TRICHLOROETHYLENE
(CAS Reg. No. 79-01-6)

INTERIM ACUTE EXPOSURE GUIDELINE LEVELS
(AEGLs)

For
NAS/COT Subcommittee for AEGLS

2009

PREFACE

Under the authority of the US Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. AEGL-2 and AEGL-3 levels, and AEGL-1 levels as appropriate, will be developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population including infants and children, and other individuals who may be sensitive and susceptible. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing odor, taste, and sensory irritation, or certain non-symptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL level, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL level. Although the AEGL values represent threshold levels for the general public, including sensitive subpopulations, it is recognized that certain individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL level.

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EXECUTIVE SUMMARY

Trichloroethylene is a colorless, highly volatile liquid at ambient temperature and pressure. It has a sweet, chloroform-like odor, which can be detected at ≥50 ppm (HSDB, 1992). The compound's main use is for vapor-degreasing and cold cleaning of fabricated metal parts. Formerly it was used on a limited scale as a medical anesthetic and analgesic with 5000-15000 ppm for producing light anesthesia and 3500-5000 ppm for analgesia (Parfitt et al., 1999).

Following exposure to trichloroethylene humans primarily experience central nervous system effects and irritation. At high concentrations cardiac arrhythmias have also occurred. Individual cases of liver and kidney damage have been reported. In animal studies neurotoxicity was the most sensitive endpoint for acute exposures. For inducing cardiac arrhythmias and liver and kidney damage very high concentrations were required in the limited acute animal studies available for these endpoints.

The AEGL-1 derivation is based on the exposure of volunteers to 300 ppm for 2 hours (Vernon and Ferguson, 1969). At this concentration marginal CNS-depression was present in only 1 out of 8 volunteers. Based on the weight-of-evidence from a range of human volunteer studies, the 300 ppm for 2h from Vernon and Ferguson (1969) was considered to be an adequate starting point. For across-duration extrapolation a human PBPK model was used (Boyes et al., 2002). Using this model the peak concentration of trichloroethylene in blood after 2 hours of exposure to 300 ppm was calculated and subsequently the external concentrations that would produce the same blood concentration for the other exposure durations, were derived. From animal experiments it was cleat that CNS related endpoints showed the highest correlation to the peak blood levels compared to the area under the curve. No interspecies uncertainty factor was needed because a human study was used. For interindividual variation among humans an intraspecies factor of 3 was used. A higher factor is not necessary because the mechanism of action (general CNS depression) is not expected to vary greatly within the human population.

The AEGL-2 derivation is based on the same study by Vernon and Ferguson (1969). At 1000 ppm for 2 hours the subjects reported light-headedness, dizziness, or lethargy. In addition reduced neurobehavioral performance was detected at this concentration, most importantly reduced performance in the pegboard test. Although significant in themselves, these effects are not escape-impairing. Thus the concentration of 1000 ppm for 2 hours is considered an adequate starting point for AEGL-2 effects in humans. For across-duration extrapolation again the Boyes et al. human PBPK model was used. No interspecies uncertainty factor was needed because a human study was used. For interindividual variation among humans an intraspecies factor of 3 was used. A higher factor is not necessary because the mechanism of action (general CNS depression) is not expected to vary greatly within the human population.

The AEGL-3 value is based on the acute mouse mortality study by Friberg et al. (1953). The NOEL from this study is 4600 ppm for 4 hours. Across-duration extrapolation was done using $C^n xT$ with n = 1.511 (derived by probit analysis from the study of Adams et al., 1951). It should be noted that this value of n is different from that previously reported by Ten Berge et al.

(1986). The PBPK calculations using the model Boyes et al. (2002) show that humans need much higher external concentrations for reaching a certain concentration in blood than do rats and presumably mice. Therefore no interspecies uncertainty factor is needed for toxicokinetic differences between mice and humans. Although a toxicodynamic difference may still exist, it is expected that this variability is small compared to the clear difference in uptake. Hence, an interspecies uncertainty factor is considered not necessary for the derivation of the AEGL-3. For interindividual variation among humans an intrapecies factor of 3 is appropriate. A higher factor is not necessary because the mechanism of action (CNS depression) is not expected to vary greatly between individuals. In the AEGL-3 derivation it is also taken into account that cardiac arrythmias may occur in humans at levels of 10,000 ppm and above and that this level will quickly result in complete narcosis. In addition, general anesthesia may be associated with vomiting, another risk factor especially in the absence of medical assistance. Therefore the level of 10,000 ppm should not be exceeded. The 10 min AEGL-3 is set at the 30 min value of 6100 ppm instead of the calculated value of 12600 ppm.

The Level of Distinct Odor Awareness (LOA) is calculated to be 337 ppm.

The calculated values are listed in the table below.

Summary of Proposed AEGL Values for Trichloroethylene in ppm [mg/m^3]

Classification	10-minute	30-minute	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGL-1	260 [1400]	180 [970]	130 [700]	84 [450]	77 [410]	Marginal CNS-effects in only 1 out of eight volunteers exposed to 300 ppm for 2 hours (Vernon and Ferguson, 1969).
AEGL-2 (Disabling)	960 [5200]	620 [3300]	450 [2400]	270 [1400]	240 [1300]	Light-headedness, dizziness, or lethargy in combination with reduced performance in neuro-behavioral test in volunteers at 1000 ppm for 2 hours (sub AEGL-2 level) (Vernon and Ferguson, 1969)
AEGL-3 (Lethal)	6100 [33000]	6100 [33000]	3800 [20000]	1500 [8100]	970 [5200]	NOEL for mortality in mice: 4600 ppm for 4 hours (Friberg et al., 1953)
LOA	337					Based on odor threshold of 28 ppm reported in the EPA Health Hazard Assessment.

References:

Adams, E.M., Spencer, H.C., Rowe, V.K., McCollister, D.D., Irish, D.D. (1951) Vapor toxicity of trichloroethylene determined by experiments on laboratory animals. *Archives of Industrial Hygiene and Occupational Medicine* **4**, 469-481.

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Substances and Risk Assessment, National Institute of Public Health and the Environment, the Netherlands, dated February, 2002. (See appendix A).

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HSDB (1992) Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, Midland, USA.

Parfitt, K., Sweetman, S.C., Blake, P.S. and Parsons, A.V. (1999) Martindale - The Complete Drug Reference, Thirty-second edition. London, Pharmaceutical Press, 1999. ISBN 0 85369 429

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1. INTRODUCTION

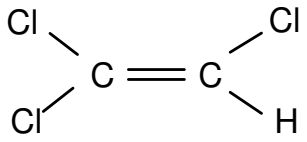
Trichloroethylene is a colorless, highly volatile liquid at ambient temperature and pressure. It has a sweet, chloroform-like odor, which can be detected at ≥50 ppm (HSDB, 1992). Industrial experience has shown the mild odor not to be an effective warning-sign of exposure. Transient mild eye irritation is seen at 200 ppm with definite eye and nose irritation occurring at concentrations of ≥1000 ppm only (Torkelson, 1994).

Commercial production of trichloroethylene started in 1920 in Germany and in 1925 in the USA. The current method of manufacture is by the chlorination of ethylene or 1,2-dichloroethane. An alternative mode of production is from ethylenedichloride or other C_2-chlorinated hydrocarbons. Manufacture of trichloroethylene in 1990 was 131 kilotonnes for Western Europe (Cf. 210 kilotons in 1980), 79 kilotons for the USA (121 kilotons in 1980) and 57 kilotons for Japan (82 kilotons in 1980). The estimated annual consumption in these areas is 65 to 103% of the production levels. The compound is mainly used for the vapour degreasing and cold cleaning of fabricated metal parts (80-95% of consumption). Other applications include industrial dry-cleaning, printing, the production of printing-ink, extraction processes, paint production and textile printing (IARC,1995).

Trichloroethylene was formerly used on a limited scale as a medical anesthetic and analgesic, an application that is still practiced in some countries (5000-15000 ppm for producing light anesthesia and 3500-5000 ppm for analgesia) (Parfitt et al., 1999).

Workers in the degreasing-industry are the group in which exposure is highest, with concentrations ranging from 1 to 100 ppm. For the general population exposure may also be via food and drinking-water but inhalation usually is the most important route (ACGIH, 1991; IARC, 1995; ATSDR, 1997).

Trichloroethylene's chemical structure is depicted below, and its physicochemical properties are presented in Table 1.



1

Table 1. Physical and Chemical Data		
Parameter	**Data**	**Reference**
Synonyms	Trichloroethene (Chem. Abstr. Name), Ethinyl chloride, ethylene trichloride	
Chemical Formula	C_2HCl_3	
Molecular Weight	131.4	
CAS Registry No.	79-01-6	
Physical State	Liquid	
Color	Colorless	
Oder threshold	--	See Appendix E for data.
Vapour Pressure	74 mm Hg at 25EC	ATSDR, 1997
Specific Gravity	1.4642 g/l (25/4EC)	IARC, 1995
Melting/Boiling/Flash Point	-73EC/87EC/none	IARC, 1995; ATSDR, 1997
Solubility	In water at 20EC: 1.070 mg/liter; idem at 25EC: 1.366 mg/liter Miscible with many common organic solvents (including alcohol, ether and chloroform)	ATSDR, 1997
Conversion factors in air	1 ppm = 5.37 mg/m^3 (at 25EC) 1 mg/m^3 = 0.18 ppm (at 25EC)	

2
3
4 ## 2. HUMAN TOXICITY DATA
5
6 ### 2.1 Acute Lethality
7
8 It is not known at which concentrations trichloroethylene will produce death in humans.
9 A number of fatal chemical accidents have been reported in literature. In most cases this
10 involved accidental inhalation in the workplace during or after degreasing operations. ATSDR
11 (1997) provides a review of the acute lethality data in humans. Without exception the available
12 case studies suffer from poor exposure characterization. The most frequently reported causes of
13 death are depression of the CNS and cardiac toxicity (Keinfeld and Tabershaw, 1954). Liver
14 toxicity has also been reported as the cause of death (e.g. Priest and Horn, 1965).
15
16 Information on exposure levels in the fatal poisonings is insufficient to reliably
17 determine LC-values. Keinfeld and Tabershaw (1954) report a concentration between 200 and
18 8000 ppm (not further specified) for one of their fatalities with no exposure information for their
19 other three cases. Similarly James (1963), who reported a fatal intoxication following repeated
20 sniffing of trichloroethylene, provides no usable exposure information. The same goes for Bell
21 (1951). Ford et al (1995) provide a review of fatal trichloroethylene exposures for the period
22 between 1975 and 1992, again without exposure information for the reviewed cases. For one

case however, a young man who died after spraying trichloroethylene on the walls of a booth that was contained within a larger room, they estimated the exposure concentration using a human PBPK model (with the concentrations as measured in blood and brain of the deceased as input). The post mortem in this subject showed mild pulmonary edema, visceral congestion and brain edema. The result of the exposure estimation was that the subject had been exposed to concentrations greater than 7500 ppm and perhaps up to 10000 ppm (exposure duration probably several hours).

A few cases have been reported of lethal poisonings due to oral exposure to unknown quantities of trichloroethylene with hepatorenal failure as the cause of death (ATSDR, 1997).

2.2 Nonlethal Toxicity

2.2.1 Controlled experiments

2.2.1.1 Neurobehavioral studies

In the 1960's and 1970's a number of neurobehavioral volunteer studies were carried out with trichloroethylene. Stewart et al. (1974a) exposed nine volunteers (three females, six males) to 0, 50 or 110 ppm for two 4-hour periods separated by a 1½ hour lunch break. There was no separate control group in this study. Using a Latin square design (each group being tested at each concentration once) this procedure was repeated on three exposure days with between-exposure intervals of four days. The test room concentrations were monitored by an infrared spectrometer and independently also by gas chromatography (measured concentrations ranged from 49 to 56 ppm and from 110-114 ppm, respectively). The subjects received a complete medical examination both prior to and after exposure. EEG tracings were recorded during all sessions at hourly intervals. The following six neurobehavioral tests were done: a complex reaction time test, a tachistoscopic perception test (ability to reproduce the pattern of images seen on a screen), a digit span test (ability to repeat a list of numbers given by voice in the correct or reverse order), a finger dexterity test (placing pins in holes), the Flanagan coordination test (ability to do pencil tracings) and a digit inspection test (speed to delete a given number from a list of random numbers). Each volunteer performed these tests soon after entering the test chambers and 1½ hours before termination of exposure in the afternoon. The only change suggestive of a neurotoxic effect was a slight impairment in the Flanagan test, present in two individuals at 110 ppm in the matutinal test only. The only consistent subjective symptom reported by the subjects was detection of the odor at both concentrations. Some subjects reported slight symptoms (headache, nausea, drowsiness, eye and throat irritation) but they did so also when they were exposed to 0 ppm, so the significance of these effects is highly doubtful.

In a previous study the same investigators had a group of five to six volunteers (probably males) to 200 ppm for 7 hours/day on 5 consecutive days. There was no separate control group in this study. The concentration of trichloroethylene in the exposure chamber was monitored continuously. Mean concentrations were 198-199 ppm. Forty percent of the subjects reported a dry throat within 2h, which in one subject led to throat irritation. Twenty percent of the subjects complained of mild eye irritation. On later days of exposure, these symptoms no longer

occurred. On day 4, fatigue was reported by all five test subjects, while three also reported drowsiness. No objective effects were found in tests for dexterity and coordination. Half of the exposed subjects noted that the performance of the neurobehavioral tests required greater mental effort. The results of this study were reported as a brief summary only, without any detailed presentation of the responses seen in the individual experiments (Stewart et al., 1970). ATSDR identified the 200 ppm test concentration from this study as an LOAEL and used this level for deriving an acute Minimal Risk Level for trichloroethylene (ATSDR, 1997).

Nomiyama and Nomiyama (1977) found effects at lower concentrations. They exposed twelve male students in groups of three to 0, 27, 81 or 201 ppm for 1-4 hours. The subjects noted the smell at 27 ppm but within three hours had lost sensitivity to the smell, even at 201 ppm. At 27 ppm and above, irritation of mucous membranes (nose, throat) and drowsiness were reported, and after 2 hours at 81 ppm headaches also occurred. After 4 hours at 201 ppm dizziness, anorexia and skin irritation were stated to have been noted but in the table with an overview of all subjective symptoms, a zero incidence is presented for these symptoms. Symptom severity was not reported (only presence or absence in subjects) and consequently the dose-response relationship in effect severity cannot be determined. Yet a further point of unclarity is that drowsiness was reported as occurring earlier at the low concentrations than at 201 ppm. Neurobehavioral parameters determined in this study were limited to flicker fusion frequency and two-point discrimination. No effect on these parameters was seen. Neither was there an effect on respiratory rate. This study has limitations in that only three subjects/concentration were tested whilst the results are reported insufficiently. Thus, the value of this study is limited.

