)

Assay	Animal species	Strain	TCE purity, stabiliser	Protocol[a]	Dose (range) or concentration	Result[b]	LEC[c], comment	Reference
Micronucleus	Mouse	B6C3F$_1$	Epoxide free	Gavage, acute	1,200 mg/kgbw	+ve	1,200 mg/kgbw, bone marrow cells	Sbrana *et al*, 1985
	Mouse	C57BL/6J	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	-ve	Splenocytes, bone marrow	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	+ve -ve	Bone marrow Lymphocytes	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h/d for 4 d	5, 50, 500 ppm	-ve	Bone marrow, lymphocytes	Kligerman *et al*, 1992
	Mouse	B6C3F$_1$	> 99%, epoxide free	I.p.	500, 1,00, 2,000, 2,500	-ve	Bone marrow	Shelby *et al*, 1993
	Mouse (♂ only)	C57BL/6J	≥ 99%	Inhalation, 6 h/d for 5 d	5, 50, 500 ppm	-ve	Germ cells	Allen *et al*, 1994
Chromosomal aberrations	Mouse	B6C3F$_1$	Epoxide free	Gavage, acute	1,200 mg/kgbw	-ve	Bone marrow	Sbrana *et al*, 1985
	Mouse	B6C3F$_1$	Epoxide free	Inhalation, 7 h/d, 5 d/wk, 10 wk	600 ppm	-ve	Bone marrow	Sbrana *et al*, 1985
	Mouse	CD-1	Undefined	Gavage, acute	1,000 mg/kgbw	-ve	Bone marrow	Loprieno and Abbondandolo, 1980
	Mouse	Not specified	Undefined	I.p.	0.5xLD_{50} single application; 1/6 LD_{50}, 5 repeated applications	-ve	Bone marrow	Čemá and Kypěnová, 1977

Table 12 *In Vivo* Mutagenicity Assays with TCE (cont.)

Assay	Animal species	Strain	TCE purity, stabiliser	Protocol[a]	Dose (range) or concentration	Result[b]	LEC[c], comment	Reference
Chromosomal aberrations	Rat	Sprague-Dawley	99.9%	Inhalation, 7 h	100-500 ppm	-ve	Bone marrow	Beliles *et al*, 1980
	Mouse	C57BL/6J	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	-ve	Splenocytes	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	-ve	Lymphocytes	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h/d for 4 d	5, 50, 500 ppm	-ve	Lymphocytes	Kligerman *et al*, 1992
SCE	Mouse	C57BL/6J	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	-ve	Splenocytes	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h	5, 500, 5,000 ppm	-ve	Lymphocytes	Kligerman *et al*, 1992
	Rat	CD	> 99%	Inhalation, 6 h/d for 4 d	5, 50, 500 ppm	-ve	Lymphocytes	Kligerman *et al*, 1992
Dominant lethal	Mouse	NMRI/BGA	99.5%	Inhalation, 24 h	50-450ppm	-ve		Slacik-Erben *et al*, 1980
	Rat	Sprague-Dawley	99.9%	Inhalation, 7h/d for 5 d	100-500 ppm	Inconclusive		Beliles *et al*, 1980
Sperm abnormalities	Rat	Sprague-Dawley	99.9%	Inhalation, 10 wk	100-500 ppm	-ve		Beliles *et al*, 1980
	Mouse	CD1	99.9%	Inhalation, 10 wk	100-500 ppm	+ve	100 ppm after 1-4 wk only	Beliles *et al*, 1980
	Mouse	$C57F_1$ x C3H	Undefined	Inhalation, 4h/d for 5d	0.02-0.2%	+ve	0.2%, 23d after exposure	Land *et al*, 1981

Table 12 *In Vivo* Mutagenicity Assays with TCE

Assay	Animal species	Strain	TCE purity, stabiliser	Protocol[a]	Dose (range) or concentration	Result[b]	LEC[c], comment	Reference
UDS	Mouse	$B6C3F_1$	Undefined	Gavage	50-1,000 mg/kgbw	-ve		Mirsalis *et al*, 1985
	Mouse	CD 1	> 99%	I.p.	1,000 mg/kgbw	-ve		Doolittle *et al*, 1987
	Rat	F344	> 99%, epoxide stabiliser free	Gavage	1,000 mg/kgbw	-ve	Kidney cells	Goldsworthy *et al*, 1988a,b; Loury *et al*, 1987
Single-strand breaks	Mouse	$B6C3F_1$	99%	I.p.	2,000 mg/kgbw	-ve		Parchman and Magee, 1982
	Mouse	NMRI	99.5%	I.p.	4-10 mmol/kgbw	+ve	At 1 h, at 6 mmol/kgbw	Walles, 1986
	Mouse	$B6C3F_1$	> 99%, epoxide free	Oral	0.76-22.9 mmol/kgbw	+ve	11.4 mmol/kgbw	Nelson and Bull, 1988
	Rat	Sprague-Dawley	>99%, epoxide free	Oral	3.9-30.4 mmol/kgbw	+ve	22.9 mmol/kgbw	Nelson and Bull, 1988
Sex-linked recessive lethal	*Drosophila*		Technical grade	Diet or s.c. injection?	Undefined	-ve		Abrahamson, 1980
	Drosophila		99.9%	Inhalation	100-500 ppm	-ve		Beliles *et al*, 1980

a i.p. = intraperitoneal; i.s. = intrasanguineous; s.c. = subcutaneous
b +ve, positive; -ve, negative; (-) negative, but reported +ve by the author
c LEC, Lowest Effective Dose (or concentration)
e 150 mg/kgbw by gavage 5 x/wk (22 administrations), plus 400 mg/kgbw on the day of sacrifice
f Purity not specified in original publication, but stated as epoxide free in Crebelli and Carere (1989)

Table 13 Human Occupational Exposure Monitoring Studies

Assay	TCE concentration	TCE purity	Result[a]	Comment	Reference
SCE/lymphocytes	Undefined	Technical	(-)	Slight increase due to some individuals (6 workers, 4 unmatched controls)	Gu *et al*, 1981
	30 ppm, as calculated from urinary TCA levels	Undefined	-ve	22 workers, smokers and non-smokers	Nagaya *et al*, 1989
	7 ppm (mean), 32 ppm (maximum)	Pure and technical	-ve	Non-smokers, 14 ♂, 16 ♀ examined	Seiji *et al*, 1990
	7 ppm (mean), 32 ppm (maximum)	Pure and technical	+ve	Smokers, 8 ♂ examined, slight increase vs. concurrent controls	Seiji *et al*, 1990
Chromosomal aberration/lymphocytes	Undefined	Technical	+ve	Increased chromosome gaps and structural aberrations correlated with longest exposure (n = 15 workers). Smoking not examined as a confounding factor	Rasmussen *et al*, 1988
	Up tp 200 ppm	Technical	(-)	Increased hypodiploid cells in 9/28 workers; correlation was found with mean maximum daily concentration. No matched controls	Konietzko *et al*, 1978
Sperm counts and morphology	Undefined	Technical	-ve		Rasmussen *et al*, 1988

a +ve, positive; -ve, negative; (-) negative, but reported +ve by the author

their high control values may be aprtly based on the scoring method used. Therefore, the data obtained in this study needs to be interpreted with caution.

A statistically significant increase was observed in micronuclei in bone marrow cells of B6C3F$_1$ mice after a single oral treatment (1200 mg/kgbw), utilising an epoxide-free TCE sample as used in Maltoni's bioassay (Sbrana *et al*, 1985; Maltoni *et al*, 1988). At the same dose level no increase in structural chromosomal aberrations was observed (Sbrana *et al*, 1985). Prolonged exposure to 600 ppm TCE (7h/d, 5 d/week, 10 weeks) did not increase the frequency of structural chromosomal aberrations in bone marrow cells (micronuclei were not scored). The negative outcome of the structural cytogenetic analysis in chronically exposed mice suggests that pure TCE is not clastogenic in mouse bone marrow. However, due to the lack of precise information on sample size and lack of peer review (only an abstract was published), it is not possible to determine the significance of the findings by Sbrana *et al* (1985).