Vernon & Ferguson (1969) exposed eight male volunteers to 0, 100, 300 or 1000 ppm for 2 hour periods. The compound was inhaled with a breathing-tube. The concentrations of trichloroethylene were sampled with a halide meter. The measured concentrations, however, were not reported. Each subject performed six tests to measure visual-motor function, doing each test three times during each 2-hour session. The majority of subjects (not further specified) reported subjective symptoms of CNS-depression, i.e. light-headedness or dizziness, or lethargy, at 1000 ppm. One subject reported subjective symptoms at 300 ppm also (not specified which symptoms). At 100 ppm no symptoms were reported. At 1000 ppm performance in tests for visual perception (Howard Dolman) and steadiness was reduced, as was performance in the pegboard test. In the latter test overt motor effects were observed as increased clumsiness and difficulty in placing pegs in holes. Some subjects at 1000 ppm however showed *improved* performance in the pegboard test. In conclusion, symptoms of CNS-depression with reduced performance in certain neurobehavioral tests occurred at 1000 ppm with no significant effects at 300 ppm (Vernon & Ferguson, 1969). In a subsequent study by the same authors, again in eight male volunteers, slight effects (statistically not significant) were seen in the Howard Dolman and steadiness tests at 300 ppm (exposure for 2 hours) with clear effects at 1000 ppm. This study also examined interaction with ingested alcohol (35 ml/70 kg body weight as a carbonated drink with ice), showing no effect at 300 ppm (exposure for 2 hours) and marked increases in subjective symptoms together with poorer performance in the Howard Dolman, steadiness and pegboard tests at 1000 ppm. The interaction with two CNS drugs, i.e. Thonzylamine hydrochloride and Meprobamate was also examined at 300 and 1000 ppm. These drugs did not

alter neurobehavioral performance compared to a placebo in combination with inhalation of trichloroethylene (Ferguson & Vernon, 1970).

Salvini et al. (1971) tested a group of six male students at 110 ppm (average concentration; measured range from 90 to 130 ppm). The subjects did a series of tests (for perception, memory, complex reaction time and manual dexterity) on two separate days, on one of which they were exposed to trichloroethylene for two 4-hour periods separated by a 1½ hour break. There was no exposure to trichloroethylene on the other day. Tests were performed at 8.30 h and 18.00 h. All subjects complained about the odor (not reported whether they did this on both days). The volunteers reported slight dizziness and transient eye irritation when the concentration rose to the top of the range. Decreases in performance in all the psychophysiological tests were reported. The study was repeated with six workers who regularly worked with trichloroethylene, and who thus were more used to its odor. Similar reductions in psychophysiological performance were recorded. The available report of this study presents only limited information. No actual test results of the neurobehavioral tests are given but only the statistical analyses of the test scores. The findings in TCE-workers are presented as a brief summary statement only (Salvini et al., 1981).

In another poorly reported experiment Winneke et al. (1976) exposed twelve volunteers to 50 ppm (nominal concentration; range: 50±11 ppm) for 3½ hours during which period the subjects were tested for neurobehavioral function. These tests included a reaction time test, a tapping test (for hand/arm speed) and the pursuit rotor test (precision of pursuit tracking by measuring the time on target). Control values were obtained individually for the same experimental situation without exposure to trichloroethylene. No effect was seen in the neurobehavioral tests. Auditorily evoked brain potentials however did show a changed pattern. The significance of this effect is unclear. In a second series of experiments by the same group of investigators, twenty-four male volunteers were subdivided into four groups of six and exposed to 50 ppm (range unreported) for 3½ hours with or without a single oral dose of alcohol (0.6 g/kg bw). One of the groups served as control and in another the subjects received the alcohol dose only. The same behavioral tests were administered as in the previous experiment, again showing no effect of trichloroethylene. Alcohol alone adversely affected neurobehavioral performance, an effect that remained uninfluenced by simultaneous exposure to trichloroethylene (Winneke et al., 1976; Winneke, 1982).

Further volunteer studies were carried out by a Dutch group of investigators (Ettema & Zielhuis, 1975; Ettema et al., 1975), who exposed healthy subjects (students) to various concentrations of trichloroethylene for 2½ hours and compared their performance in a range of neurobehavioral tests with that after moderate amounts of alcohol (oral administration). A total of 47 young adult males (aged between 19 and 27 years) were randomly divided into three groups of 15 or 16 individuals. They were exposed to 0, 150 or 300 ppm (nominal concentrations) trichloroethylene for 2½ hours. During this exposure they performed a range of neurobehavioral tests: a test for binary choice tasks (for auditory signals and for visual signals), the Bourdon-Wiersma test (identifying those groups of dots on paper that contain four or five dots), identification test (detecting small differences in columns of similar figures or words), and memory test (repeating lists of common words). Any signs of toxicity reported during the

exposure period were recorded. Heart rate was followed continuously throughout the exposure period (cardiotachometer). A suppression of sinus arrhythmia (indicating a greater functional exertion to perform the same task), was seen at both dose levels, this effect however being greater at 150 ppm than at 300 ppm. The only change observed in neurobehavioral function was a slightly reduced performance (not statistically significant) in the Bourdon-Wiersma test at 300 ppm. The study authors consider this change to be suggestive of a borderline effect on mental capacity but also note the large interindividual variability which warrants caution in the interpretation of this result. In separate experiments (in 40 volunteers) oral doses of alcohol (20 g/pp, about 0.3 mg/kg bw) resulted in definite impairment in both the binary choice tests and the Bourdon-Wiersma test. These experiments had been preceded by a pilot study in which thirty four male volunteers had been exposed to 0, 75 or 300 ppm for 2½ hours with the same test method as above. There was no effect at 75 ppm and at 300 ppm the only change relative to the controls was suppression of sinus arrhythmia's (Ettema & Zielhuis, 1975; Ettema et al., 1975).

The same group of investigators used a group of twenty-four male students (26-29 years old) for further studying the combined effects of alcohol and trichloroethylene (Windemuller & Ettema, 1978). Groups of six subjects were exposed to 0 or 200 ppm (nominal concentrations) for 2½ hours, or 0.35 g/kg body weight ethanol or to a combination of these treatments. Neurobehavioral testing comprised a binary choice test using visual stimuli and the pursuit rotor test (following the movement of an illuminated point) and was done immediately after cessation of exposure. Breathing rate was monitored throughout the experiment, as was heart rate. No effect on neurobehavioral performance was found. Heart rate and breathing rate also were not affected. Concentrations of ethanol and trichloroethylene in blood showed that alcohol inhibited trichloroethylene metabolism (blood trichloroethylene levels in the combination group were markedly higher than in the group exposed to trichloroethylene alone). A further experiment reported in the same publication involved fifteen male students (additional to the twenty-four mentioned above), each serving as his own control for a single exposure to 200 ppm trichloroethylene, alone or in combination with alcohol. Again trichloroethylene produced no effect on neurobehavioral function (binary choice, pursuit rotor test). In the group only dosed with alcohol, a slight impairment in the pursuit rotor test was noted, a change that occurred to a more marked degree in the combination group (Windemuller & Ettema, 1978).

Further neurobehavioral tests were done by Triebig et al. In two subsequent studies with exactly the same test conditions, they exposed groups of seven students (each consisting of three males and four females) to 0 or 100 ppm for 6 hours/day on 5 consecutive days. Trichloroethylene concentrations were measured continuously using a IR-spectrophotometer, showing mean concentrations ranging from 99.8 to 135 ppm. Neurobehavioral function was tested by a battery of standardized achievement tests and self-report rating scales (in one study) or with tests for intelligence, memory and attention and self-report rating scales (in the other study). These tests were administered daily both at the beginning and at the end of exposure and at one day after the last exposure. No effect was found (Triebig et al.,1976, Triebig et al., 1977).

Gamberale et al. (1976) exposed a group of fifteen healthy adult males to concentrations of 0, 100, and 200 ppm for periods of 70 minutes (each group subsequently serving as control, low-dose and high-dose group, Latin square design). Measured exposure concentrations varied

from 94 to 103 ppm and from 192 to 197 ppm (method of analysis not reported). The taste and smell of the gas were disguised by putting menthol crystals in the tube of the mouthpiece through which the mixture was inhaled. During the last twenty minutes of each session neurobehavioral performance tests were carried out, comprising three tests for reaction time and one for short-term memory. Self-estimates of intoxication and mood were collected as the final step of each session. The only change observed was a decreased performance in one of the tests for reaction time, the addition reaction time (in which subjects have to add up three displayed numbers as quickly as possible). Only a brief statistical summary stating this result is available with no specification what effects occur at 100 or 200 ppm (no tables with results) (Gamberale et al. (1976).

Finally, Konietzko et al. (1975a and 1975b) exposed a group of 20 volunteers (male students and research assistants) to 95.8 ± 8.2 ppm trichloroethylene (measured concentration) for 4 hours and submitted these subjects to a battery of six psychomotor tests (tests for manual dexterity, hand reaction time, hand steadiness, tracing of a line, aiming and tapping). These tests were carried out prior to and directly after exposure. In addition EEG-recordings were made for each subject once every hour. Control scores for all parameters were obtained from the same subjects at 1 week before exposure. No effect was observed (Konietzko et al., 1975a, 1975b).

2.2.1.2. Other endpoints

Stewart et al. (1974b), in their series of volunteer studies, found that some subjects developed very marked skin rashes after consuming alcoholic beverages. This was seen after repeated exposure (5 days/week, 7½ hours/day for about three weeks) to 200 ppm trichloroethylene (not tested at lower concentrations) in 4/4 subjects. It was also seen in 2/3 subjects that had been exposed daily to the same concentration for 2½ hours instead of 7½ hours. The rashes disappeared spontaneously. They could be elicited (by drinking alcoholic beverages) up to three weeks after stopping trichloroethylene treatment (Stewart et al., 1974b). The same effect was already reported by Sbertoli and Brambilla (1962) in two workers after degreasing metal parts using trichloroethylene. This effect may be due to accumulation in the blood of acetaldehyde (a compound that is known to produce this effect) with trichloroethylene competitively inhibiting acetaldehyde dehydrogenase and thus preventing the breakdown of ethanol.

Konietzko et al (1975c) exposed 20 healthy male volunteers (students and research assistants, mean age 27 years) to 95.8 ± 8.2 ppm trichloroethylene (measured concentration) for 4 hours while continuously monitoring ECGs. Only 1/20 subjects showed a change: an abnormal ECG with ventricular extrasystoles that began after 15 minutes (after commencing exposure) and lasted one hour (Konietzko et al. 1975c). In a later study Konietzko and Reill (1980) exposed 20 healthy male volunteers (age 18-44 years) for 4 hours and measured serum marker enzymes for liver dysfunction prior to exposure and at 0, 4 and 20 hours post-exposure. Control values were obtained from the same subjects on an exposure-free day. No indication for liver damage was found (Konietzko and Reill, 1980).

1 **Table 2: Summary of human volunteer studies**

Exposure	Effects	Remarks	Reference
9 volunteers (3 f, 6 m), 0, 50 or 110 ppm for two 4 hour periods per day, on three exposure days (with 4-d intervals in-between)	No effect in five neurobehavioral tests, very slight effect in one further test (Flanagan coordination test); no effect on EEG; the subject detected the odor at both concentrations	Subjects reported slight subjective symptoms but did this also when exposed to 0 ppm. No separate control group in this study	Stewart et al. (1974a)
Five or six volunteers (m), 200 ppm for 7 hours/day of five consecutive days	No effect in tests for dexterity and coordination. Subjective symptoms: dry throat, mild eye irritation. At day 4: fatigue, drowsiness	No control group in this study; results reported as a brief summary only	Stewart et al. (1970)
Four groups of three volunteers (m), 0, 27, 81 or 201 ppm for 1-4 hours	Subjects noted the smell at ≥27 ppm, eye and throat irritation and drowsiness at ≥27 ppm, headache at ≥81 ppm, dizziness, anorexia and skin irritation at 201 ppm; no effect on respiratory rate, no effect on flicker fusion frequency and two-point discrimination	Study report limited, limited no. of subjects per concentration, smell indicated exposure. Effects were not dose-related, effects were reported sooner at low levels compared to high levels. Overall study of limited value.	Nomiyama and Nomiyama (1977)
Eight volunteers (m), 0, 100, 300 and 1000 ppm for 2 hours	Subjective symptoms (light-headedness, dizziness, lethargy) and reduced performance in test for visual perception and pegboard test (increased clumsiness) at 1000 ppm	No separate control group; subjective symptoms reported very briefly only; study indicates NOAEL of 300 ppm for acute neurological effects	Vernon and Ferguson (1969)
Idem, with alcohol consumption	Increase in subjective symptoms and increased reduction of neurobehavioral performance (compared to Vernon & Ferguson, 1969) at 1000 ppm	Idem	Ferguson and Vernon (1970)
Six volunteers (m), 210 ppm for two 4-h periods on one day	Odor complaints, slight dizziness, transient eye irritation, reduced performance in all neurobehavioral tests (perception, memory, complex reaction time, manual dexterity)	Report insufficient, Stewart et al (1974b) could not reproduce the results	Salvini et al. (1971)
Twelve volunteers, 50 ppm for 3.5 hours	No effect on neurobehavioral function (reaction time test, tapping test, pursuit rotor test)	Report limited	Winneke et al. (1976)
Four groups of six volunteers (m), 50 ppm for 3.5 hours, with or without alcohol (including one control group, one alcohol-only group)	No effect in neurobehavioral tests (reaction time test, tapping test, pursuit rotor test), in alcohol-only group reduced neurobehavioral performance	Report limited	Winneke et al (1982)
Three groups of 15 or 16 volunteers (m), 0, 150 or 300 ppm for 2.5 hours	No effect on neurobehavioral performance (binary choice test, Bourdon-Wiersma-test, identification test, memory test), no clinical symptoms, suppression	Study supports NOAEL of 300 ppm for acute neurological effects	Ettema and Zielhuis (1975), Ettema et al. (1975)

Exposure	Effects	Remarks	Reference
	sinus arrhythmia's suggestive of borderline effect on mental capacity at 300 ppm only		
Four groups of six volunteers (m), 0 or 200 ppm for 2.5 hours, with and without alcohol consumption	No effect on neurobehavioral function (binary choice test, pursuit rotor test) in trichloroethylene or alcohol groups, no clinical signs		Windemuller and Ettema (1978)
Fifteen volunteers (m), 200 ppm trichloroethylene	No effect on neurobehavioral function (binary choice test, pursuit rotor test) after trichloroethylene exposure, slightly reduced performance after alcohol+trichloroethylene and alcohol alone, no clinical signs		Idem
Groups of seven volunteers (3m, 4 f), 0 or 100 ppm for 6 h/day for 5 consecutive days	No effect on neurobehavioral function (standard battery achievement tests, self-report rating scales, tests for intelligence, memory and attention)	Two studies with exactly the same test conditions	Triebig et al. (1976 and 1977)
Fifteen volunteers (m), 0, 100 or 200 ppm, Latin square design	No clinical symptoms, no effect in two psychomotor tests (simple reaction time choice reaction time), no effect in a short-term memory test, reduced performance in reaction time addition test (not specified at which exposure levels this effect occurred)	Report very limited (no actual test results given)	Gamberale et al. (1976)
Twenty volunteers (m), 95 ppm for 4 hours	No effect on EEG, no effect in neurobehavioral function tests (tests for manual dexterity, hand reaction time, hand steadiness, tracing of a line, aiming and tapping)	No separate control group	Konietzko et al. (1975a and 1975b)

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2.2.2 Case Reports

The case reports of intoxications with trichloroethylene derive from its use as an anesthetic agent, its inhalation by solvent abusers and its use in industry. These data indicate that the primary target organ is the central nervous system. In addition there are reports of liver damage and renal damage. Cardiac arrhythmias have been reported during trichloroethylene anesthesia. Information on exposure concentrations mostly is very limited, with at best only rough estimates of what was the actual exposure level. This applies even to the cases of side effects during anesthesia where the duration of exposure was unknown, as was the concentration gradient over the exposure period.

Longley and Jones (1963) report acute narcosis in two male workers after exposure to trichloroethylene in a large tank. This accident occurred after spillage of trichloroethylene over the floor of the tank, which led to concentrations of about 3000 ppm (estimated concentration based on a later simulation of the exposure conditions). The men had already been working with trichloroethylene for several hours before the actual accident happened and thus were already mildly intoxicated when the peak exposure due to the spillage occurred (Longley & Jones, 1963). Kostrzewski et al. (1993) describe the case of three male workers who accidentally inhaled trichloroethylene when they entered a tank that contained an unknown amount of the compound. Loss of consciousness occurred within five minutes but the men remained in the tank for 20 to 30 minutes, at which time they were rescued from the tank and brought to hospital. Upon regaining consciousness the subjects complained of headache, vertigo and burning/tearing. Increases in serum ASAT and ALAT indicated that liver damage had occurred; these increases were seen up to three days after exposure. Trichloroethylene concentrations in blood were measured, as were concentrations of the principal metabolite trichloroacetic acid in urine (both up to discharge from hospital after five to thirteen days). Based on these biokinetic data and previously published data on kinetics from inhalation volunteer studies, it was estimated that the subjects had been exposed to about 15000 mg/m^3 (2800 ppm). Similar clinical findings – i.e. marked nervous system effects with indications for slight liver effects – had already been reported by Cotter (1950) and also by Armstrong (1944) (the latter studied patients who had been anesthetized with trichloroethylene).

The use of trichloroethylene as an anesthetic agent has led to a number of publications in which the safety of this practice is discussed (Waters et al., 1943; Brittain, 1948; Pembleton, 1974; Crawford and Davies, 1975; Langton-Hewer, 1975). According to Parfitt et al. (1999) concentrations in the range of 5000 to 20000 ppm are needed for producing light anesthesia. Langton-Hewer (1975), in his historical overview on the use of trichloroethylene as an anesthetic, states that anesthesia requires a blood concentration of about 100 mg/liter and that this can be obtained by sustained inhalation of 7000 ppm (exposure period not further specified). Mostly when trichloroethylene is used as an anesthetic, this is done in combination with N_2O.

Adverse side effects of trichloroethylene anesthesia on the whole have been few. In some patients cranial neuropathies were observed but this was believed to occur only when a closed-circuit gas system with soda lime was used in which toxic decomposition products (dichloroacetylene) were formed. Cases of cardiac arrhythmias have also been reported. Barnes

and Ives (1944) recorded the electrocardiograms of 40 patients (30 males and 10 females, age between 10 and 74 years) throughout 15 to 95 minutes periods of anesthesia with trichloroethylene. The concentration to which these patients were exposed is not reported. Clinically (by taking the pulse) cardiac disturbances were detected in 12/40 patients. Electrocardiographically however, arrhythmia's were observed in 33/40 patients. The arrhythmia's consisted of two groups. During the first twelve to fifteen minutes relatively minor arrhythmia's (principally sinus bradycardia and auroventricular nodal rhythm) were seen in the majority of the patients. Later on alternating ventricular premature contractions occurred in 11 patients, in 4 of whom this led to multifocal ventricular tachycardia. Orth and Gillespie (1945) carried out a similar study in 14 patients. Cardiac irregularities were observed in 13/14 patients. In some patients heart rates were slower and in others faster. The most frequently observed changes were multifocal ventricular extrasystoles. For seven patients trichloroethylene concentrations in the inspired vapor were measured, showing concentrations of 7500 to 12500 ppm. Cardiac irregularities were stated to occur at 10000 to 12500 ppm only. The duration of treatment and the time-course of the concentration were not reported. The latter is important because the medical procedure often involves intermittent application only ("as needed") to maintain unconsciousness during the later stages of the anesthetic period (see for instance Kaczmarek, 1951). Thus, Pembleton (1974) exposed his patients (n=522) initially to 10000 ppm which was reduced to 5000 ppm after a few minutes and to 2000 or 1000 ppm later on (not specified). In 36 patients cardiac arrhythmia's were found (electrocardiographical detection). These were extrasystoles mainly. In the evaluation of his results, this author notes that arrhythmia's have been reported with other anesthetics as well and that despite the arrhythmia's seen with trichloroethylene, use of the agent gave satisfactory outcomes of the medical treatment as a whole. The data suggest that the cases where the cardiac arrhythmia's lead to cardiac arrest are very few. Boulton and Sweet (1960) indicate that there are only eleven fully documented cases in which unexplained primary cardiac failures occurred during trichloroethylene anesthesia. This after large scale use in the UK for sixteen years. In conclusion the data on the side effects of trichloroethylene anesthesia indicate that cardiac arrhythmia's will occur when subjects are exposed to concentrations of about 10,000 ppm and higher, even when exposure is only for a few minutes. What is the minimum effective concentration when exposures last longer (one hour for instance), cannot be ascertained from these data. Nor can for such exposure durations a clear minimum effective concentration for narcosis be determined from these studies.

Some authors report organic lesions in the nervous system after accidental industrial exposure. Sagawa et al. (1973) reported complete loss of sensation in the trunk and legs and visual disturbances in a young woman after exposure to several thousand ppm of trichloroethylene for an unknown period. They interpreted these symptoms as indicating cranial neuropathy. Another patient, involved in the same accident, also had become unconsciousness, but showed much milder effects. Feldman (1970) reports trigeminal sensory loss in a subject who was exposed for several minutes to high concentrations while he inspected a degreasing tank. He experienced nausea, vomiting and dizziness but not anesthesia. Extensive sensory loss occurred over the face with numbness also in the mouth and the pharynx. The subject also had blurred vision, suggesting the involvement of the optic nerve or the visual area of the cortex. Complete numbness over a large area of the face was maintained for three months, with areas of

hyperanalgesia for over a year. Eighteen years after exposure Feldman et al. (1985) again examined this patient and found indications for long-term residual oculomotor and ciliary reflex dysfunction as well as impaired neuropsychological performance.

Nephrotoxicity after acute intoxication with trichloroethylene appears to be rare. Gutch et al. (1954) reported acute renal failure in a man after exposure to high concentrations of trichloroethylene for several hours. David et al. (1985) report a similar case after exposure for eight hours. Their patient's acute renal failure was ascribed to acute allergic interstitial nephritis and with secondary tubular necrosis. A recent report of renal damage is that by Brüning et al. (1998) who demonstrated tubular damage in a 17-year-old man who had ingested 70 ml of trichloroethylene in a suicide attempt. The man showed fever, tremor, general restlessness, and sinus tachycardia and lost consciousness at 5 hours after drinking trichloroethylene. The typical standard clinical parameters for nephrotoxicity, i.e. glucose, total protein excretion, serum creatinine and BUN, were negative but biomarkers for tubular damage (excretion of α_1- and β_2-microglobulins and β-N-acetylglucosaminidase, urinary protein electrophoresis) were positive. The authors suggest that the reactive intermediates formed in the kidney via the glutathione pathway of trichloroethylene biotransformation may be responsible for the acute renal effects. The patient regained consciousness after 5 days and was transferred to a psychiatric department.

2.2.3 Chronic Studies

No controlled studies are available. Numerous health surveys have been carried out in workers exposed to trichloroethylene. In these studies the information on exposure levels was limited or even lacking entirely. In addition, as is usual with this kind of studies, exposure concentrations show wide fluctuations over time. Thus, the value of these data as to the dose-response relation of trichloroethylene upon repeated exposure, is limited. Below only the most meaningful studies are summarized.

Bardodej and Vyskocil (1956) noted widespread CNS-symptoms in two groups of workers involved in either degreasing operations (n=55) or in textile cleaning (n=12). Atmospheric concentrations in dry-cleaning were estimated to be in the range of 0.16 to 3.4 mg/liter (30-612 ppm) and in degreasing 0.028 to 0.83 mg/liter (5-154 ppm) (not further specified). No control group was used in this study. The most common CNS-symptoms were headache, sleepiness, feelings of inebriation, nausea and tinnitus. In addition there were signs of local irritation to the eyes (lachrymation), skin (reddening) and respiratory tract.

Takamatsu (1962) carried out two health surveys in a group of 50 industrial degreasers, most of whom had been employed for 2.5 years at the time of the first survey. No control group was used in this study. The concentrations in the degreasing-room at the time of the survey were in the range of 100 to 600 ppm whereas in the adjacent room 50 to 100 ppm was measured. In the most heavily exposed group, most workers complained of CNS-symptoms (vertigo, fatigue, headaches, and sleeplessness). In addition many had visual disturbances. Blood albumin was decreased, blood gamma-globulin was increased and urine analysis showed the presence of albumin and urobilinogen in 30 to 36% of the workers. Similar, but less marked effects were noted in the subjects exposed to 50 to 100 ppm. In the second survey in the same factory the

workers were divided into three groups, based on exposure level. For one group (n=8) the concentrations were 150 to 250 ppm, for another group (n=14) 50 to 100 ppm and for the third group (n=16) less than 50 ppm. CNS-symptoms similar to those in the first survey and alterations in blood albumin and gamma-globulin were present in the high exposure group and to a lesser degree also in the intermediate group. The group exposed to the lowest trichloroethylene levels showed no effects.

El Ghawabi et al. (1973) clinically examined 30 male workers from an Egyptian printing-factory. Mean workplace concentrations ranged from 38-172 ppm. It is not clear whether these concentrations represent time-weighted averages. Most of the workers had been employed for more than three years. The incidences of symptoms were compared to a control group of 30 unexposed workers. Among the exposed workers the incidences of a wide range of symptoms were increased: headache, dizziness, sleepiness, nausea and vomiting, lachrymation, reduced libido, skin rashes, itching and fatigue. Electrocardiographic examinations were carried out in 25 of the exposed workers, the results of which showed no abnormalities.

Landrigan et al. (1987) carried out two medical surveys among trichloroethylene exposed workers (n=9-12) . In the first survey the 8 hours TWA concentrations ranged from 117 to 357 mg/m^3 (22-66 ppm) with 5-15 minutes peak concentrations of 413 to 2000 mg/m^3 (77-370 ppm). An increased incidence of CNS-symptoms was noted (fatigue, lightheadedness, sleepiness) and also eye irritation. The second survey was carried out three months later, after measures for exposure reduction had been taken (interval between exposure reduction and re-evaluation unknown). The 8 hours TWA concentrations now ranged from 37 to 144 mg/m^3 (6.9-26 ppm) with 5-15 minutes peak concentrations of on average about 400 mg/m^3 (74 ppm). Similar symptoms were found as in the first survey, with only a marginal reduction in the number of complaints. The low number of subjects limits the value of this study. It should be noted that there are large differences between TWA and peak levels in this study.

Ruijten et al. (1991) evaluated nerve function in a group of 31 male printing-workers. A group of 28 non-exposed workers from the same printing-works served as controls. All exposed subjects had previously worked with an ink containing trichloroethylene which had been replaced by a water-based ink at three years before this study. The exposure concentrations at the time of the study were estimated to be about 17 ppm. In the period of eight years before the introduction of the water-based ink, it was estimated to have been about 35 ppm and prior to that 70 ppm. Individual cumulative exposures were calculated, based on work history. Neurological function tests were carried out (autonomic , trigeminal and peripheral nerves) showing slight reduction in sural nerve conduction velocity and a slight prolongation in the sural nerve refractory period. In addition there was an increase in masseter reflex latency (a measure of trigeminal nerve function) but blink reflex (also a measure of trigeminal nerve function) was not prolonged. No effect on peripheral nerve motor function or on autonomic nerve function was found. In questionnaires that the workers filled in, no increase in symptoms of neuropathy was reported. The observed changes are slight and their health implications are uncertain. The authors consider that the observed changes may reduce the body's reserve capacity to cope with other noxious influences.

Rasmussen et al. (1993a, 1993b) evaluated 99 degreasers for effects on the liver, kidney and nervous system. The workers were categorized in four exposure groups, based to the number of hours of exposure. Group I (reference group) with less than 1 year of full time exposure, group II, 1 to 2.8 years, group III 2.9 to 6.7 years and group IV 6.8 to 35.6 years. Present or recent exposure was estimated from metabolite concentrations in blood and urine. From historical data on trichloroacetic acid concentrations in urine, it was concluded that the highest exposure group in the preceding decades had been exposed to concentrations of less than 50 ppm. Neurological and neuropsychological examinations were made: psychometric tests, medical history of symptoms of mental impairment, clinical signs for demential behavior. Based on these tests the prevalence of the psycho-organic syndrome (OPS) was determined for each group, OPS being defined as a mild syndrome characterized by cognitive impairment, personality changes, and reduced motivation and initiative. Liver and kidney function were evaluated by laboratory tests on blood and urine samples: aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, bilirubin, protein, prothrombin, urinary N-acetyl-beta-glucosaminidase. Statistically significant exposure response relationships for elevated serum gamma-GT and urinary NAG were found but this relationship was no longer significant when age and smoking were taken into account. The neurological examinations showed a prevalence of OPS of 10% for low exposure, 39% for medium exposure and 63% for high exposure. After adjustment for known potential confounders, logistic regression analysis showed an increased risk of developing OPS only in the highest exposure group, with an odds ratio of 11.2 (95%-confidence interval 1.9 to 66.6). Of the 42 workers diagnosed as having OPS, 31 had been predominantly exposed to trichloroethylene and seven exclusively exposed to trichloroethylene.