No effects of acute TCE exposure (i.p., by inhalation or gavage) were observed in mouse bone marrow chromosome aberration studies by Černa and Kypenova (1977), Beliles *et al* (1980) and Loprieno and Abbandandolo, 1980). High doses were used in 2 studies, viz.: 1,000 mg/kg (Loprieno and Abbandandolo, 1980) and 0.5 LD_{50} (Černa and Kypenova, 1977).

Kligerman *et al* (1992) exposed C57BL/6 mice and CD rats for 6 h to TCE (99%+ purity) concentrations of 5, 500 or 5000 ppm. Splenocytes were isolated from the mice 19 h after exposure and cultured for a period of 46-51 h. No significant increases in the frequencies of chromosomal aberrations, SCE or micronuclei in binucleate cells were observed. The bone marrow of the mice was also sampled 19 h after exposure and no significant increase in the frequency of micronucleated polychromatic erythrocytes was observed. Blood samples were obtained from the rats 18 h after exposure to TCE and were cultured for periods of 48 to 70 h. No significant increases in the frequencies of chromosomal aberrations, SCE or micronuclei in binucleate cells were observed. The bone marrow of the rats was also sampled, 18 h after termination of exposure. A statistically significant and concentration-related increase in the incidence of micronucleated polychromatic erythrocytes was observed at all concentrations tested. The maximum increase in rats exposed to 5000 ppm was approximately 4 times the control value. TCE was also cytotoxic in rats causing a significant concentration-related decrease in the ratio of polychromatic to normochromatoc erythrocytes. The authors also stated that "from the size of the micronuclei observed, it is believed that the induced micronuclei were the result of aneuploidy events". In a follow-up study, Kligerman *et al* (1992) exposed groups of CD rats to TCE at 5, 50 or 500 ppm for 6 h/d for a period of 4 d. All the endpoints examined, as detailed above, were negative.

Shelby *et al* (1993) conducted 2 bone marrow micronucleus assays on male B6C3F$_1$ mice. In the first assay 5-6 mice received i.p. 3 doses of TCE at 500, 1,000 or 2,000 mg/kg at 24 h intervals. The mice were killed 24 h after the third dose. No deaths and no statistically or biologically significant increase in the incidence of micronucleated erythrocytes were observed. In the second assay, 5-6 male mice received 3 i.p. doses of TCE at 2,000 or 2,500 mg/kg, again given 24 h apart. One animal died at each of the dose levels tested indicating that the maximum tolerated dose level was used. No statistically or biologically significant increase in the incidence of micronucleated erythrocytes were observed.

Studies in Man

Contradictory results were found in man occupationally exposed to TCE with respect to SCE induction (Gu *et al*, 1981; Nagaya *et al*, 1989; Seiji *et al*, 1990). When confounding factors like smoking are taken into account, no effect could be detected at levels of approximately 30 ppm in air, calculated from urinary TCA levels (Nagaya *et al*, 1989; Seji *et al*, 1990). In a study by Rasmussen *et al* (1988), a slight increase in chromosomal aberrations was observed in cultured lymphocytes of 15 workers exposed to TCE during metal degreasing when compared to a large population (669) of non-exposed people. The significance of these findings is unclear as increased levels of chromosomal aberrations were confined to 3 workers only and no information is given about actual exposure levels and confounding factors like smoking.

5.2.4 Germ Cells Effects

Animal Studies

In a dominant-lethal assay, no reduction of fertilization rate and no significant increase of pre- and post-implantation losses were observed after a 24-h inhalation exposure of male NMRI/BGA mice to TCE vapours up to 450 ppm (Slacik-Erben *et al*, 1980). Inconclusive results were found in rats after 5 d of inhalation exposure to pure TCE (Beliles *et al*, 1980). The high background of dead implantations in all groups during the first weeks of testing did not allow for any reliable conclusion. Allen *et al* (1994) exposed male C57B1/6J mice by inhalation to 5, 50 or 500 ppm TCE for 6 h/d for 5 days. The authors concluded that 5 consecutive days of exposure to TCE during preleptotene through early pachytene stages of meitoic cell development did not result in increased frequencies of micronuclei in spermatids.

In another, non-validated type of study (Land *et al*, 1981), significant increases in the number of morphologically abnormal spermatozoa were observed in C57F1xC3H mice after inhalation exposure for 4 h/d during 5 d to a TCE sample of undefined composition.

In a 10-week inhalation study in mice, abnormal sperm was found at 1 and 4 weeks after treatment and not after 10 weeks suggesting that spermatogonia were either unaffected or that lesions were repaired. No abnormal sperm was observed in a 10-week inhalation study with rats (Beliles *et al*, 1980). Crebelli and Carere (1989) state that, due to the possible involvement of non-genotoxic mechanisms, these results should not be considered as evidence for TCE mutagenicity at the germ cell level.

Studies in Man

No effects on sperm counts and sperm morphology were observed in workers exposed to TCE during metal degreasing (Rasmussen *et al*, 1988).

5.2.5 DNA Damage

In a study with B6C3F$_1$ mouse, acute TCE exposure did not induce UDS in an *in vivo/in vitro* hepatocyte DNA-repair assay, but significantly increased hepatic cell proliferation (Mirsalis *et al*, 1985). A similar result was obtained in CD-1 mice where oral administration of TCE significantly increased the percentage of hepatocytes in the S-phase but did not cause DNA repair (Doolittle *et al*, 1987).

The ability of trichloroethylene (purity: > 99%, epoxide stabiliser free) to induce unscheduled or replicative DNA synthesis (RDS) was also investigated in an *in vivo/in vitro* rat kidney cell assay. Administration of TCE (1,000 mg/kgbw) to male Fischer 344 rats did not induce an increase in UDS in the target tissue. At the same dose level TCE did not increase RDS when administered to male rats for 1, 2, 3, or 12 weeks (Goldsworthy *et al*, 1988a,b; Loury *et al*, 1987).

Contrasting results were reported in mouse liver DNA fragmentation studies. No significant fragmentation was seen in B6C3F$_1$ mice by alkaline sucrose gradient centrifugation after i.p. injection of an LD_{50} TCE dose (2 g/kgbw) (Parchman and Magee, 1982), whereas a linear increase in DNA single-strand breaks (SSB) was detected with the DNA unwinding technique in the liver and kidney but not in the lung of NMRI mice (Walles, 1986). One possible explanation for this discrepancy can be given by the different time intervals between treatment and killing (6 h vs. 1 h in the B6C3F$_1$ and NMRI study, respectively) since it was shown that TCE-induced SSB are quickly

repaired (Walles, 1986). The relevance of this endpoint in connection with TCE induced hepatocarcinogenesis is not clear.

TCE induced single-strand breaks in hepatic DNA of male B6C3F$_1$ mice and Sprague-Dawley rats *in vivo* using a alkaline unwinding assay (Nelson and Bull, 1988). Doses of TCE of 22-30 mmol/kgbw were required to produce strand breaks in DNA in rats, whereas a dose of 11.4 mmol/kgbw was sufficient to increase the rate of alkaline unwinding in mice. The effects on DNA were attributed to metabolites of TCE: TCA, DCA, and chloral hydrate inducing DNA single-strand breaks at lower dose levels. Other authors (Macky *et al*, 1990a,b; Chang *et al*, 1992) were unable to reproduce these results (Section 5.3.3).

5.2.6 DNA Synthesis

Stott *et al* (1982) compared the effects of oral TCE treatment on liver DNA synthesis in B6C3F$_1$ mice and Osborne-Mendel rats, which are susceptible and non-susceptible to TCE hepatocarcinogenesis, respectively. In this study repeated gavage of a TCE dose, which is reported to induce tumours after chronic administration, resulted in increased DNA synthesis in mice (220% of the control group) and, to a lesser extent, in rats (175% of the control group). The same treatment produced very low DNA binding in the mouse liver and severe histopathological changes (hepatocellular swelling and necrosis) in mouse but not in rat hepatocytes.