Seldén et al. (1993) carried out a cross-sectional study in a group of 29 metal degreasers, focussing on potential nephrotoxicity. Eighty-six percent of the 8-hours TWA concentrations were well below 50 mg/m^3 (9 ppm). The mean cumulative exposure time was 6.2 years. Urine was examined for N-acetyl-beta-glucosaminidase (marker of tubular damage). No effect was found.

Brüning et al. (1999) studied renal toxicity in 39 male workers chronically exposed to trichloroethylene for 6 to 20 years (average 16 years). They determined alpha-1-microglobulin and glutathione transferase-alpha (as markers of proximal tubular damage) and glutathione transferase-gamma (as a marker of distal tubular damage) in urine . In addition, serum creatinine, serum urea, urinary creatinine and total urinary protein were measured. Exposure levels were ranked semi-quantitatively by applying a system that integrates total exposure time and frequency and severity of acute symptoms. The highest exposed workers were thought to have had exposures in excess of 500 ppm. A group 46 office workers from the same factory served as controls. Urinary excretion of alpha-1-microglobulin and glutathione transferase-alpha were increased in the exposed workers, suggesting slight renal damage in these subjects.

2.3 Developmental/Reproductive Toxicity

No human reproductive studies are available. Developmental toxicity was examined in several epidemiological studies (chronic exposure). Taskinen et al. (1989) studied the

association between paternal occupational exposure to trichloroethylene and spontaneous abortions. This was a nested case-control study on 120 cases of spontaneous abortions and 25 cases of congenital malformations and 251 controls based on a file of 6000 Finish workers who had been biologically monitored for exposure to solvents. No quantitative exposure data are available. Paternal exposure to trichloroethylene was not a risk factor for spontaneous abortions. For congenital malformations the number of cases was too small to allow an analysis specifically for trichloroethylene.

The same group of investigators examined the association between maternal occupational solvent exposure and spontaneous abortions (Lindbohm et al., 1990). A total of 73 cases was identified for each of which three controls were selected. Trichloroethylene exposure had an odds ratio of 0.6 indicating that it is not a risk factor for spontaneous abortion. In yet a further investigation by this group (Taskinen et al., 1994), the 7316 pregnancies from a group of 9186 female Finnish laboratory workers were analyzed for a possible relation between adverse outcome and solvent exposure. The analysis for spontaneous abortions involved 206 cases and 329 controls; for congenital malformations this was 36 cases and 105 controls. Seven women with a spontaneous abortion had been exposed to trichloroethylene compared to nine among controls (odds ratio 1.6). For congenital malformations the number of cases was too small allow an analysis specifically for trichloroethylene.

2.4 Genotoxicity

Several studies are available in which workers were monitored for cytogenetic effects. Konietzko et al (1978) found increased incidences of hypodiploid cells in peripheral lymphocytes from nine workers exposed to mean maximal trichloroethylene concentrations of 206 ppm, an effect that was absent in workers exposed to mean maximal concentrations of 116 ppm. Nagaya et al. (1989) and Seiji et al. (1990) searched for sister chromatid exchanges in peripheral lymphocytes of workers exposed to trichloroethylene at estimated concentrations of 7 or 30 ppm, both research groups finding no effect. Rasmussen et al. (1988) found increased incidences of chromosome aberrations and hyperdiploid cells in the peripheral lymphocytes of 15 metal degreasers exposed to unknown concentrations of trichloroethylene. In the sperm of twelve of these workers the frequencies of abnormal sperm heads and the number of sperm with two fluorescent Y bodies were not increased.

Two recent German studies were focussed on detecting mutations in the von Hippel-Lindau tumor suppressor gene in renal cancer patients who had been exposed to trichloroethylene occupationally. The Brauch et al (1999) study included 44 renal carcinoma patients with trichloroethylene exposure and 107 renal carcinoma patients without exposure. DNA analyses showed that 75 % of the exposed patients had VHL mutations; there was a significantly positive association between the number of mutations in each patient and the presumed level of trichloroethylene exposure. Of those with VHL mutations 39% had C>T missense mutation at nucleotide 454. The presence of this mutation was also determined in the non exposed patients and none was found. The overall results of the DNA analyses of the non-exposed subjects (no of subjects with VHL mutations in this group) were not reported. These results suggest that trichloroethylene may trigger a unique mutation pattern in renal tumor

patients. The causal role of these genetic events in tumor formation, however, is unclear. This study was a follow-up to the Brüning et al. (1997) study in which VHL mutations had been demonstrated in twenty-three renal cell carcinoma patients with a known history of trichloroethylene exposure.

2.5 Carcinogenicity

No studies on acute exposure are available. Human carcinogenicity after chronic exposure to trichloroethylene has been studied in a large number of occupational epidemiology studies. This includes cohort studies as well as case-control studies. These data have been reviewed by IARC (1995) , McLauglin & Blot (1997) and Wartenberg et al. (2000). IARC identified three cohort studies as being the most informative, i.e. Axelson et al. (1994), Anttila et al. (1995) and Spirtas et al. (1991). In each of these studies excess relative risks for cancer of the liver and biliary tract were found (relative risks: 1.4, 1.9 and 1.9 respectively). The total number of cases however was small. The relative risk for non-Hodgkin lymphomas was increased similarly (relative risks 1.5, 1.8 and 1.3 respectively). In none of these studies it was possible to correct for potential confounding factors. The available case-control studies mostly deal with exposure to groups of chemicals and thus are of limited value as to possible trichloroethylene carcinogenicity. IARC concluded that the available epidemiological evidence provides *limited evidence* for carcinogenicity of trichloroethylene in humans. Wartenberg et al. (2000), who in their review used a data base that was very similar to IARC (1995) (only with an extended follow-up for the Spirtas et al.-cohort), provide an analysis that is consistent with that of IARC but they suggest a stronger association between trichloroethylene and cancers at several sites in the body. This includes renal cancers for which they conclude that there is fairly strong evidence for an increased risk. For these cancers they report an average risk of 1.7 across the five studies they classified as Tier I cohort studies, however without discussing the merit of the study with the highest risk of 8.0, i.e. the study by Henschler et al. (1995). This was a retrospective cohort that probably was initiated after the observation of a cluster (without deleting the cluster tumors from the analysis) and therefore IARC attached little weight to it. In sum the evidence that trichloroethylene causes renal cancer in humans is very weak only – as was concluded by IARC (1995) and also by McLauglin & Blot (1997).

2.6 Summary

Lethality data in humans are limited and do not provide any detailed information on LC-values. The central nervous system is the main target organ for acute trichloroethylene toxicity. In addition there are reports of cardiac toxicity, liver effects and renal effects.

The most common CNS-symptoms are headache, dizziness, sleepiness and nausea. Neurobehavioral functioning has been investigated in a number of volunteer studies. Mild subjective symptoms and nose and throat irritation were reported by volunteers at 200 ppm, applied for 7 hours/day on the first day of 5 subsequent days. On later days of exposure these symptoms no longer occurred (Stewart et al., 1970). CNS-depression and effects on neurobehavioral functions were seen in volunteers by Vernon and Ferguson (1969) after exposure to 1000 ppm for 2–hour periods. At 300 ppm only marginal effects were observed

(exposure for 2 hours). At the latter concentration Ettema & Zielhuis (1978) also observed slight to marginal effects on neurobehavioral function after exposure for 2½ hours.

Chronic toxicity was investigated in several occupational epidemiology studies. Because of incomplete exposure characterization and fluctuations in workplace trichloroethylene concentrations these studies provide only limited information on the chronic dose response relation. The central nervous system was the primary target organ in these studies; indications for renal effects were also found.

Cancer epidemiology studies provide limited evidence for a carcinogenic effect of trichloroethylene in humans. The results of occupational studies for genotoxicity endpoints are inconclusive.

3. ANIMAL TOXICITY DATA

3.1 Acute Lethality

3.1.1 Rat

Adams et al. (1951) studied the dose response relation for lethality in Wistar rats. The rats (sex not reported) were exposed to 3000, 4800, 6400, 9600, 12000 and 20,000 ppm for different periods, ranging from 0.3 hours to 14 hours. The mortality rates were as follows:

At 20,000 ppm								
Exposure duration (h)	5.0	4.0	3.0	1.0	0.8	0.6	0.4	0.3
Mortality	20/20	7/10	3/10	1/10	1/5	4/20	2/20	0/20
At 12,000 ppm								
Exposure duration (h)	8.0	7.0	6.0	5.0	2.0	1.0	0.6	-
Mortality	9/10	5/5	4/5	9/10	2/5	2/5	0/20	-
At 9600 ppm								
Exposure duration (h)	2.0	1.0	0.8	-	-	-	-	-
Mortality	8/15	3/20	0/20	-	-	-	-	-
At 6400 ppm								
Exposure duration (h)	4.0	2.0	1.4	1.0	-	-	-	-
Mortality	4/10	1/10	0/10	0/20	-	-	-	-
At 4800 ppm								
Exposure duration (h)	8.0	6.0	4.0	1.4	-	-	-	-
Mortality	6/30	4/9	3/10	0/20	-	-	-	-
At 3000 ppm								
Exposure duration (h)	14.0	8.0	-	-	-	-	-	-
Mortality	2/10	0/30	-	-	-	-	-	-

From Adams et al. (1951)

The only acute effect noted in the animals was depression of the central nervous system, which was apparent as unsteady gait, stupor, unconsciousness and failure of respiration or, possibly, of cardiac function. Full anesthesia was apparent at concentrations of 4800 ppm and above but not at 3000 ppm. Post-mortem examinations in this study were done in separate groups of animals exposed to concentrations in the same range as in the above mortality series (not further specified). Only minor changes were found: slightly increased liver weight, cloudy swelling in the liver (without fat globules). Chemical analysis showed a slight increase in liver lipid content (Adams et al., 1951).

Vernot et al. (1962) determined 1-hour LC_{50}-values for a large number of chemicals. The results are reported as a summary table only. For trichloroethylene in male rats a value of 26,300 ppm (23,700-29,200) is given and for female rats 25,700 ppm (22,300-29,500).

Siegel et al. (1964) examined the acute toxicity of dichoroacetylene and trichloroethylene and its mixtures in male NMRI:O(SD) rats. In a four-hour whole body exposure regimen they tested trichloroethylene concentrations of 6750, 8000 and 14,700 ppm (presumably nominal concentrations). The mortality ratios were as follows: 1/16, 3/16 and 10/16. An LC_{50} of 12,500 ppm was calculated. The only symptom reported was depression of the nervous system (no post mortem results given).

Bonnet et al. (1980) determined the six-hour LC_{50} in male Sprague Dawley rats for a number of chlorinated solvents, including trichloroethylene. Whole animals were exposed but the administered concentrations are not reported. The LC_{50} was 5918 ppm (5718-6214 ppm). The only symptoms reported were hypotonicity, somnolence and stereotypic behavior. Post mortem showed no macroscopic lesions in lungs, liver and kidneys.

3.1.2 Mouse

Friberg et al. (1953) investigated trichloroethylene lethality in female white mice (strain not reported). Groups of 8 animals were exposed for 4 hours. The authors give only a very short summary of their study with no details on the experimental conditions and no information on intoxication symptoms and post-mortem results. The mortality pattern was as follows:

Concentration (ppm)	Mortality
3750	0/8
4600	0/8
5350	2/8
7600	2/8
8600	6/8
9300	7/8
11,500	5/8
12,000	6/8
14,100	5/8
14,750	8/8

From Friberg et al. (1953)

The LC_{50} calculated from these data was 8450 ppm (Friberg et al., 1953).

Gradiski et al. (1978) determined the six-hour LC_{50} in female OF_1 mice for a number of chlorinated solvents, including trichloroethylene. Whole animals were exposed but the administered concentrations are not reported. The reported LC_{50} was 5857 ppm (5489-6250 ppm). No information is given on symptomology or on post-mortem results.

3.1.3 Dog

Baker (1958) studied the effect of trichloroethylene by exposing 15 dogs to 30,000 ppm. The published information on this study is very limited. Within 5 minutes the dogs showed salivation and uncontrolled limb movements. Loss of consciousness, convulsions and death were seen within about 20 minutes. The actual number of dogs that died is not reported. Post-mortem examination probably was limited to the central nervous system, in which no lesions were found.

3.2 Nonlethal Toxicity

3.2.1 Rat

Neurotoxicity

The effect of trichloroethylene on the behavioral activity of rodents following single inhalation was examined in several studies carried out in the 1960s and 1970s. Grandjean (1963) tested for swimming-performance in six rats immediately after exposure to trichloroethylene for six hours. Tests were done both without and with load (a 27 gram weight attached to the rats' tail). The same animals were used for testing 0, 400 and 800 ppm. In different groups of rats motor activity was determined during and after a five hours' exposure. Concentrations of 0, 400, 800 and 1600 ppm were tested, each test involving 12 rats which served, in alternating arrangement, once as controls and once as subjects. All concentrations were nominal concentrations introduced into to the exposure chambers, with probably no measurements of actual concentrations. Swimming-performance was decreased at 800 ppm. Motor activity during exposure was decreased at all concentrations but the difference with the control exposure was significant at 1600 ppm only. At one hour after cessation of exposure (the end of the experiment), this effect was still present. Grandjean and Bättig (1964) report several tests including one for spontaneous alternation behavior in twelve rats in a T-maze after exposure to 0, 200, 400, 600, 800 or 1600 ppm for four or eight hours (nominal concentrations). The number of alternations was reduced at 400, 600 and 800 ppm after exposure for eight hours (at 1600 ppm no effect but motor speed was reduced at that level). After exposure for four hours to 200 and 400 ppm no effect was observed. Locomotion speed in the maze showed an inconsistent pattern with a slight decrease at 200 ppm (4 hours exposure), increases at 400 ppm and 600 ppm (4 or 8 hours of exposure), no change at 800 ppm (8 h) and again a decrease at 1600 ppm.

More recently Kishi et al. (1993) studied the relation between blood trichloroethylene concentrations and behavioral changes in rats after a single inhalation exposure for 4 hours. In this study only a single group of 8 male rats (Wistar) was used. Prior to treatment the rats had

been trained in a Skinner box to press a lever in response to a light stimulus to avoid receiving a shock. The test animals were sequentially exposed to 0, 250, 500, 1000, 2000 and 4000 ppm in ascending order, with 10 to 20 day intervals between exposures. The animals were exposed during the dark cycle of the light/dark schedule (the active time for rats). Exposure chambers concentrations were determined with a gas chromatograph but the results of these analyses were not reported. Concentration-related decreases in shock avoidance performance were observed at all dose levels. Exposure to 250 ppm affected performance within 140 minutes; at 500 ppm this was 80 minutes and at 1000 and 2000 ppm 20 minutes. At 4000 ppm all rats became ataxic and no longer responded to the stimuli; one rat died whereas the other seven showed some recovery post exposure. Blood analyses showed that concentrations of 10 to 40 µg/ml (found at the end of the exposure period at 250 and 500 ppm) are associated with slight decreases in performance and some CNS-symptoms. Concentrations of 100 µg/ml and higher (found after 4 hours at 2000 or 4000 ppm) were associated with marked CNS-depression and anesthesia.

Niklasson et al. (1993) examined the effect of acute exposure to several solvents, including trichloroethylene, on the central vestibular system in male and female pigmented rats by recording eye movements upon different stimuli. Test concentrations were selected based on observation of loss of righting reflex and depression of avoidance response in preliminary tests. In the main experiment the rats (total number: 18 females and 10 males) were exposed to actual trichloroethylene concentrations of 14,800, 22,700, 32,700 and 38600 mg/m^3 (2700, 4200, 6100 and 7200 ppm) for one hour. Each rat was used in an initial control experiment with no solvent exposure and one or two subsequent experiments with exposure to trichloroethylene at one or two levels of concentration. Provocation and recording of saccades (quick reposition of the eyes) began 10 minutes after initiation of exposure. Thereafter the mean slow-phase eye velocities of nystagmus (rapid involuntary movement of the eye ball) were recorded during vestibular and/or optokinetic stimulation.

Reductions in the ability to compensatory eye movements following optokinetic stimulation were seen, as were reductions of maximal slow-phase eye velocities following vestibular stimulation. In addition, nystagmus was prolonged and the ability to suppress the vestibular response upon simultaneous optokinetic and vestibular stimulation was impaired. These changes occurred at all dose levels, to a dose-related degree. Changes in the generation of saccadic eye movements were also seen in the exposed animals (not clearly reported exactly in which groups). The authors interpret their findings for the solvents they tested, as indicating that they affect the cerebro-vestibular reflex circuit. They hypothesize that changes in the lipid membrane of neuronal cells leading to altered receptor protein function with consequent effects on neurotransmission, are the underlying cause for the effects.

Bushnell (1997) of the US-EPA examined the concentration-time relationship for the acute neurotoxic effects of trichloroethylene in male Long-Evans rats. Various combinations of concentration (C) and time (t), designed to yield a constant C x t product, were tested. A constant response for these combinations would support Haber's rule. The second step in this research involved calculating the empirical relationship between C and t which produces a constant change in behavior (standard effect). A single group of twelve rats was trained to perform a signal detection task, i.e. pressing a lighted lever to obtain a food pellet whilst

ignoring a second unlighted lever that is simultaneously offered (signal trial) or pressing an unlighted lever and ignoring the simultaneously offered, previously lighted lever (blank trial). Each rat was exposed to 0, 400, 600, 800, 1200, 1600, 2000 and 2400 ppm, twice at each concentration (nominal values; actual concentrations well within ±10% of nominal values). Exposures were done in three 2-week series, the first two of which were done subsequently and the third after 6 weeks. Within the first week of such a 2-week series, rats were exposed to air only on day 1 and to increasing concentrations on the subsequent three days (one concentration/day); within the second week of the 2-week series this procedure was repeated with exactly the same concentrations being tested. Thus, in the course of the study each of the seven concentrations was tested. Daily exposures lasted 0.33, 0.67 or 1.00 hour. Two measures of performance of the task were calculated: the sensitivity index (SI) that represents the ability of the animal to discriminate signal trials from blank trials and the response time (RT) that is the time between insertion of levers into the exposure chamber and the lever press. These parameters were analyzed statistically, including matched-pair t-tests to compare the effects of exposures at constant C x t product but different values of C and t (e.g. 800 ppm x 1 hr, 1200 ppm x 0.67 hr and 2400 ppm x 0.33 hr). In addition, the concentration-effect function for SI and RT from each rat at each duration of exposure was fitted with a cubic polynomial function (least square regression). From these functions the criterion concentrations were calculated, for SI the concentration that produced a 0.1 unit decrease in this parameter and for RT at the concentration producing a 100 msec increase above the rats mean RT in air. These cut-off points were chosen – given the lack of normative clinical criteria for impaired function for these endpoints - based upon existing data from this and other tests for attention in rats. The obtained values for $SI_{0.1}$ and RT_{100} were compared across exposure durations.

The results showed that the sensitivity index was decreased after exposures to ≥1200 ppm that lasted 0.67 or 1.0 hours; the NOAEL for this effect was 800 ppm for 1.0 hour. The mean response time was increased after exposures to 2400 ppm that lasted 0.67 or 1.0 hours; the NOAEL for this endpoint was 2000 ppm for 1.0 hours. The effect of trichloroethylene was not constant over different C x T combinations. Concentration contributed more to the effect than did exposure duration. Measured at 0.33, 0.67 and 1.00 hours, the $SI_{0.1}$-values were 2298, 1823 and 1373, respectively; for RT_{100} the corresponding values were 2317, 2118 and 1983, respectively. When plotted in logarithmic C x T space these criterion concentrations formed lines with slopes of 2.2 for $SI_{0.1}$ and 7.1 for RT_{100} (slope based on the 0.33 and 1.00 hours criterion concentrations). Thus, in the formula $C^n xt = k$, based on $SI_{0.1}$ the empirical value for n is 2.2 and based on RT_{100} n is 7.1. These different values for n indicate that the underlying mechanism for these effects (i.e. signal detection and reaction speed) is not the same (Bushnell, 1997). In a subsequent publication (Bushnell and Oshiro, 2000) report that in the above experiment the rats developed some tolerance to the disruption of the signal detection behavior across the weeks of exposure. Thus, in the response in the later series of exposures (to the higher concentrations) an additional, uncontrolled-for variable was operative. The values for n in the formula $C^n xt = k$ that would have been found if all exposures would have been done in previously unexposed rats, are higher than those derived by Bushnell (1997).

Also at the US-EPA, Crofton and Zhao (1997) carried out a similar study to evaluate the extrapolation of neurotoxic effects from high-concentration short-term exposures to the

neurotoxicity produced by longer exposures. They exposed four groups of 8-12 male Long-Evans rats for either 1 day (4000, 6000, 8000 ppm), 1 week (1000, 1600, 2000, 2400, 3200, 4000 ppm), 4 weeks (800, 1600, 2300, 3200 ppm) or 13 weeks (800, 1600, 2400, 3200 ppm). The animals were exposed whole body during 6 hours/day, 5 days/week. Between 3 and 5 weeks after exposure the animals were monitored for trichloroethylene ototoxicity (hearing-loss) by determination of the auditory threshold for a 16 kHz tone (by reflex modification audiometry). The dB15-concentrations were calculated for every exposure duration, being the concentration needed to produce a 15 dB increase in the auditory threshold. The 95% lower bounds on the dB15-concentrations were also determined, these being the corresponding Benchmark Concentrations (as defined by Crump). A benchmark effect of 15 dB was chosen because this level of hearing-loss is generally accepted as being clinically significant when occurring in humans. The results showed that concentration contributes more than linearly to the effects. The dB 15–concentrations were 6218, 2992, 2592 and 2160 ppm for the 1-day, 1-week, 4-week and 13-week exposures respectively. The corresponding BMCs were 5223, 2108, 1418 and 1707 ppm respectively (Crofton and Zhao, 1997).

A number of animal neurotoxicity studies of longer duration is available. The induction of medium-frequency hearing-loss was seen in rats after subacute and semichronic inhalation (studies by Rebert et. al, 1991 and 1993; Crofton and Zhao, 1993; Jaspers et al., 1993; Crofton et al., 1994). For the induction of this effect relatively high concentrations were required. Rebert et al. (1991) estimated a threshold concentration of about 2000 ppm when rats were exposed for 12 weeks (12 hours/day, 6 days/week). Other neurological endpoints effects were seen at lower concentrations after subacute to subchronic exposure. Arito et al. (1994) found decreased wakefulness at 50 and 100 ppm in a 6-week study in rats (exposure 8 hours/day, 5 days/week). The same investigators had observed this effect also at ≥300 ppm in a 3-day study (exposure for 8 hours/day). At 3000 ppm for 3 days (8 hours/day) and at 6000 ppm for 3 days (4 hours/day) they found more severe effects, i.e. incapacitation of postural maintenance and occasional seizures of the hind limbs (Arito et al., 1993). ATSDR used the LOAEL of 50 ppm from the 6-week study (Arito et al., 1994) in its derivation of an intermediate duration MRL. In an early study by Bättig and Grandjean (1963) swimming-behavior in rats was impaired at 400 ppm (8 hours/day, 5 days/week for 44 weeks), the only dose level tested in this study.

Hepatotoxicity

Cornish and Adefuin (1966) exposed groups of six male Sprague-Dawley rats to several chlorinated solvents, including trichloroethylene, and measured the activity of the liver damage marker enzymes serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and serum isocitric dehydrogenase (SICD) at 24 hours after exposure. Histopathology was also done. Test concentrations of 100, 2000, 5000, and 10,000 ppm were administered for 8, 4, 4 and 1.5 hours, respectively. Chamber trichloroethylene concentrations were measured and were reported to be within ± 5% of the nominal values. Each of the exposure concentrations was also tested after pretreatment with ethanol (5 mg/kg bw p.o. at 16 to 18 hours before exposure). This was done to determine the influence of liver enzyme induction on solvent hepatotoxicity. Increases in serum marker enzyme actvity was seen only at 10000 ppm (with and without alcohol pretreatment) and at 5000 ppm with alcohol pretreatment. At 10000 ppm 3/6 rats died in both groups. The report of the histopathology results is limited to

the statement that widespread degenerative lipidic infiltration of the liver was seen at 5000 ppm without alcohol pretreatment and that rats at this concentration with alcohol pretreatment, in addition showed early centrilobular necrosis with slight acute cellular inflammation.

A similar study was done by Carlson (1974) in male albino rats (strain not reported) with pretreatment with phenobarbital or 3-methylcholanthrene. In one group of five rats, exposed to 10400 ppm for 2 hours (actual concentration) after pretreatment with phenobarital, liver glucose-6-phosphatase activity was decreased and SGOT- and SGPT-activities were increased. In the trichloroethylene-treated control group no such changes were seen. Histopathology showed edema, cell enlargement and focal necrosis in the rats treated only with trichloroethylene whereas the pretreated rats showed massive necrosis. Other groups of five rats were exposed to 6900 ppm trichloroethylene for 2 hours, with or without pretreatment with 3-methylcholanthrene. The results were similar as those described above, with changed liver enzyme activities in the combination group only and slight histological damage in the trichloroethylene-treated control and marked damage in the combination group.

Enhancement of trichloroethylene hepatotoxicity by pretreatment with known liver enzyme inducers was also observed by Moslen et al. (1977a and 1977b). They tested a concentration of 10,000 ppm for 2 hours (a treatment expected to be anesthetic) in male Sprague-Dawley rats after pretreatment with phenobarbital, Aroclor 1254, hexachlorobenzene, 3-methylcholanthrene or pregnenolone-16-α-carbonitrile. As shown by increased SGOT and liver histopathology the toxic response was increased due to increased trichloroethylene metabolism. Also the anesthesia recovery times were increased when enzyme inducers were given, a finding that suggests that trichloroethylene metabolites play a crucial role in the narcotic effect produced by the compound.

In rat studies of longer duration liver effects have been observed in some instances only, with the observed effects being of a mild nature or even lacking toxicological significance entirely. Adams et al (1951) found only liver enlargement without histological effects at 3000 ppm after exposure for five weeks (7 hours/day, 5 days/week). In an 8-month study in rats these authors found the same effect also at 400 ppm (NOAEL 200 ppm). Prendergast et al. (1967) found no liver effects (including histopathology) after continuous exposure to 35 ppm for 90 days, nor after 6 weeks' exposure to 712 ppm. Kjellstrand et al. (1981) observed increased liver weight after continuous exposure for 30 days to 150 ppm (no liver histopathology carried out). Koizumi et al. (1984) observed dose-related increases in the activity of delta-aminolevulinic acid dehydratase in liver and blood after continuous exposure to trichloroethylene at 50, 400 or 800 ppm for 48 hours or 10 days. Serum glutamic pyruvic transaminase (SGPT) was increased at 800 ppm only. The authors state that the effects occurred without apparent liver injury (post mortem findings not further reported).

Nephrotoxicity

Chakrabarti and Tuchweber (1988) studied the acute nephrotoxic potential of trichloroethylene in male Fischer 344 rats. Groups of six animals were exposed to 0, 1000 or 2000 ppm for 6 hours (nominal concentrations). Monitoring of the exposure chamber concentrations was done with a gas chromatograph. The results of these analyses, however,

were not reported. Urine was collected up to 24 hours after exposure, at which time the animals were killed. The urinary activities of N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyltranspeptidase were determined. Urinary glucose and proteins were also measured, as was serum urea nitrogen. In addition renal cortical slice uptake of p-aminohippurate (PAH; a measure of proximal tubular function) was determined upon sacrifice. Each of the urinary parameters was increased at both exposure levels. An exception was NAG which was increased at 2000 ppm only. The capacity of renal slices to accumulate PAH was significantly reduced at both exposure levels. These results indicate an impairment of both tubular and glomerular function.

In inhalation studies of longer duration in rats (Prendergast et al., 1967; Kjellstrand et al., 1981) only increased kidney weight have been observed (without changes in routine light microscopical histopathology).

Pulmonary toxicity

A series of experiments was carried out in rats and mice to investigate the effect on lung tissue (Odum et al., 1992). This research was carried out to elucidate the mechanism of the apparently species-specific (mouse only) formation of lung tumors. Female rats (Alpk:APfSD) were exposed to 0, 500 or 1000 ppm trichloroethylene for 6 hours and were subsequently sacrificed at 24 hours. Lung cells were studied by light microcopy. No effect was observed (Odum et al., 1992).

Cardiac toxicity

White and Carlson (1979) examined the effect of trichloroethylene on heart rhythm in groups of five epinephrine-treated male Sprague-Dawley rats. Exposure to trichloroethylene lasted one hour during which the animals received increasing intravenous doses of epinephrine until arrhythmia's occurred (maximum dose 4 μg/kg body weight). The influence of alterations in drug metabolism was examined by pre-treatment with liver enzyme inducers and liver enzyme inhibitors. Only a single trichloroethylene concentration of 25,000 ppm (nominal value) was tested in this study (concentration selected based on preliminary experiments in which rats proved rather insensitive to trichloroethylene cardiotoxicity). Trichloroethylene had a very weak effect only, the group pre-treated with the enzyme inhibitor SKF 525A being the only one in which cardiac arrhythmia's could be induced.