5.2.7 DNA Binding *In Vivo*

No covalent binding of TCE or its metbolites to microsomal protein, RNA or DNA has been demonstrated *in vivo* by Parchmann and Magee (1982) and by Stott *et al* (1982).

TCE binding to macromolecules (DNA, RNA, proteins) was measured in lung, liver, kidney and stomach of male Wistar rats and Balb-c mice, 22 h after injection of radiolabeled TCE (Mazullo *et al*, 1992). A weak interaction with DNA was reported for all of the tissues analysed (approximately 1 adduct in 10^7 nucleotides), which did not reflect the known target organ or species dependent carcinogenicity of TCE. Furthermore, the pattern of binding was unusual, the levels bound to RNA being significantly higher than those bound to either DNA or protein. No distinction was made between covalent binding and the incorporation through the C-1 pool. Consequently, these results cannot be taken as evidence of DNA alkylation *in vivo* by TCE or its metabolites.

5.2.8 *Drosophila*

Abrahamson (1980) and Beliles *et al* (1980) have reported negative studies with TCE in *Drosophila* (Table 10).

5.2.9 Summary of *In Vivo* Studies

TCE showed positive effects in host-mediated assays only in yeasts which are known to be sensitive to TCE *in vitro*. A single host-mediated assay utilising *S. typhimurium* TA98 was negative. It is known that TCA (a major metabolite of TCE) induces protein denaturation due to reduction of pH, which could be the major cause for the effects seen in fungal assays.

Positive results were reported in a mouse bone marrow micronucleus study using a non-standard scoring method. A second positive finding was found in a poorly described study. Two more recent bone marrow micronucleus studies in mice using high inhalation or gavage doses were negative. A single, positive result with TCE in a rat bone marrow micronucleus assay following inhalation exposure appeared to be an effect of aneuploidy. *In vivo* tests for chromosome aberration assays were negative in mice as well as in rats.

A micronucleus assay in germ cells of mice following inhalation exposure to TCE for 5 days was clearly negative. Dominant lethal assays with TCE in mice were also negative. Two sperm abnormality tests in mice showed slight, transient effects after TCE exposure by inhalation.

Acute or repeated TCE exposure produced an increase in hepatic cell proliferation in the mouse but no DNA binding, damage or repair.

Studies in man did not provide evidence of chromosomal damage at TCE levels up to 30 ppm. Other human monitoring studies were inappropriate for evaluation.

There is no conclusive evidence from animal and human studies that pure TCE is genotoxic *in vivo*, either in mammalian cells or in germ cells.

5.3 METABOLITES (Tables 14-17)

5.3.1 TCE Epoxide

TCE epoxide, a putative metabolite of TCE which could never be isolated, is negative in *S. typhimurium* TA1535 and in *E. coli* WP2 uvrA (Kline *et al*, 1982). Weak mutagenic activity was observed in a forward mutation assay with *S. pombe* and V79 cells (Loprieno and Abbondandolo, 1980) and in a morphological transformation test (DiPaolo and Doninger, 1982).

5.3.2 Chloral Hydrate

Chloral hydrate, the TCE metabolite, was found mutagenic in *S. typhimurium* TA100 but inactive in TA 1535, TA1537 and TA98 (Waskell, 1978; Bignami *et al*, 1980; Haworth *et al*, 1983). Leuschner and Leuschner (1991) reported, in modern, well-conducted studies, negative results with chloral hydrate (purity 99.4%) in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 up to 5,000 mg/plate, with and without metabolic activation.

In experiments without exogenous metabolic activation, positive results were obtained in *S. coelicolor* (forward and reverse mutations) and in two forward mutation assays in *A. nidulans* (Bignami *et al*, 1980; Crebelli *et al*, 1985). In *S. cerevisiae*, chloral hydrate was weakly active only in the presence of exogenous activation in assays for gene conversion in strain D7 (Bronzetti *et al*, 1984) and reversion (*trp* locus) in strain XV185-14C (Nestmann and Lee, 1985). Borderline increases in gene convertants were also observed with strain D7 in cells recovered from lungs, but not from liver or kidneys, in an intra-sanguineous host-mediated assay with mice receiving an acute oral dose of chloral hydrate (500 mg/kgbw) (Bronzetti *et al*, 1984).

Chloral hydrate has been assayed for its ability to induce chromosome number variation in human lymphocytes in culture. Aneuploidy induction has been detected by means of *in situ* hybridization on interphase nuclei with a chromosome Y-specific DNA probe. A dose-dependent increase in the number of hyperdiploid nuclei was found at chloral hydrate concentrations ranging from 250 to 750 mg/ml (Vagnarelli *et al*, 1990). In another test using human lymphocytes, a slight increase in sister-chromatid exchanges was found after incubation with chloral hydrate (Gu *et al*, 1981).

Russo *et al* (1984) reported aneuploidy in germ cells of mice following i.p. administration of chloral hydrate at 82.7, 165.4 and 413.5 mg/kgbw.

Table 14 Bacterial Mutagenicity Assays with TCE Metabolites

	Species	Strain, type	Protocol	Metabolic activation	Dose (range)	Result[a]	Comment	Reference
TCE epoxide	*S. typhimurium*	TA 1535	Preincubation assay	No	0.25-5 mM	-ve		Kline *et al*, 1982
	E. coli	WP2 uvrA	Preincubation assay	No	0.25-5 mM	-ve		Kline *et al*, 1982
Chloral hydrate	*S. typhimurium*	TA100, TA1535, TA1537, TA98	Plate incorporation	Aroclor-induced rat liver S9	Up to 10 mg/plate	+ve	TA100, ±S9	Waskell *et al*, 1978; Bignami *et al*, 1980; Haworth *et al*, 1983
	S. typhimurium	TA98, TA100, TA1535, TA1537, TA1538	Plate incorporation	Aroclor-induced rat liver S9	Up to 5,000 µg/plate	-ve	GLP study	Leuschner and Leuschner, 1991
Trichloroethanol	*S. typhimurium*	TA100, TA1535	Spot test	Aroclor-induced Rat liver S9	2-20 µl/plate	(-)	-S9, TA100, TA1535	Bignami *et al*, 1980
	S. typhimurium	TA100, TA1535, TA1537, TA98	Plate incorporation	Rat liver S9	Up to 7.5 mg/plate	-ve		Waskell *et al*, 1978
Trichloroacetic acid	*S. typhimurium*	TA98, TA100	Plate incorporation	± S9	Up to 5 mg/plate	-ve		Moriya, 1983
	S. typhimurium	TA100, TA1535, TA1537, TA98	Plate incorporation	Rat liver S9	Up to 0.45 mg/plate	-ve		Waskell *et al*, 1978
Dichloroacetate	*S. typhimurium*	TA98, TA100, TA1535, TA1537, TA1538	Plate incorporation	Aroclor-induced rat liver S9		(-)	±S9, TA98	Herbert *et al*, 1980
S-1,2-Dichloro-vinyl-L-cysteine	*S. typhimurium*	TA100		Rat kidney S9		+ve	+S9	Dekant *et al*, 1986b
	S. typhimurium	TA2638	Plate incorporation	Aroclor-induced rat liver S9	Up to 10 nmol/plate	+ve	+S9, from 1 nmol/plate	Commandeur *et al*, 1991
S-2,2-Dichloro-vinyl-L-cysteine	*S. typhimurium*	TA2638	Plate incorportion	Aroclor-induced rat liver S9	Up to >100 nmol/plate	+ve	+S9	Commandeur *et al*, 1991

a +ve, positive; -ve, negative; (-) not considered to be biologically significant, but reported +ve by the authors