3.2.2 Mouse

Hepatotoxicity

In an early study Heim et al. (1966) studied the effect of acute exposure to 18000 ppm trichloroethylene (a narcotic concentration) on normal glycolytic carbohydrate metabolism in the livers of male NMRI mice. After exposure for either 30 or 60 minutes the animals were killed and a number of biochemical parameters of glycolysis were determined. The results indicated a impairment of normal liver intermediary metabolism both after 30 minutes and 60 minutes of exposure. Liver fatty acid content was slightly increased. No histopathology was done in this study.

In a similar study from the same period Ogata et al. (1968) exposed groups of female Cb mice to 800 ppm trichloroethylene for three hours. At 0, 4, 8 and 20 hours after treatment the mice were killed and the livers were excised and weighed. Liver ATP, total lipid and triglycerides were determined. No histopathology was done. ATP contents were decreased and lipids and triglycerides were increased.

Gehring (1968) examined the hepatotoxic potential of several chlorinated solvents in female Swiss-Webster mice relative to their lethal and narcotic potencies. In whole body exposure chambers groups of 9-20 mice were exposed to 5500 ppm for different periods. The number of minutes needed to produce the endpoint in question (lethality, narcosis, hepatotoxicity) was recorded. Hepatoxicity was evaluated by measuring SGPT in blood samples that were obtained at 24 hours after the test started. The study design is not reported clearly and the results for trichloroethylene are reported very briefly only. The results showed that the time needed to produce liver damage was considerably longer than that needed for anesthesia with the durations needed to produce significant SGPT-increases being in close range of the lethal durations.

Kjellstrand et al. (1983a and 1983b) tested for the effect on the liver in seven strains of mice after continuous exposure for 30 days. Increased liver weights were observed in all strains. In NMRI mice additional testing was done to determine the dose response relation with a tested dose range of 37 to 3700 ppm and exposure periods of 30 or 120 days. Some groups were exposed continuously and others intermittently. Increased liver weights and enlarged hepatoctyes and cytoplasmic vacuolization were seen at all dose levels. At 30 or 120 days post-exposure the effects were present to very slight degree only. These results show the greater sensitivity of mice to the action of trichloroethylene on the liver when compared to rats and gerbils (similar tests were carried out by the same authors in these latter two species). The authors suggest that the observed changes are not toxicologically significant but represent a "transient abnormal state".

Neurotoxicity

Complete anesthesia occurred in female white mice after about 5 min of exposure to 12000 ppm and about 10 min to 7000 ppm (Friberg et al., 1953). In another study in female Swiss-Webster mice about 50% of the animals were anesthetized after 46 minutes exposure to 5500 ppm (Gehring, 1968).

Wolff (1976) studied the effect on spontaneous locomotor activity in mice after single inhalation exposure. The tests were carried out in circular exposure chambers with infrared light beams as the device for detecting motor activity. In a first series of experiments groups of 5 AB mice (sex not reported) were tested at measured concentrations of 600, 1010 and 1400 ppm (exposure for 2 hours) with significant reductions being observed at all levels. In a second series male ICR mice were tested at measured concentrations of 120, 692 and 1108 ppm (exposure presumably again 2 hours) with significant reductions in activity at 692 and 1108 ppm and no effect at 120 ppm.

Kjellstrand et al. (1985) exposed male mice (NMRI) to trichloroethylene for one hour (during the night, the active period for mice) and determined their general motor activity using a Doppler radar unit. Control measurements were made during exposure to air. A total of 54 mice was used in this experiment. The distribution of the test animals across dosing groups is not reported clearly. The four highest concentrations were tested first and subsequently lower concentrations were tested with new test animals being taken into the experiment. Test concentrations were: 3600, 2300, 1800, 1200, 1100, 900, 700, 570, 480 and 380 ppm (measured concentrations). At high concentrations motor activity increased initially (during the first five minutes); this effect was gradually lost when the concentration was lowered and at 900 ppm no effect was found. At lower concentrations motor activity was slightly decreased, an effect that was gradually lost below 700 ppm. At the end of the exposure period a rapid change in motor activity was seen with initially hyperactive animals becoming hypoactive. The authors believe that the rate of change in the test concentrations was an important determining factor in this experiment. They consider a direct effect on the nerve cell membrane to be the most probable explanation for the effects.

For mice no subacute or (semi)chronic neurotoxicity studies are available for the inhalation route.

Pulmonary toxicity

A series of experiments was carried out in rats and mice to investigate the effect on lung tissue (Odum et al., 1992). This research was carried out to elucidate the mechanism of the apparently species-specific (mouse only) formation of lung tumors. Female CD-1 mice were exposed to 20, 100, 200, 450, 1000 or 2000 ppm for 6 hours and were sacrificed after 24 hours. The only clinical sign of toxicity reported was mild anesthesia at the higher doses (not specified). Light microscopy on lung cells revealed dose-dependent vacuolization of the Clara cells at all dose concentrations. At 20 ppm the effect was slight with only a small number of cells that were affected; at 200 ppm most Clara cells were affected and at ≥450 ppm the vacuolation was accompanied by pyknosis of the bronchiolar epithelium. At 2000 ppm focal loss of bronchiolar epithelium was evident with exudate in the lumen. Similar investigations for the metabolites chloral and trichloroethanol suggest that the effects seen with trichloroethylene are due to its metabolite chloral (Odum et al., 1992). Similar cell-specific toxicity was also found in by Villaschi et al. (1991) who exposed male B6C3F1 mice to 500, 1000, 2000, 3500, or 7000 ppm for 30 minutes. They sacrificed the animals after 2 or 24 hours or after 2, 5 or 7 days. The lesions were found to be reversible, recovery being complete after 7 days.

As already indicated, this research was aimed at detecting interspecies differences in the pulmonary response to trichloroethylene. As Green (2000), who summarizes the data available on this issue, points out, mouse lung Clara cells have a high capacity for the formation of chloral hydrate from trichloroethylene. Rats have a much lower capacity (27-fold lower, so comparative in vitro studies indicate) and in humans it is much lower still (600-fold lower than in mice, in vitro data suggest). Thus, the pulmonary cytotoxicity seen in mouse after inhalation of trichloroethylene has little relevance for human health.

3.2.3 Rabbit

In a poorly reported acute experiment Truhaut et al. (1972) exposed rabbits (number not reported) to several concentrations of trichloroethylene by direct introduction into the pulmonary tree during variable periods. A concentration of 9250 ppm produced complete anesthesia in about 50 minutes. At 6900 ppm a slight increase in general activity was noted (exposure duration not reported).

In two neurotoxicity studies of longer duration Blain et al. (1992 and 1994) observed changes in visually evoked potentials and in electroretinal responses to flash stimulation determined weekly in rabbits exposed to 350 and 700 ppm for 12 weeks (4 hours/day, 4 days/week). Multiple regression showed that the effects on visually evoked potentials correlated with trichloroethanol concentrations in blood and not with trichloroethylene concentrations in blood. The authors note that this supports the hypothesis (first proposed by Ertle et al., 1972) that trichloroethanol is responsible for the neurotoxic effect by trichloroethylene.

White and Carlson (1979) examined the effect of trichloroethylene on heart rhythm in epinephrine-treated male New Zealand rabbits (2-7 animals per group). Exposure to trichloroethylene lasted one hour during which the animals received increasing intravenous doses of epinephrine until arrhythmia's occurred (maximum epinephrine dose 4 μg/kg body weight). The influence of alterations in drug metabolism was examined by pre-treatment with liver enzyme inducers and with liver enzyme inhibitors. Only a single trichloroethylene concentration of 3000 ppm (nominal value) was tested in this study (concentration selected based on preliminary experiments in which rabbits appeared as a sensitive species for trichloroethylene cardiotoxicity). Cardiac arrhythmia's developed in saline-treated rabbits in response to 2 μg epinephrine/kg body weight after 15 minutes of exposure to trichloroethylene. Rabbits treated with enzyme inhibitors responded to lower doses of epinephrine and had higher concentrations of trichloroethylene in blood. Rabbits treated with the enzyme inducer phenobarbital had fewer arrhythmia's and had lower concentrations of trichloroethylene in blood. Pre-treatment with the inducer Aroclor 1254 had no effect.

In a subsequent experiment (White and Carlson, 1981a) rabbits (n=3 per group) were exposed to 2000, 4000, 6000 or 8000 ppm trichloroethylene for 1 hour, again with intravenous doses of epinephrine as inducer of cardiac arrhythmia's. Arrhythmia's developed at ≥4000 ppm (NOAEL 2000 ppm). Blood concentrations of trichloroethylene were found to increase over all dose levels whereas concentrations of the metabolites trichloroethanol and trichloroacetic acid were similar at ≥4000 ppm (indicating saturation of metabolism). In separate groups that received chloral hydrate (the intermediate in trichloroethylene metabolism) much higher concentrations of trichloroethanol and trichloroacetic acid were found, without arrhythmia's being inducible. This, so the authors conclude, indicates that trichloroethylene rather than its metabolites sensitizes the rabbit myocardium to epinephrine-induced cardiac arrhythmia's. In yet a further experiment the same authors (White and Carlson, 1981b) showed that ingestion of ethanol at 0.5 hour before exposure to trichloroethylene (6000 ppm for 1 hour), potentiated the cardiac effect of trichloroethylene. This potentiation is thought to arise through the competitive

inhibition of the biotransformation of trichloroethylene-derived chloral hydrate to trichloroethanol, resulting in increased concentrations of parent compound in blood.

3.2.4 Gerbil

No acute studies are available. Long-term trichloroethylene neurotoxicity was studied by Haglid et al. (1981) in Mongolian gerbils. They observed ultrastructural changes in brain neurons (Purkinje and Golgi cells) and increased concentrations glial cytoplasmic protein S100 after exposure to 60 or 320 ppm for 3 months (continuous exposure) followed by 4 months without exposure. In a subsequent study (Kyrklund et al., 1983) this group found slight changes in the lipid composition of the cerebral cortex, hippocampus and brain stem after continuous exposure to 50 or 150 ppm for 12 months. The toxicological significance of the changes seen in these studies is unclear.

3.2.5 Dog

Reinhardt et al. (1973) examined the effect of trichloroethylene (and several other industrial solvents) on heart rhythm by studying the effect on epinephrine-induced arrhythmias. Male Beagle dogs (number not reported) were given two intravenous doses of epinephrine, one before and one during a ten-minutes exposure to 5000 or 10,000 ppm trichloroethylene. The test was positive for cardiac sensitization when an arrhythmia developed for the combination of the epinephrine dose with exposure to trichloroethylene (with no response after the pre-exposure epinephrine dose). At 5000 ppm, of the twelve exposures one was positive whereas at 10,000 ppm this was seven out of twelve.

3.3 Developmental/Reproductive Toxicity

Inhalation data on reproductive toxicity are limited. Two studies were reported in which the effect on sperm morphology was examined after subacute exposure. Of the first study, carried out by Beliles et al. (1980) for the NIOSH, no original report is available. In this study male rats (Sprague Dawley) and male mice (CD) were exposed to 0, 100 or 500 ppm for 7 hours/day on five subsequent days. Each group consisted of 12 animals. At 1, 4 or 10 weeks after treatment, groups of 4 animals were sacrificed and their sperm was sampled. No effect on sperm morphology was seen in rats. However the positive control (TEM) also showed no response in rats. In mice the incidence of abnormal sperm was increased at 500 ppm after week 1 and 4 (incidences of 18.9% and 23.5% respectively, versus 6.8% and 8.1% in the control group). At 100 ppm the incidence of abnormal sperm was increased after 4 weeks only (incidence 14.3%). The latter increase may be due to chance since in another control group (from a similar test for tetrachloroethylene) the control incidence was 13.9%.

A second study of similar design was carried by Land et al. (1981). They exposed groups 5, 10 or 15 male mice (strain: C57B1OxC3H) to 0, 200 or 2000 ppm for 4 hours/day on five subsequent days. Sperm samples were taken from the cauda epididymus at 28 days after the first exposure. The percentage of abnormal sperm was increased at 2000 ppm (2.43±0.15 versus 1.42±0.08 in controls) but not at 200 ppm.

The effect on female reproduction after inhalation was studied by Dorfmueller et al. (1979). They exposed female rats to 0 or 1800 ppm for two weeks (6 hours/day, 7 days/week) prior to mating. At day 21 of pregnancy the animals were killed and fetuses were removed for examination. No adverse effect was observed.

The oral reproductive toxicity of trichloroethylene has been extensively studied in rats and mice. This included NTP studies using the RACB (Reproductive Assessment by Continuous Breeding) protocol in rats and mice. In Swiss CD-1 mice significant hepatic and renal toxicity (increased weights and microscopic lesions), reduced sperm motility and increased pup mortality during lactation were seen. No effect on reproductive performance was observed. The effects were seen only at the highest dose level of 700 mg/kg bw/day (NOAEL 300 mg/kg bw/day) (NTP, 1985). In rats (F344) slight reductions in the number of litters and litter size were noted at 150 and 300 mg/kg bw/day. Slight growth retardation and increased weights of liver and kidneys were seen at the same dose levels (NOAEL 75 mg/kg bw/day) (NTP, 1986).

The developmental toxicity was examined in several limited inhalation studies. Schwetz et al. (1975) exposed pregnant rats (Sprague-Dawley) and pregnant mice (Swiss Webster) to 0 or 300 ppm for 7 hours/day from day 6 through day 15 of pregnancy. At day 21 of pregnancy the animals were killed and fetuses were examined. No adverse effects were found. Dorfmueller et al. (1979) also used only one test concentration. They exposed groups of 30 female rats (Long Evans hooded) to 1800 ppm for two weeks prior to mating until day 21 of pregnancy or during pregnancy only. Half of the animals were killed at day 21 of pregnancy with standard examination of fetuses. The other half were kept until the offspring was 100 days old. The activity levels of the offspring were assessed on days 10, 20 and 100 after birth (using automated electronic methods). No effect on litter size, fetal weight and postnatal activity levels was found. Postnatal body weight was slightly decreased in the groups treated before pregnancy. In the group treated during pregnancy there was only a slight increase in minor visceral and skeletal abnormalities. These changes are indicative of developmental delay by trichloroethylene. The Beliles et al. study (mentioned above) included testing for the effect of trichloroethylene on pregnancy in rats and rabbits. Again only one dose level was tested. Groups of 20-28 Sprague Dawley rats were exposed to 0 or 500 ppm for 7 hours/day from day 0-18 of pregnancy or day 6-18 of pregnancy. A further group of rats was exposed for three weeks prior to mating also. No clear evidence of maternal or developmental toxicity was observed. In rabbits the same concentration was tested from day 0–21 or day 7-21 of pregnancy. A further group of rabbits was exposed also for three weeks prior to mating. In 4 fetuses from two litters, both of the group that was treated during pregnancy on day 0-21, hydrocephalus was found. This is suggestive of a teratogenic effect by trichloroethylene, the study-authors concluded, while adding that the evidence is not conclusive. Of this study no original study report was available (Beliles at al., 1980). Yet a further developmental inhalation study was published by Healy et al. (1982), again with only one test concentration. They exposed mated female Wistar rats (n=32) to 0 or 100 ppm for 4 hours/day from days 8 to 21 of pregnancy. The number of females with total litter loss was increased (7/32 versus 2/31 in controls). In addition, fetal weights were decreased and the incidence of minor skeletal variants was increased in the trichloroethylene exposed group.