Table 15 Fungal Mutagenicity Assays with TCE Metabolites

Test compound	Species	Strain, type	Protocol	Metabolic activation	Dose (range)	Result	Comments	Reference
TCE epoxide	*S. pombe*					+ve		Loprieno and Abbondandolo, 1980
Chloral hydrate	*S. coelicolor*	A3	Spot and plate test	No	2-10 mg/plate	+ve	At poor survival rate	Bignami *et al*, 1980
	A. nidulans	35	Spot and plate test	No	1-10 mg/plate	+ve	In plate test only	Bignami *et al*, 1980
	A. nidulans	35x17	Plate test	No	5-10 mM	+ve	Segregation	Crebelli *et al*, 1985
	S. cerevisiae	D7	Suspension test	± S9	5-20 mM	(-)	Gene conversion	Bronzetti *et al*, 1984
Chloral	*S. cerevisiae*	XV185-14C	Plate test	± S9	Up to 25 µl/plate	(-)	Reversion	Nestmann and Lee, 1985
Trichloroethanol	*A. nidulans*	35	Spot and plate test	No	5-10 µl/plate	(-)	In spot test only	Bignami *et al*, 1980
	S. coelicolor	A3	Spot and plate test	No	10-40 µl/plate	-ve		Bignami *et al*, 1980
	A. nidulans	35x17	Plate test	No	5.12-10.24 mM	+ve	Segregation	Crebelli *et al*, 1985

+ve, positive (reproducible and dose-related increase in mutant rate/number); -ve, negative; (-), ≤ 2 x background level, but reported +ve by author

Table 16 Other *In Vitro* Mutagenicity Assays with TCE Metabolites

Test compound	Assay	Strain, type	Experimental procedure	Metabolic Activation	Dose (range)	Result[a]	Comments	Reference
TCE-epoxide	Point mutation	V79				+ve		Loprieno and Abbondandolo, 1980
	Transformation	Syrian hamster embryo cell	Liquid incubation (30 min)	No	1.1-5 mM	+ve	At 5 mM (73% survival)	DiPaolo and Daninger, 1982
Chloral hydrate	Chromosome aberration	Human lymphocytes				+ve	Increased number of hyperdiploïd nuclei	Vagnarelli *et al*, 1990
	SCE	Human lymphocytes	Liquid incubation	No	54 mg/l	(-)		Gu *et al*, 1981
Trichloroethanol	SCE	Human lymphocyte	Liquid incubation	No	178 mg/l	(-)		Gu *et al*, 1981
Trichloroacetic acid	Chromosome aberration	Human lymphocytes		±	2,000-5,000 mg/ml	-ve +ve	Neutralised TCA as free acid (±S9)	Mackay *et al*, 1991
	Single-strand breaks	Rat and mouse hepatocytes	Alkaline unwinding assay, 4 h incubation	No	1-10 mM	-ve		Chang *et al*, 1992
	Single-strand breaks	Human lympho-blastic cell		No	0.1-10 mM	-ve		Chang *et al*, 1992
Dichloroacetic acid	Single-strand breaks	Rat and mouse hepatocytes;	Alkaline unwinding assay,	No	1-10 mM	-ve		Chang *et al*, 1992
		human lympho-blastic cell	4 h incubation	No	1-20 mM	-ve		Chang *et al*, 1992
S-1,2-Dichloro-vinyl-L-cysteine	DNA repair	Porcine kidney cells				+ve	Weakly +ve	Vamvakas *et al*, 1989

a +ve, positive (reproducible and dose-related increase in number of revertants); -ve, negative; (-) negative: approximately 2 x background level, but reported +ve by the authors

Table 17 *In Vivo* Mutagenicity Assays with TCE Metabolites

Test compound	Assay	Species, strain	Route	Dose (range)	Result	Comment, LEC	Reference
Chloral hydrate	l.s. host mediated with *S. cerevisiae*	Mouse	Oral	500 mg/kgbw	(-)	Slight effect in lung cells but not liver or kidney cells	Bronzetti *et al*, 1984
	Cytogenic evaluation of germ cells	Mouse, C57BlxC3H	I.p.	82.7-413.5 mg/kgbw	+ve	Aneuploidy from 82.7 mg/kgbw	Russo *et al*, 1984
	Micronucleus/bone marrow	Mouse, C57BlxC3H	I.p.	315-2,500 mg/kgbw	-ve		Bruce and Heddle, 1979
	Micronucleus/bone marrow	Mouse, NMRI	I.p.	500 mg/kgbw	-ve		Leuschner and Leuschner, 1991
	Micronucleus/germ cells	Mouse, C57Bl/6J (♂ only)	I.p.	41, 83 or 165 mg/kgbw	+ve -ve	During spermatogon-ial stem cell or preloptene sperma-tocyte stages During leptotene-zygotene or dia-kinesis-metaphase stages	Allen *et al*, 1994
	Sperm abnormality	Mouse, C57BlxC3H	I.p.	315-2,500 mg/kgbw for 5 d	-ve		Bruce and Heddle, 1979
	Chromosome aberration/ bone marrow	Rat, Sprague-Dawley	Oral	100-1,000 mg/kgbw	-ve		Leuschner and Leuschner, 1991
	Single-strand breaks	Mouse, B6C3F$_1$	Oral	0.01-20 mmol/kgbw	+ve	In hepatic DNA	Nelson and Bull, 1988
		Rat, Sprague- Dawley	Oral	0.6-20 mmol/kgbw	+ve	In hepatic DNA from 1.8 mmol/kgbw	Nelson and Bull, 1988
Trichloroacetic acid/ trichloroacetate	Chromosome aberration/ bone marrow	Mouse, Swiss	I.p./oral, acute/repeated	125-500 mg/kgbw acute, i.p., oral; 100 mg/kgbw i.p. for 5 d	+ve	TCA	Bhunya and Behera, 1987

Table 17 *In Vivo* Mutagenicity Assays with TCE Metabolites (cont.)

Test compound	Assay	Species, strain	Route	Dose (range)	Result	Comment, LEC	Reference
Trichloroacetic acid/ trichloroacetate	Micronucleus	Mouse, Swiss	I.p.	125-500 mg/kgbw for 2d	(-)	TCA, effect not dose-realetd	Bhunya and Behera, 1987
	Micronucleus	Mouse, C57BL/6JfBL10/Alpk	I.p.	337-1,300 mg/kgbw	-ve	Neutralised TCA	Mackay *et al*, 1990b
	Sperm abnormality	Mouse	I.p.	125-500 mg/kgbw	+ve	TCA	Bhunya and Behera, 1987
	Single-strand breaks	Mouse, B6C3F$_1$	Oral	0.0006-0.10 mmol/kgbw	+ve	Neutralised TCA, from 0.006mol/kgbw	Nelson and Bull, 1988
	Single-strand breaks	Rat, Sprague-Dawley	Oral	0.6-20 mmol/kgbw	+ve	Neutralised TCA, from 0.6 mmol/kgbw	Nelson and Bull, 1988
	Single-strand breaks	Mouse, B6C3F$_1$	Oral	1-10 mmol/kgbw	(-)	Effect at 4h but not at 1h in liver	Chang *et al*, 1992
	Single-strand breaks	Rat, Sprague- Dawley	Oral	1-10 mol/kgbw	-ve		Chang *et al*, 1992
	Single-strand breaks	Mouse, B6C3F$_1$	Oral	500 mg/kgbw 1, 2 or 3 d	-ve	TCA and neutralised TCA, 1 and 4 h after dosing	Mackay *et al*, 1990b
Dichloroacetate	Single-strand breaks	Mouse, B6C3F$_1$	Oral	Single 1-10 mmol/kgbw	(-)	Liver peroxisome proliferation at 4 h but not at 1 h	Chang *et al*, 1992
	Single-strand breaks	Mouse, B6C3F$_1$	Drinking water	0.5-5 g/l for 7-14 d	-ve		Chang *et al*, 1992
	Single-strand breaks	Rat, F344	Oral	Single 1-10 mmol/kgbw	-ve	Peroxisome proliferation	Chang *et al*, 1992
	Single-strand breaks	Rat, F344	Drinking water	0.05-0.5 g/l for 30 d	-ve		Chang *et al*, 1992
	Single-strand breaks	Mouse, B6C3F$_1$	Oral	0.006-60 mmol/kgbw	+ve	From 0.006 mmol/kgbw	Nelson and Bull, 1988
	Single-strand breaks	Rat, Sprague- Dawley	Oral	0.10-20 mmol/kgbw	+ve	From 0.25 mmol/kgbw	Nelson and Bull, 1988

+ve, positive; -ve, negative; (-) negative, but reported +ve by the author

Chloral hydrate was negative in bone marrow micronucleus and sperm abnormality assay in mice after i.p. administration on 5 consecutive doses from 315 to 2,500 mg/kgbw (Bruce and Heddle, 1979).

Allen *et al* (1994) exposed male C57BL/6J mice by i.p. injection to chloral hydrate (purity ≥ 99%) at dose levels of 41, 83 or 165 mg/kgbw. Chloral hydrate, at all dose levels, was positive for spermatid micronuclei induction when treatments corresponded to spermatogonial stem cell or preleptotene spermatocyte stages of development; negative results were obtained after treatments of leptotene-zygotene or diakinesis-metaphase stages.

Negative results were also observed in a mouse micronucleus test at 500 mg/kgbw after i.p administration, and in a rat bone marrow chromosome aberration assay at 1,000 mg/kgbw after oral administration (Leuschner and Leuschner, 1991).

Chloral was investigated for its potential to form DNA-protein cross-links (DPX), a lesion produced by other aldehydes. Chloral did not form DPX in rat liver nuclei at concentrations up to 250 mM for 30 min at 37°C, while chloro-acetaldehyde (47 mM) and acetaldehyde (200 mM) did form cross-links. Experiments with the aldehyde-trapping reagents thiosemicarbazide and semicarbazide showed that chloral did not react, in contrast with aldehydes that form DPX. This indicates a very strong hydration of chloral. Mice given 800 mg/kgbw [^{14}C]chloral after pretreatment with 1,500 mg/kgbw TCE for 10 d had no detectable covalent binding of ^{14}C to DNA in the liver. These results do not support a genotoxic theory of carcinogenesis for TCE mediated through chloral (Keller and Heck, 1988).

Chloral hydrate induced single-strand breaks in hepatic DNA of male B6C3F$_1$ mice and Spraque-Dawley rats *in vivo* using a alkaline unwinding assay (Nelson and Bull, 1988).

5.3.3 Trichloroethanol and Trichloroacetic acid

In assays without exogenous metabolic activation trichloroethanol was found to be weakly mutagenic in *Salmonella typhimurium* TA1535 and TA100 and in forward mutation assays in *A. nidulans* (Bignami *et al*, 1980), where it induced chromosome malsegregation (Crebelli *et al*, 1985). However, Waskell (1978) did not find any increased in revertants with *Salmonella typhimurium* TA100, TA1535, TA1537, TA98 both with and without rat liver metabolic activation. Trichloroacetic acid was not mutagenic in *Salmonella typhimurium* TA98 and TA100, both with and without activation (Waskell, 1978; Moriya *et al*, 1983).

In an *in vitro* cytogenetic assay with human lymphocytes, neutralised TCA at dose levels of 2,000 and 5,000 mg/ml did not induce a significant increase in the frequency of chromosomal damage, either in the absence or presence of rat liver S-9 mix. TCA as a free acid on the contrary, induced statistically significant increases in the incidence of chromosomal damage at dose levels of 2,000 (with S-9) and 3,500 mg/ml (with and without S-9) (Mackay *et al*, 1990a, 1991). Trichloroethanol was found to induce a slight increase in sister-chromatid exchange in human lymphocytes (Gu *et al*, 1981).

In vivo cytogenetic assays in the mouse (bone marrow chromosomal aberrations and micronucleus) and the sperm-head abnormality test with TCA gave a positive response after either i.p. or oral administration of single or fractionated doses (Bhunya and Behera, 1987). Neutralised TCA, administered in two equal i.p. doses to C57BL/6J;BL10/Alpk mice (337, 675 and 1,080 mg/kgbw in males; 405, 810 and 1,300 mg/kgbw in females), did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow cells (Mackay *et al*, 1990b).

Trichloroacetate induced single-strand breaks in hepatic DNA of male B6C3F$_1$ mice and Spraque-Dawley rats *in vivo* using a alkaline unwinding assay (Nelson and Bull, 1988).

On the contrary, no evidence of induction of single-strand breaks in hepatic DNA was obtained in male B6C3F$_1$ mice by Mackay *et al* (1990b). The animals were given 1, 2, or 3 daily doses of TCA (500 mg/kgbw by gavage) as a neutralised solution (sodium salt) and killed 1 h after the final dose. Some mice were given a single dose of TCA (500 mg/kgbw) as the acid or as a neutralised solution and killed 24 h later. Studies on liver growth parameters (hyperplasia, peroxisome proliferation) revealed that TCA induced small but significant increases in both parameters (Mackay *et al*, 1990b).

An alkaline unwinding assay was used to quantitate the induction of DNA strand breaks in the livers of rats and mice treated *in vivo*, in rodent hepatocytes in primary culture, and in CCRF-CEM cells, a human lymphoblastic leukemia cell line, following treatment with tri-, di-, and monochloroacetic acid and their corresponding aldehydes, tri- (chloral hydrate), di- and monochloro-acetaldehyde. None of the chloroacetic acids induced DNA strand breaks in the livers of rats at 4 h following a single administration of 1-10 mmol/kgbw. TCA (10 mmol/kgbw) did produce a small amount of strand breakage in mice (7% at 4 h) but not at 1 h. TCA also failed to induce DNA strand breaks in splenocytes and epithelial cells derived from the stomach and duodenum of mice treated *in vivo*. None of the three chloroacetaldehydes induced DNA strand breaks in either mouse or rat liver. Both the chloroacetic acids and the chloroacetaldehydes were ineffective in inducing DNA strand breaks in cultured rat and mouse hepatocytes at concentrations below those that yielded

cytotoxicity. The chloroacetic acids were also ineffective in the CCRF-CEM cells. These studies provide further evidence that the chloroacetic acids lack genotoxic activity not only in rodent liver, a tissue in that they induce tumours, but in a variety of other rodent tissues and cultured cell types (Chang *et al*, 1992).

5.3.4 Dichloroacetic acid

Dichloroacetic acid (as a salt) demonstrated weak mutagenic activity in *Salmonella typhimurium* TA98 (Herbert *et al*, 1980) or in TA100 and TA1535 (Waskell, 1978); but the effect of impurities on these results cannot be ruled out.

No DNA strand-breaks were found after single exposure of B6C3F_1, mice (i.p., 1-10 mmol/kgbw) or Fisher 344 rats (gavage, 1-5 mmol/kgbw). Chronic exposure to DCA in the drinking water (mice, 7-14 d; rats, 30 weeks) did not induce appreciable DNA damage (Chang *et al*, 1992). Direct *in vitro* exposure of rat and mouse hepatocytes to DCA did not reveal any DNA damage (Chang *et al*, 1992). Also no effect on DNA was found after exposure of human CCRF-CEM cells to DCA (Chang *et al*, 1992).

These results were in contrast to that observed earlier by Bull and coworkers (Nelson and Bull, 1988) who reported dose-dependent increases in rats and mice liver DNA strand-breaks after DCA exposure (gavage, effect from 0.23 mmol/kgbw in rats, from 0,006 mmol/kgbw in mice). The different results may be explained by the difference in the DNA unwinding assay used.