Oral developmental neurotoxicity studies suggest that via this route trichloroethylene may adversely influence brain development. In a limited rat drinking-water study in Sprague Dawley rats by Isaacson and Taylor (1989), the number of myelinated fibers in the hippocampus was decreased in the offspring at day 21 after birth. This was seen at maternal dose levels of 28 and 56 mg/kg bw/day (estimated from drinking-water consumption levels). In mice Fredriksson et al. (1993) observed a reduced rearing rate (raising of the front legs, resting on haunches) at an age of 60 days after oral application of 50 or 290 mg/kg bw/day on postnatal days 10 until 16.

Another oral developmental rat study indicates that via this exposure route trichloroethylene may induce fetal heart defects. This study was prompted by the observation of an increased risk for these effects in an epidemiological community survey. After exposure of rats via drinking-water before and during pregnancy, increased rates of fetal heart defects were seen at both of the widely spaced dose levels (0.18 and 132 mg/kg bw/day). This increase did not show a clear dose response relation (incidences 8.2 and 9.2% versus 3% in controls) (Dawson et al. 1993).

3.4 Genotoxicity

Numerous studies have been carried out in a variety of test systems, both in vitro and in vivo. These data have been reviewed by ECETOC (1994), IARC (1995), ATSDR (1997) and WHO (2000). For many assays the interpretation of results is hampered by the presence in the tested samples of impurities known to be mutagenic (epoxide stabilizers).

In brief the following results have been observed. In a number of studies in bacteria, purified trichloroethylene was weakly mutagenic in the presence of metabolic activation. In other studies, however, purified trichloroethylene produced no such response. In fungi and yeasts a weak positive response was found when the purified compound was tested with activation, but negative results were also observed, notably also when non-purified trichloroethylene was tested.

In mammalian cells *in vitro*, tests for gene mutations have shown a weak positive response with activation. A single test for chromosome aberrations in mammalian cells *in vitro* did not show an effect.

The *in vivo* mutagenicity studies include several host-mediated assays in mice with yeasts as test organism. In these limited tests (mostly only one dose level tested) again both positive and negative results were found. Trichloroethylene purity could not explain the conflicting results.

Micronucleus tests and cytogenetic tests for chromosome aberrations in vivo have been carried out in mice and rats. A consistent finding in these studies was the absence of chromosome aberrations in both bone marrow cells and lymphocytes or splenocytes. Micronuclei in bone marrow were found by some investigators but not by others. In a study by Kligerman et al. (1994) the incidence of micronuclei in bone marrow was increased slightly but significantly in rats after single inhalation exposure to 5, 500 and 5000 ppm for 6 hours. The incidence of chromosome aberrations in bone marrow showed no increase. In the same study

trichloroethylene inhalation produced no effect in mice. The study-authors suggest the induction of micronuclei without chromosome aberrations, as seen in rats, to be due to spindle effects.

An in vivo test for gene mutations was done in a transgenic mouse model called Muta-mouse (Douglas et al., 1999). In this study no mutations of the LacZ-gene in liver, lungs and bone marrow were observed after inhalation of 0, 200, 1000 or 3000 ppm trichloroethylene 6 hours/day for 12 days. This assay has not been fully validated yet, but the result nevertheless indicates that trichloroethylene is not an in vivo mutagen.

The results of a limited number *in vivo* tests for effects on germ cells (carried out in mice and rats) were inconclusive. In the *Drosophila* sex-linked recessive lethal assay trichloroethylene produced no effect.

In studies for DNA damage in mice and rats *in vivo*, trichloroethylene induced single strand breaks in liver DNA. This effect was found by some investigators but other investigators found no evidence for DNA damage. A result potentially relevant for the evaluation of the hepatocarcinomas seen in mice is the finding that trichloroethylene produced an increased rate of hepatic DNA synthesis in mice but not in rats. *In vivo* studies in mice and rats have shown a very low level of binding of trichloroethylene to DNA in all tissues examined (ECETOC, 1994; IARC, 1995; ATSDR, 1997; WHO, 2000).

In conclusion, the available genotoxicity data for trichloroethylene do not show a consistent pattern. The results indicate that if trichloroethylene has any mutagenic potential at all that potential is very low. Further, the results suggest that exposure to trichloroethylene produces numerical chromosome aberrations (aneuploidy) *in vivo*, which effect may be due to the metabolite chloral hydrate, a known aneugen.

In a recent review by Moore and Harrrington-Brock (2000) the genotoxicity data for the different trichloroethylene metabolites are summarized. For chloral hydrate its aneugenic potential was clearly demonstrated in vitro whereas in vivo several groups of investigators found an effect (i.e. presence of micronuclei in sperm or bone marrow) whereas other groups found no effect. Chloral hydrate was also positive for point mutations and chromosome aberrations in vitro. The in vivo data for these endpoints however were inconclusive. The conclusion for this compound was that its genotoxic activity is a high-dose effect. This applies also to its aneugenic potential.

The metabolite dichloroacetic acid was mutagenic in vitro. In vivo the compound had a low potential for inducing micronuclei and also for mutations in the liver (in the Lac I locus of the Big Blue Mouse). Thus dichloroacetic acid is a mutagen but its potency in vivo is very low only. For another genetic endpoint, i.e. DNA strand breaks, dichloroacetic acid was positive in liver cells in both rats and mice in vivo with the effect being stronger in mice than in rats.

For trichloroacetic acid no or only very weak mutagenic activity was found in vitro; in vivo results were conflicting. For trichloroethanol only a negative result in bacteria is available (no further data). Two minor metabolites, both formed through the glutathione pathway of trichloroethylene biotransformation, are *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-(1,2-dichlorovinyl)-glutathione (DCVG). DCVC and DCVG produce point mutations in bacteria (the Salmonella typhimurium Ames-test). In the latter assay DCVC was the most potent mutagen of

all trichloroethylene metabolites. Further data on the genotoxicity of these two metabolites are lacking and therefore no further conclusions can be drawn as to the genotoxic risk that may arise as a consequence to their formation in trichloroethylene biotransformation (Moore & Harrington-Brock, 2000).

3.5 Carcinogenicity

Many animal bioassays haven been performed with trichloroethylene. These data have been reviewed by IARC (1995) and ATSDR (1997). The following tables give an overview of the relevant studies and their results. Several of the studies show limitations in study design, reducing their value for the evaluation of trichloroethylene carcinogenicity. Major deficiencies are indicated in the table. In the older studies the tested samples contained impurities that are known to produce carcinogenicity in animals and the positive results (tumors in liver and lungs) have been attributed to the action of the impurities and not that of trichloroethylene. In subsequent studies with purified trichloroethylene, however, similar results were found.

Table 3: Inhalation Carcinogenicity Bioassays for Trichloroethylene

Species & strain	Treatment	Observed increased[a] tumor incidence	Reference
Mouse (m,f) B6C3F1	0, 100, 300 and 600 ppm, 7 hours/day, 5 days/week for 78 weeks; observation for rest of lifespan; trichloroethylene purity 99.9%, epoxide-free	Pulmonary adenomas in females only (4/90, 6/90, 10/90 & 15/90) Hepatomas (not specified) in females (3/90, 4/90, 4/90 & 9/90) and males (14/90, 19/90, 27/90 & 21/90)	Maltoni et al. (1988)
Mouse (m,f) Swiss	Idem	Pulmonary adenomas and carcinomas in males only (10/90, 11/90, 23/90 & 27/90); Hepatomas (not specified) in males only (4/90, 2/90, 8/90 & 13/90)	Maltoni et al. (1988)
Mouse (m,f) NMRI	0, 100 & 500 ppm, 6 hours/day, 5 days/week for 78 weeks; observation until week 130;trichloroethylene purity, epoxide-free	Lymphomas in females only (9/29, 18/28 & 17/30) (authors note high spontaneous incidence of this type of tumor in this strain of mice)	Henschler et al. (1980)
Mouse (f) ICR	0, 50, 150 & 450 ppm, 7 hours/day, 5 days/week for 104 wks; observation until week 107; purity 99.8% + 0.02% benzene + 0.02% epichlohydrin	Pulmonary adenocarcinomas (1/49, 3/50, 8/50 &7/46)	Fukuda et al. (1983)
Rat (m,f) Wistar	0, 100 & 500 ppm 6 hours/day, 5 days/week for 78 weeks, observation until week 156; trichloroethylene purified, epoxide-free	No increase observed	Henschler et al. (1980)
Rat (m,f) Sprague-Dawley	Identical to study in ICR mice given above	No increase observed	Fukuda et al. (1983)
Rat (m,f) Sprague-	0, 100, 300 & 600 ppm, 7	Renal adenocarcinomas in males	Maltoni et al.

Dawley	hours/day, 5 days/week for 104 weeks; observation for rest of lifespan; purity 99.9% epoxide-free	only & at high dose only (4/130 versus 1/130 in controls) (renal tubular cell cytomegaly & karyomegaly in medium-& high-dose males); Leydig cell tumors in testis (6/135, 16/130, 30/130 & 31/130)	(1986)
Hamster (m,f) Syrian	Identical to study in NMRI mice given above	No increase observed	Henschler et al. (1980)

[a] The presentation of numbers of tumors: control incidence followed by low to high dose incidences.

Table 4: Oral Carcinogenicity Bioassays for Trichloroethylene

Species & strain	Treatment	Observed increased[a] tumor incidence	Reference
Mouse (m,f) B6C3F1	Gavage, gavage 5 days/week; 1169 & 2339 mg/kg bw (m), 869 & 1739 mg/kg bw (f) for 78 wks, sacrifice after 90 weeks; trichloroethylene stabilised with 0.09% epichlorohydrin + 0.19% Epoxybutane	Hepatocellular carcinomas in males (1/20, 26/50 & 31/48) and females (0/20, 4/50 & 11/40); marginal increase in lung adenomas in females (1/20, 4/50 & 7/47) (in males 0/20, 5/50 & 2/48)	NTP (1976)
Mouse (m,f) B6C3F1	Gavage, 5 days/week, 0 & 1000 mg/kg bw for 103 weeks; trichloroethylene epichlorohydrin-free	Hepatocellular carcinomas in males (8/48 & 30/50) and females (2/48, 13/49); (toxic effects: renal cyto-megaly in all TRI-treated males & females)	NTP (1990)
Mouse (m,f) Ha:ICR	Gavage 5 days/week; 0, TWA 1900 mg/kg bw (m), TWA 1400 mg/kg bw (f) for 78 weeks; TRI +/- epichlorohydrin & epoxybutane	Papillomas & carcinomas in forestomach in groups given trichloroethylene+epichlorohydrin+ epoxybutane; no increase observed in groups that received pure trichloroethylene	Henschler et al. (1984)
Rat (m,f) Osborne-Mendel	Gavage 5 days/week; 549 & 1098 mg/ kg bw for 78 weeks with observation until week 110	No increase observed but value of study reduced because survival was decreased due to toxic nephropathy (both sexes, both dose levels)	NTP (1976)
Rat (m,f) F344/N	Gavage 5 days/week; 0, 500 & 1000 mg/kg bw for 104 wks; trichloroethylene purity >99.9%, epoxide-free	Renal tubular adenocarcinomas in males only (0/33, 0/20 & 3/16); nephropathy in all treated groups (m&f), NTP considers study inadequate (survival too low)	NTP (1990)
Rat (m,f) Sprague-Dawley	Gavage, 5 days/week; 0, 50 & 250 mg/kg bw for 52 wks; observation for rest of lifespan; trichloroethylene purity >99.9%, epoxide-free	No increase observed (cytokaryomegaly in renal tubular cells in 250 mg/kg males only)	Maltoni et al. (1986)
Rat (m,f) ACI, August, Osborne Mendel, Marshall	Gavage, 5 days/week; 0, 500 & 1000 mg/kg bw for 104 wks; trichloroethylene purity	Study judged as inadequate by NTP; nevertheless to be noted: in Osborne Mendel rats: renal tubular	NTP (1988)

	>99.9%, epoxide-free	cell adenomas in males only (0/50, 6/50 & 1/50); in Mar- shall rats: interstitial cell tumors (17/46, 21/48& 32/48)	

[a] The presentation of numbers of tumors: control incidence followed by low to high dose incidences.

As the table above shows, liver adenomas and carcinomas were seen in two mouse inhalation studies (in strains B6C3F1 and Swiss) and two mouse oral studies (both in B6C3F1). Pulmonary adenomas and carcinomas were observed in three mouse inhalation studies (B3C6F1, Swiss and ICR) and lymphomas in one mouse inhalation study (NMRI). In rats an increase in interstitial testicular tumors was observed after inhalation in one study (in Sprague-Dawley rats) and after oral application in another (Marshall rats). Slight but significant increases of renal tumor incidences were seen in two oral rat studies (F344/N, Osborne Mendel) and in one rat inhalation study (Sprague Dawley).

Of these tumors, the significance of the malignant lymphomas seen in one mouse study only, is questionable in view of the lack of a clear dose-response for this effect, the high spontaneous incidence in this particular strain of mice and the absence of a similar effect in all other studies. The interstitial testicular tumors in rats were seen in strains not known to be particularly sensitive for this kind of tumor and therefore are judged to be relevant for human health (conclusion in agreement with WHO, 2000). Mechanistic data on how trichloroethylene induces these tumors are lacking but the genotoxicity data (summarized in the previous section) indicate a non-genotoxic mode of action.

The tumors in mouse liver, mouse lung and rat kidney have led to additional mechanistic studies on the mode of action through which these tumors arise and possible interspecies differences therein. The liver tumors in mice are probably due to the high levels of trichloroacetic acid (TCA) and dichloroacetic acid (DCA) formed in this species at high trichloroethylene exposure levels. Rats have a much lower ability for this metabolic conversion. Limited in vitro tests in human hepatocytes indicate that humans resemble rats in this respect. In the formation of the mouse liver tumors peroxisome proliferation and increased cell proliferation are thought to play a role (non-genotoxic mechanism). There is some evidence (from comparative in vitro studies) that human hepatocytes are much less sensitive to peroxisome proliferation by TCA than are mouse and rat hepatocytes. Thus, the weight of evidence indicates that the liver tumors seen in mice are not significant for humans. The mode of action for these tumors probably is non-genotoxic. These are the conclusions of WHO (2000), Bull (2000) and Moore and Harrington-Brock (2000). It should however be noted that these reviews were focused on environmental exposures to relatively low concentrations of trichloroethylene. Whether the conclusions also apply for scenarios involving acute exposure of humans to high concentrations of trichloroethylene, is uncertain.