5.3.5 Cysteine Conjugates

The cysteine conjugate of TCE, S-(1,2-dichlorovinyl)-L-cysteine was found to be mutagenic in *Salmonella typhimurium* TA100 in the presence of rat kidney S9 (Dekant *et al*, 1986b). An NADPH cofactor mixture was not required, which suggests that activation was due to the enzyme cysteine conjugate β-lyase (Green and Odum, 1985). The mutagenicity of the two isomers of L-cysteine- and of N-acetyl-L-cysteine-S-conjugates was tested in strain TA2638 *Salmonella typhimurium* in the presence of rat kidney S9. All compounds were mutagenic but the 1,2 dichlorovinyl-isomer and its N-acetyl derivative were more potent than the 2,2-dichlorovinyl-isomers (Commandeur *et al*, 1991). The S-(1,2 dichlorovinyl)-L-cysteine is a weak inducer of DNA repair in mammalian kidney cells (Vamvakas *et al*, 1989).

5.3.6 Summary of Studies with TCE Metabolites

TCE epoxide, a putative metabolite of TCE which has never been isolated from biological sytems, showed mutagenic effects in yeasts but not in bacteria. It also showed slight activity in cell transformation assays and in an *in vitro* chromosome aberration assay.

With chloral hydrate, both negative and positive results were observed in bacterial mutagenicity assays. Chloral induced aneuploidy in some fungal strains and chromosome aberrations in mammalian cells *in vitro*. It also induced aneuploidy in germ cells and chromosome aberrations and single-strand breaks in hepatic DNA (*in vivo*).

TCA was not mutagenic in bacteria. *In vitro* and *in vivo* tests showed clastogenic effects of TCA only when the acidic form was used, but not with the neutralised form. Single-strand breaks in hepatic DNA were observed in one study, but could not be confirmed in two other studies.

DCA did not show mutagenic activity in bacteria: results of two DNA strand breaks assays in hepatic DNA were controversial.

The cysteine conjugates of TCE appeared to be mutagenic *in vitro* (Ames test).

5.4 OVERALL SUMMARY AND APPRAISAL

The mutagenic potential of TCE has been studied widely. In many of the reported studies the purity of the test sample is not stated, although potentially mutagenic epoxide stabilisers were almost certainly present. The relevance of the various studies to the mutagenicity of TCE itself is therefore confounded.

The results of studies of the mechanism of carcinogenic action of TCE in mice have demonstrated that the rates and products of metabolism are different in mice to those seen in rats and in human systems *in vitro*. The mouse is thus not a good model for man for the assessment of TCE toxicity. The same reservations apply to the interpretation of mutagenicity studies conducted in mice.

TCE has been found to be non-mutagenic or only marginally mutagenic in several point mutation assays. A contribution to the positive findings from the presence of mutagenic stabilisers contained in the samples of TCE tested cannot be ruled out in most of these assays. Furthermore, many single studies have been carried out at high doses with no evidence that the results were

reproducible. No significant positive finding in point mutation assays has been reproducibly demonstrated either in or between laboratories using the pure chemical.

A reproducible aneuploidy response has been observed in some fungi strains. However, one of the major metabolites of TCE, TCA, is known to cause protein denaturation and has also been shown to cause chromosome aberrations *in vitro* due to a reduction in pH which is independent of the acid used. This response is therefore considered to be artefactual.

Positive findings with TCE have been reported in 2 poorly described mouse bone marrow micronucleus assays, following gavage administration of high doses. Two more recent studies in mice using high inhalation or gavage doses gave clear negative results. A single, positive result with TCE in a rat bone marrow micronucleus assay appeared to be an effect of aneuploidy. At the doses used, extensive metabolism to chloral hydrate and subsequently to TCA will have occurred in the test animal. Extensive testing of TCA in the micronucleus assay has failed to demonstrate a positive effect. The significance of the positive findings to the overall mutagenicity assessment of TCE is, therefore, unclear. *In vivo* tests for chromosome aberrations were negative in mice as well as in rats. *In vivo* assays with TCE for germ cell mutagenicity were also negative.

Chloral (hydrate) has been reported to be both mutagenic and clastogenic, yet a recent study with highly purified chloral hydrate reported clear negative results in the Ames, mouse bone marrow micronucleus and rat bone marrow cytogenetics assays. Single reports of TCA induced DNA single strand breaks or chromosomal damage were not reproduced by two other laboratories or were shown to be due to pH effects caused by un-neutralised TCA. S-dichlorovinylcysteine, the precursor to the minor metabolite S-dichlorovinyl-N-acetylcysteine, is known to be mutagenic in the Ames bacterial mutation assay when activated by rat kidney fractions. The significance of this observation *in vivo* has yet to be determined

Studies in man exposed to TCE did not provide evidence of clastogenicity. Other human monitoring studies were inappropriate for evaluation.

Thus overall there is no convincing or conclusive evidence that pure TCE is genotoxic.

SECTION 6. MECHANISM OF TUMOUR FORMATION

6.1 MOUSE LIVER TUMOURS

TCE causes peroxisome proliferation in the mouse liver, a biochemical response associated with cancer induction in rodents with a wide range of chemicals (Reddy *et al*, 1980). This effect cannot be demonstrated in rats exposed to TCE (Elcombe *et al*, 1985). TCA, a major metabolite of the cytochrome P-450 pathway has been shown to cause peroxisome proliferation in both rats and mice. The lack of a response in rats exposed to TCE has been attributed to saturation of metabolism in this species, limiting the maximal levels of TCA to below those required to induce peroxisome proliferation.

Increases in hepatic DNA synthesis and mitosis, but not unscheduled DNA synthesis (UDS), have been reported in mice dosed with TCE either by gavage or by inhalation (Stott *et al*, 1982; Mirsalis *et al*, 1985; Dees and Travis, 1993). Small increases in DNA synthesis were seen in rat liver in one study (Stott *et al*, 1982). A marked species difference was identified in similar experiments in which TCA was given to rats and mice at equivalent dose levels in drinking water. Watson *et al* (1993) found that TCA induced a 4-fold increase in replicative DNA synthesis in mouse liver, whereas in rat liver a marked reduction was observed (to 10% of control). TCE has also been shown to act as a tumour promoter in the mouse liver (Randall and Sipes, 1984).

Inhibition of gap-junction-mediated intercellular communication is a cellular effect exhibited by a number of non-genotoxic carcinogens and tumour promoters. Klaunig *et al* (1989) assessed the effects of TCE and its metabolites, TCA, trichloroethanol (TCEth) and chloral hydrate (CH) on gap-junction-mediated intercellular communication in cultured $B6C3F_1$ mouse and F344 rat hepatocytes. TCE and TCA inhibited intercellular communication in both 24-h old and freshly plated mouse hepatocytes but not in rat hepatocytes. TCE appeared to require cytochrome P-450 metabolism by the mouse hepatocytes since treatment with SKF-525A prevented the inhibition of intercellular communication by TCE. The inhibitory effect of TCA on intercellular communication was unaffected by treatment with SKF-525A. While the species dependent effect of TCE on intercellular communication may be correlated with different rates and extent of metabolism of TCE by rat and mouse hepatocytes, the inhibiting effect of TCA only on mouse hepatocytes suggests that other intrinsic factors in the male mouse make this species more susceptible to the effects of TCE and TCA on gap junction mediated intercellular communication. These findings may account, in part, for the observed species difference in susceptibility to TCE induced liver carcinogenesis. TCEth and CH had no effects on hepatocyte intercellular communication in either rat or mouse cells.

Both TCA and DCA (a minor TCE metabolite), have been shown to cause liver tumours when administered to $B6C3F_1$ mice in drinking water (Herren-Freund *et al*, 1987, Bull *et al*, 1990; DeAngelo *et al*, 1991). In contrast, neither metabolite induced liver tumours in F344 rats at equivalent dose levels to those used in the mouse studies (DeAngelo *et al*, 1992). Based on these results, Watson *et al* (1993) concluded that both peroxisome proliferation and DNA synthesis are required to induce tumours in mice administered TCA.

The relative roles of TCA and DCA in the development of mouse liver tumours have been compared (Larson and Bull, 1992a,b; Bull *et al*, 1993). These authors reported higher blood levels of DCA in mice than in rats exposed to TCE, and suggested a role for DCA in the development of mouse liver tumours. The apparent species differences in the blood levels of DCA were not substantiated by the levels of DCA excreted in the urine of rats and mice in a number of studies (Green and Prout, 1985; Dekant *et al*, 1986a). It should also be noted that the blood levels of TCA are at least an order of magnitude higher than those of DCA. Thus, although DCA is a known mouse liver carcinogen, the contribution of this metabolite to the development of liver tumours in mice exposed to TCE remains to be established.

The apparent strain differences between mice in the hepatocarcinogenicity of TCE may be explained by strain differences in metabolism to TCA. In Swiss and $B6C3F_1$ mice in which liver tumours are induced (US-NCI, 1976; Maltoni *et al*, 1988; US-NTP, 1986), TCA accounted for 7-12% of the dose (Green and Prout, 1985), whereas in NMRI mice where liver tumours were not induced (Henschler *et al*, 1980), this metabolite was only 0.1% of the dose (Dekant *et al*, 1984). Equally the levels of DCA differed, being 2% in Swiss and $B6C3F_1$ mice and 0.1% in NMRI mice.

In conclusion, there is strong evidence that the liver tumours seen in mice exposed to TCE are a result of its metabolism to TCA, which stimulates peroxisome proliferation and DNA synthesis, and inhibits intercellular communication. Peroxisome proliferation is not seen in rats because metabolism to TCA is limited by metabolic saturation. In addition, TCA, even at high dose levels, does not stimulate DNA synthesis in this species, but suppresses it, nor does TCA inhibit intercellular communication in the rat. All of these responses are consistent with the observed species and strain differences and with a non-genotoxic mode of action. The role of the minor metabolite DCA has yet to be established.

Comparisons of the metabolism of TCE to TCA in rat, mouse and human hepatocytes confirmed the species differences between rats and mice seen *in vivo* and demonstrated that the rate of metabolism in human hepatocytes was significantly lower than that in the rat (Elcombe, 1985;

Knadle *et al*, 1990). Furthermore, although peroxisome proliferation could be induced *in vitro* in rat and mouse hepatocytes by TCA, this response could not be induced in human hepatocytes (Knadle *et al*, 1990). It is concluded therefore, that the liver tumours seen in mice exposed to TCE are caused by a combination of factors which are unique to Swiss and the $B6C3F_1$ mouse and do not occur in either rats or man.

6.2 MOUSE LUNG TUMOURS

A number of studies have shown that inhaled TCE is cytotoxic to the Clara cells of the mouse bronchiolar epithelium (Forkert *et al*, 1985; Forkert and Birch, 1989; Villaschi *et al*, 1991; Odum *et al*, 1992). The possible significance of these findings for tumour development in mice has been examined in a series of *in-vivo* and *in-vitro* tests reported by Odum *et al* (1992). The cytotoxicity, which is characterised by vacuolation of the Clara cell (a specific cell type in the lung), was observed in female CD-1 mice exposed for a single six h period to TCE at concentrations ranging from 20 to 2,000 ppm. The effects were dose-related, with very few cells affected at 20 ppm whereas most Clara cells were affected at exposures of 200 ppm and above. Mice exposed to concentrations of 100 ppm TCE and above showed a reduction in the cytochrome P-450 activity at the Clara cell, while glutathione-S-transferase activities were generally unaffected. The effect was specific to the Clara cell, no damage being apparent to other cell types in the lung. This response was not seen in the rat at equivalent dose levels.

Metabolism studies using isolated mouse lung Clara cells *in vitro* showed that a similar metabolic pathway for TCE existed to that known in the liver, ie. via chloral to trichloroethanol and TCA. Trichloroethanol is conjugated in the liver with glucuronic acid to form the major metabolite of TCE, approximately 80% of total metabolism. However, Clara cells were found to be deficient in the enzyme responsible for the metabolism of chloral to trichloroethanol and the further conjugation of trichloroethanol with glucuronic acid was virtually absent. As a result chloral accumulated in the Clara cell. When tested *in vivo*, it was shown that chloral was the only metabolite of TCE to generate the mouse lung Clara cell lesion, trichloroethanol and TCA having no effect.

Chloral is the first metabolic product of cytochrome P-450 metabolism of TCE, and mouse lung Clara cells possess high levels of this enzyme. The accumulation of chloral in mouse lung Clara cells due to its inefficient detoxification leads to an attractive hypothesis for a mechanism of lung tumour formation in the mouse. Similar damage to mouse, but not rat Clara cells, is induced by the mouse lung carcinogen methylene chloride. In this case the damage is known to lead to cell proliferation (Foster *et al*, 1992). Thus, there seems to be a strong correlation between this type of

damage and the development of lung tumours in the mouse. The rat lung contains fewer Clara cells and lower levels of cytochrome P-450 than the mouse lung, thus explaining the lack of a response in this species with either chemical.

The few Clara cells found in human lung tissue show no smooth endoplasmic reticulum (Smith *et al*, 1979) and are thus presumed not to possess significant cytochrome P-450 activity, ie. that required to generate chloral from TCE. Thus, the lesion observed in the mouse lung Clara cell resulting from production and accumulation of chloral would not be expected to occur in man. On this basis, it is presumed that the finding in the mouse has no direct significance in human health hazard assessment.

6.3 RAT KIDNEY TUMOURS

TCE has been reported to cause a low incidence of renal adenoma and adenocarcinoma in the male rat, but not in the mouse, following either inhalational exposure or gavage dosing (US-NTP, 1983 and 1988; Maltoni *et al*, 1988). Contrary to these findings, Henschler *et al* (1980) reported that there were no dose related increases in any tumour type in Wistar rats exposed to 500 ppm TCE for 78 weeks.

A number of potential mechanisms, cytotoxicity, protein droplet nephropathy and conjugation by the glutathione/β-lyase pathway, which are known to be involved in the development of rat specific renal tumours, have been investigated for TCE (Goldsworthy *et al*, 1988b; Green *et al*, 1990). Of these cytotoxicity appears to be the most significant factor. Renal tumours have not been induced by TCE in the absence of significant nephrotoxicity. Protein droplet nephropathy was not seen in male rats given high doses of TCE (2,000 mg/kgbw) by gavage, daily for 42 days.

Analysis of urine from rats and mice dosed by gavage (Dekant *et al*, 1986b; Green *et al*, 1990; Birner *et al*, 1993), and from human beings exposed to TCE by inhalation, identified trace amounts of S-1,2-dichlorovinyl-N-acetylcysteine, confirming the formation of this nephrotoxic and potentially mutagenic metabolite by the glutathione/β-lyase pathway. Where they have been quantified (Green *et al*, 1990; Birner *et al*, 1993), the levels of this metabolite in rat and mouse urine were extremely small (< 0.1% of the dose), even at the high dose levels used in these studies. In the study by Birner *et al* (1993) the levels of the conjugate in mouse urine were higher than those in rat urine and cannot therefore be considered to be causally related to the rat specific renal tumours.

Equally, when this metabolite was dosed to rats at levels equivalent to 10 mg/kgbw/d for 46 weeks, i.e. a dose that is two orders of magnitude higher than the levels found in animals dosed with TCE, there was no evidence of renal cancer at the termination of the study at 87 weeks (Terracini and Parker, 1965). It should also be noted that where glutathione conjugation has been studied as a competitive pathway with cytochrome P-450, as is the case with TCE, it has been shown to be a significant pathway only at high dose levels following saturation of the cytochrome P-450 enzymes (Green *et al*, 1988, 1990).

In conclusion, there is a clear correlation between nephrotoxicity and the low incidences of renal tumours seen in rats exposed to TCE. These tumours have not been seen in the absence of kidney damage. A role for the cysteine conjugate of TCE in this nephrotoxicity, or in the role of the development of kidney tumours by a genotoxic mechanism, has yet to be established. Nevertheless, all of the evidence suggests that kidney tumours will not occur in the absence of frank nephrotoxicity. There is no evidence of renal toxicity in man exposed chronically to TCE (Seldén *et al*, 1993), despite the fact that there are reports of toxicity in other organs, e.g. liver, suggesting that substantial exposure has occurred in the past. The lack of evidence for nephrotoxicity in man suggests that metabolism of TCE to the nephrotoxic cysteine conjugate occurs at levels which are not toxicologically significant. Consequently, it is concluded that the renal tumours seen in rats at nephrotoxic dose levels have no apparent relevance to human health assessment at reasonably foreseeable levels of exposure.

6.4 OTHER TUMOURS

An increased incidence of benign testicular Leydig cell tumours was observed following inhalation of TCE in male Sprague-Dawley rats (Maltoni *et al*, 1988). Adenomas of the Leydig cells (interstitial cells) in the testicular tissue are very common in all strain of rats. The spontaneous incidences are variable from one strain to another and may be as high as 100% in Fischer 344 rats. They appear late in life and are not life-threatening to the rat. Because Leydig cells produce and secrete sexual hormones (e.g. testosterone, dihydro-androtestoterone, oestradiol), the high incidence of hyperplasia and tumours of these testicular cells in the old rat is undoubtedly related to senile endocrine disturbances (Mostofi and Price, 1973).

Various conditions have been shown to induce Leydig cell hyperplasia/tumours in the rat. Most have been summarised by Mostofi and Bresler (1976) and include: senility *per se* as in Fischer rats, oestrogenic treatments, transplantation of testes to spleen, irradiation, injection of cadmium salts etc. In addition, increased incidences of Leydig cell tumours were described in a large number of

long-term rat studies with substances covering a variety of chemical structures including such a common substance as lactose (Bär, 1992).

The Leydig cell tumour occurrence in man is extremely low, in contrast to the high spontaneous incidence in rats (Mostofi and Price, 1973), and represents less than 3% of all of the recorded neoplasia. This makes the relevance of the rat findings to man highly questionable.

SECTION 7. ASSESSMENT OF HUMAN CARCINOGENIC HAZARD

Five cohort epidemiological studies of populations occupationally exposed to TCE have shown no evidence of an association between exposure to high levels of TCE and the occurrence of cancer in man. In addition, two uncontrolled case-studies of primary liver cancer and one colon cancer case-control study have shown no association with exposure to TCE. All these studies failed to indicate an increased risk of cancer in general or any specific type of cancer in workers exposed to significant levels of TCE.

These important findings contrast sharply with the results of animal studies where evidence of carcinogenicity has been demonstrated in two species. In order to understand the relationship of these two, apparently conflicting, sets of evidence, one must turn to an understanding of the mechanisms of carcinogenic activity of TCE in experimental animals and their relevance to human beings.

A key aspect in this process is the conclusion that there is no convincing or conclusive evidence that pure TCE is genotoxic. Other mechanisms leading to the expression of carcinogenic activity in experimental animals must therefore be operative.

7.1 RELEVANCE OF MOUSE LIVER TUMOURS

The occurrence of an increased incidence of mouse liver tumours (hepatocellular carcinomas and adenomas) is the most frequently reported and significant observation in lifetime cancer bioassays of TCE. These tumours have been observed in both male and female Swiss and B6C3F_1 mice following exposure by either inhalation or gavage, although they have not been reported in other strains of mice (i.e. NMRI and ICR).

There is strong evidence that the liver tumours seen in mice exposed to TCE are a result of its metabolism to TCA, which stimulates peroxisome proliferation, DNA synthesis, and inhibits intercellular communication. Peroxisome proliferation is not seen in rats because metabolism to TCA is limited by metabolic saturation. In addition, TCA, even at high dose levels, does not stimulate DNA synthesis in this species, but suppresses it, nor does TCA inhibit intercellular communication in the rat. All of these responses are consistent with the observed species and strain differences and with a non-genotoxic mode of action.

Comparisons of the metabolism of TCE to TCA in rat, mouse and human hepatocytes confirmed the species differences between rats and mice seen *in vivo* and demonstrated that the rate of metabolism in human hepatocytes was significantly lower than that in the rat (Elcombe 1985; Knadle *et al*, 1990). Furthermore, although peroxisome proliferation is induced *in vitro* in rat and mouse hepatocytes by TCA, this response is not induced in human hepatocytes. It is concluded therefore, that the liver tumours seen in mice exposed to TCE are caused by a combination of factors which are unique to mice and do not occur in either rats or man.

7.2 RELEVANCE OF MOUSE LUNG TUMOURS

Increased incidences of lung tumours have been observed in the female HA:ICR mouse and the female $B6C3F_1$ mouse. This effect was not observed in the Swiss mouse nor in males of any of the mouse strains tested. The effect also appears to be species specific.

Studies by Odum *et al* (1992) have demonstrated a specific lung lesion following inhalation of TCE, characterised by vacuolation of the Clara cell. Chloral, produced by cytochrome P-450 metabolism, was the only metabolite of TCE that caused this mouse lung lesion. *In vitro* studies using isolated mouse lung Clara cells have shown that chloral accumulates in these cells due to an inefficient detoxification to trichloroethanol, which leads to an attractive hypothesis for a mechanism of lung tumour formation in the mouse. The rat lung contains fewer Clara cells and lower P-450 levels than the mouse lung, thus explaining the lack of response in this species.

The few Clara cells found in human lung tissue show no smooth endoplasmic reticulum and are thus presumed not to possess significant P-450 activity, ie. that required to generate chloral from TCE. Thus, the lesion observed in the mouse lung Clara cell resulting from production and accumulation of chloral would not be expected to occur in man. On this basis, it is presumed that the finding in the mouse has no direct significance in human health hazard assessment.

7.3 RELEVANCE OF RAT KIDNEY TUMOURS

A numerically small increase in the incidence of renal tubular adenocarcinomas has been observed in male Sprague-Dawley, Fischer 344 and Osborne-Mendel rats. These findings were confined to males and no increased incidences were found in any of the other tested rat strains, although almost all rats exposed to high TCE levels had tubular cell cytomegaly.

There is a clear correlation between nephrotoxicity and the low incidences of renal tumours seen in rats exposed to TCE. These tumours have not been seen in the absence of kidney damage. A role for the cysteine conjugate of TCE in this nephrotoxicity, or in the role of the development of kidney tumours by a genotoxic mechanism, has yet to be established. Nevertheless, all of the evidence suggests that kidney tumours will not occur in the absence of frank nephrotoxicity. There is no evidence of renal toxicity in man exposed chronically to TCE (Seldén *et al*, 1993) despite the fact that there are reports of toxicity in other organs, e.g. liver, suggesting that substantial exposure has occurred in the past. The lack of evidence for nephrotoxicity in man suggests that metabolism of TCE to the nephrotoxic cysteine conjugate occurs at levels which are not toxicologically significant. Consequently, it is concluded that the renal tumours seen in rats at nephrotoxic dose levels have no relevance to human health assessment at current occupational exposure levels.

7.4 OVERALL ASSESSMENT

Five cohort epidemiological studies of populations occupationally exposed to TCE have shown no evidence of an association between exposure to high levels of TCE and the occurrence of cancer in man. In addition, two uncontrolled case-studies of primary liver cancer and one colon cancer case-control study have shown no association with exposure to TCE. All these studies failed to indicate an increased risk of cancer in general or any specific type of cancer in workers exposed to significant levels of TCE.

These important findings contrast sharply with the results from animal studies where evidence of carcinogenicity in the liver and lung of mice and in the kidney of rats (males only) has been demonstrated. There is no convincing or conclusive evidence that pure TCE is genotoxic. Furthermore, a substantial number of biochemical studies have identified mechanisms for the development of these tumours which do not require a direct interaction between TCE or its metabolites and DNA. In each case the mechanism is thought to be linked to species specific metabolism of TCE and to a range of biochemical responses which are either specific to rodents or are not seen at dose levels relevant to human exposure. The excess tumour incidences in the liver, lung or kidney in either mice or rats exposed to TCE are, therefore, considered to be of no relevance to human carcinogenic hazard assessment.

Taking all of this information into account, it is concluded that exposure to TCE does not present a carcinogenic hazard to man.

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