The lung tumors in mice, so the evidence summarized by Green (2000) indicates, are due to cytotoxicity, increased cell division and aneuploidy caused by the metabolite chloral hydrate that is formed in the Clara cells. Mouse lung Clara cells have a high capacity for the formation of chloral hydrate from trichloroethylene. Rats, in which species no lung tumors were seen, have a much lower capacity (27-fold lower, so comparative in vitro studies indicate) and in humans it

is much lower still (600-fold lower than in mice, in vitro data suggest). Thus, these tumors most likely constitute a species-specific finding with little relevance for human health.

The possible modes of action for the kidney tumors in rats were reviewed by Lash et al. (2000b). Most of the available experimental data are focused on the hypothesis that formation of dichlorovinyl cysteine (DCVC) via the glutathione pathway of trichloroethylene biotransformation leads to tumor formation. In the kidneys DCVC would be converted by β-lyase to reactive metabolites known to be mutagenic and nephrotoxic. The presence of these reactive metabolites themselves has not been demonstrated in animals or humans but N-acetyl-DCVC, the ultimate metabolite of an alternative route in the glutathione pathway, has been detected in urine of mice, rats and humans. The data indicate that in all species glutathione conjugation is a minor biotransformation route only but, as Lash et al. (2000a) point out, such a minor route can nevertheless be toxicologically relevant. A further point of unclarity is that no correlation could be established between the rates of glutathione conjugation and activation of DCVC by β-lyase and kidney toxicity and tumorigenicity in the different species. Another possible mode of action for the renal tumors is the increased excretion of formic acid in urine that would lead to nephrotoxicity and eventually to tumors. Whether this mode of action occurs in humans is unknown. In sum, at present no definitive conclusion can be drawn concerning the mode of action for the renal tumors.

IARC (1995) concluded that the results of the available animal bioassays provide *sufficient evidence* for carcinogenicity to animals.

Inhalation quantitative cancer risk estimates have been published by the WHO (2000). These estimates were done using the linearized multistage model, the model formerly used by the US-EPA. The Leydig cell tumors in rats were the most sensitive endpoint with an extra cancer risk of 4.3×10^{-7} per microgram/m^3 of lifetime exposure. New preliminary estimates – as part of the trichloroethylene cancer risk assessment that is in progress at the US-EPA - are presented by Rhomberg (2000). This exercise included the use of relevant PBPK-models. For the renal tumors as observed in rats after inhalation (the study by Maltoni et al., 1986), the estimated extra cancer risks varied from 2.2×10^{-10} to 2.8×10^{-8} per microgram/m^3 of lifetime exposure. These risk values were derived by linear extrapolation from either the modeled ED_{01} (the dose producing a 1% increase in tumor risk under the bioassay conditions) or the LED_{01} (the 95% lower confidence limit of this dose). Either the external dose was used as the dose metric, or the kidney reactive thiol dose. Similarly, for the lung tumors after inhalation extra cancer estimates of 6.5×10^{-10} to 7.5×10^{-7} per microgram/m^3 of lifetime exposure were produced. Of these, the PBPK-produced risk estimates were $\leq 1.6 \times 10^{-8}$. In the latter estimates the lower chloral production in humans (compared to mice) was taken into account and thus they are probably more realistic than the estimates based on the external dose. The inhalation liver tumor risk estimates presented by Rhomberg were derived by route to route extrapolation using a human inhalation pharmacokinetic model to estimate the human liver dose of TCA or DCA. Thus, inhalation liver cancer risk estimates of 4.2×10^{-8} to 1.6×10^{-4} per microgram/m^3 of lifetime exposure were generated. The corresponding risk estimates based on externally administered dose ranged from 3.1×10^{-7} to 8.9×10^{-6}.

It should be noted that the above cancer risk estimates are all linear estimates. If trichloroethylene's mode of action is non-genotoxic, as the genotoxicity data indicate, a linear extrapolation probably overestimates the real cancer risk. This applies to the lung, liver and testes tumors. For the renal tumors, however, genotoxicity might still play a role since they could be due to the action of kidney-specific genotoxic metabolites.

3.6 Summary

The acute lethality of trichloroethylene was examined in rats and mice. The 4-hour LC_{50}-values in these species were 12500 and 8450 ppm, respectively. Six-hour LC_{50}-values (rats, mice) were 5918 and 5857 ppm, respectively. The 1-hour LC_{50} in rats was about 26000 ppm. Adams et al. (1951) determined no-effect-times for mortality in rats. In their broadest time series, that with a test concentration of 20,000 ppm, there were no deaths after 0.3 hours of exposure whereas after 0.4 hours there were 2/20 deaths. Similarly, at 9600 ppm there were no deaths after 0.8 hours (no-effect) and 3/20 deaths after 1.0 hours (lowest effect). From the study by Friberg et al. (1953) a mouse NOAEL for mortality of 4600 ppm for a 4-hour exposure, can be derived (LOAEL 5350 ppm).

The main symptom of intoxication was depression of the nervous system (unsteady gait, stupor, narcosis). Post mortem results were not reported in most lethality studies. Where available they indicate only slight liver effects.

Several acute animal neurotoxicity studies are available. These studies revealed effects on neurohavioral performance (swimming-behavior, eye movements, shock avoidance, signal detection behavior, visual function, T-maze alternation, ototoxicity). Important experiments with regard to the concentration time relationship for acute trichloroethylene neurotoxicity are those by Bushnell (1997) and Crofton and Zhao (1997). The results of these rat studies are summarized in Boyes et al. (2000). With signal detection and reaction speed as endpoints and exposure durations from 0.33 to 1.0 hours, it was observed that the effect of trichloroethylene was not constant across different CxT combinations. Concentration contributed more to the effect than did duration. For the formula $C^n xT$ empirical values for n were derived, i.e. 2.2 for signal detection and 7.1 for reaction speed. However, due to the tolerance that the test animals developed during the study, the real values of n will be somewhat higher. The NOAEL for signal detection was 800 ppm (1 hour exposure) with an LOAEL of 1200 ppm (0.67 hour exposure). The NOAEL for response time was 2000 ppm (1 hour exposure) with an LOAEL of 2400 ppm (0.67 hour exposure). For the induction of hearing-loss in rats after single exposure to trichloroethylene for six hours, a benchmark concentration for a 15 dB loss was derived of 6218 ppm. The corresponding 95% lower bound on this concentration was 5223 ppm.

Limited acute studies in rats indicate liver damage after exposure for 4 hours to 5000 ppm or to about 10,000 ppm for 2 hours. The NOAEL for this effect is not known but in an old study of longer duration (i.e. 5 weeks) no histological liver damage was observed after exposure to 3000 ppm (7 hours/day, 5 days/week). The potential of trichloroethylene for inducing cardiac sensitization was studied in limited studies in rats, rabbits and dogs. A low potential was observed in rats with only a slight effect at 25000 ppm. In rabbits the LOAEL was 3000 ppm (exposure for 15 minutes) and the NOAEL 2000 ppm (exposure for 1 hour). In dogs the LOAEL

for cardiac sensitization was 5000 ppm for 10 minutes (NOAEL unknown).

Animal data on the reproductive toxicity of trichloroethylene after inhalation are too limited for drawing conclusions. Via the oral route, however, the compound was shown to possess only a low potential for disrupting reproductive function. Limited developmental studies in rats suggest that trichloroethylene when inhaled throughout pregnancy may delay development. The result of one rabbit study suggests teratogenic potential but the evidence is not conclusive.

Trichloroethylene has low mutagenic potential, so the available data indicate. The compound induces numerical chromosome aberrations (aneuploidy) in vivo, an effect due to the metabolite chloral hydrate that is a known aneugen. From the available animal carcinogenicity data IARC concluded that there is *sufficient evidence* for carcinogenicity to animals. However, according to the results of mechanistic studies some of the tumor types seen, are less relevant for humans. This applies to the lung and liver tumors in mice. The renal tumors (seen in low incidences in some rat studies), however, may be relevant for humans and the same goes for the rat testicular tumors. Based on the genotoxicity results, the most likely mode of action for trichloroethylene tumorigenicity is non-genotoxic. For the renal tumors, however, this remains uncertain because potentially genotoxic metabolites may be formed in this organ.

Inhalation quantitative cancer risk estimates have been published by the WHO (2000). The Leydig cell tumors in rats were the most sensitive endpoint with an extra cancer risk of 4.3 x 10^{-7} per microgram/m^3 of lifetime exposure. Rhomberg (2000) has presented preliminary estimates varying from 2.2x10^{-10} to 1.6x10^{-4} per microgram/m^3 of lifetime exposure. Specifically for the renal tumors, extra cancer risks varied from 2.2 x 10^{-10} to 2.8 x 10^{-8} per microgram/m^3 of lifetime exposure (linear extrapolation).

4. SPECIAL CONSIDERATIONS

4.1 Metabolism and Disposition

Trichloroethylene biokinetics have been studied extensively in both experimental animals and humans. Comprehensive reviews of these data are available (IARC, 1995; ATSDR, 1997; Lash et al. 2000a).

Due to its high volatility and lipophilicity trichloroethylene is readily absorbed in the lungs. In rats during single inhalation exposure for 2 hours (50 or 500 ppm) uptake was above 90% during the first 5 minutes and decreased over the next 30 minutes to near steady-state levels of about 70% (Dallas et al., 1991). Several human volunteer studies (Bartonicek, 1962; Ahlmark and Forssman, 1951; Nomiyama & Nomiyama, 1974a, 1977; Åstrand and Övrum, 1976; Monster et al., 1976 and 1979; Vesterberg et al., 1976) indicate pulmonary uptake percentages varying from 28 to 80%. Test concentrations in these studies ranged from 50 to 370 ppm and exposure periods from 30 minutes to 5 hours, with each subject being exposed more than once in the course of the test series. In two experiments (Åstrand and Övrum, 1976; Monster et al., 1976) it was observed that bodily exercise increases the absolute amount of

pulmonary uptake of trichloroethylene (the percentage uptake, however, declined).

On the distribution of trichloroethylene few human data are available. The compound's anesthetic properties indicate that it (and its metabolites) readily crosses the blood-brain barrier. Animal data and PBPK-modeling indicate distribution primarily into richly perfused tissues (including liver, kidneys, lungs, brain). The compound has a preference for fatty tissues from which it is subsequently released. The latter process is relatively slow and thus will prolong exposure of target organs after single exposure.

Concentrations of the parent compound in blood after single exposure are higher in rats than in mice. In rats at the end of a 4-hour exposure to 529-600 ppm, in blood 25.8-35.5 microgram/ml was present whereas in mice concentrations of up to 7.3 microgram/ml were observed after 368-748 ppm for 4 hours. Levels of oxidative metabolites of trichloroethylene showed a reciprocal pattern with higher concentrations in mice (Fisher et al., 1991). This difference reflects the higher capacity of mice for oxidative metabolism of trichloroethylene, a feature observed in many experiments. Limited human data and animal data indicate that trichloroethylene crosses the placenta (IARC, 1995; Lash et al., 2000a).

Concentrations of trichloroethylene in blood after inhalation exposure were measured in some volunteer studies. Monster et al.(1979) measured a mean concentration of about 1.5 mg/liter (estimated from graphical presentation of data) in five volunteers at the end of an exposure to 70 ppm for 4 hours. The mean concentration of trichloroethanol in blood in these subjects was about 3.5 mg/litter. Sato et al. (1977) exposed four males to 100 ppm trichloroethylene for 4 hours and measured a trichloroethylene concentration in blood of about 2 mg/litter at the end of exposure (concentration estimated from graphical presentation of data). Åstrand and Övrum (1976) exposed three groups five males to 100 to 200 ppm trichloroethylene for several periods of 30 minutes, with and without a work load. At rest mean trichloroethylene concentrations in arterial blood were 1.1-1.3 mg/liter and 1.3-2.1 mg/liter at 100 and 200 ppm, respectively at the end of the exposure. With a 50 watt workload mean concentrations of 2.7-3.3 mg/liter were observed at 100 ppm (highest values in this range seen in later exposure series) and 5.2-6.0 mg/liter at 200 ppm. With work loads of 100 and 150 watt mean concentrations up to 9.0 mg/liter were observed at 200 ppm (not tested at 100 ppm). In a similar experiment by Vesterberg et al. (1976) only trichloroethanol concentrations were measured in blood. After a 30 minute exposure of the five male volunteers to 100 ppm trichloroethylene, with the subjects at rest during the exposure, trichloroethanol concentrations in blood had a mean of 0.8 mg/liter. After a further exposure to 100 ppm for 90 minutes with a 50 watt work load trichloroethanol concentrations in these subjects had risen to a mean of 2.2 mg/liter. At 200 ppm a mean concentration in blood of 1.4 mg/liter was observed after exposure for 30 minutes, when the five subjects remained at rest. After a further exposure to this concentration for 90 minutes during which the subjects received an increasing work load (50 to 150 watt), the mean rose to 3.1 mg trichloroethanol/liter. Müller et al. (1975) exposed six male volunteers to 100 ppm trichloroethylene for 6 hours and measured blood trichloroethylene and trichloroethanol on several occasions during exposure and up to 20 hours afterwards. This was done both with and without simultaneous alcohol ingestion (i.e. 0.45 g/kg bw at 15 minutes before exposure and 0.037 g/kg at 15 minutes intervals during exposure). Trichloroethylene in blood steadily rose

during exposure with a highest value of about 1.5 mg/liter after 6 hours when no alcohol was given (concentration estimated from graphical presentation of data). With alcohol ingestion the peak rose to about 2.7 mg/liter. The corresponding trichloroethanol concentrations were about 4.5 mg/liter (without ethanol) and about 2.0 mg/liter (with ethanol). In another experiment by this research group, three groups of five or six male volunteers were exposed to trichloroethylene for 6 hours/day on 5 consecutive days, either constantly at 50 ppm, at 250 ppm for 12 minutes/hour (average 50 ppm) or at 100 ppm constantly. Blood concentrations of trichloroethanol were measured three times per day. The concentration directly after the first exposure were about 1.7 mg/liter in the two 50 ppm groups and about 3.2 mg/liter in the 100 ppm group (concentrations estimated from graphical presentation of data). On subsequent days of exposure these levels rose to about 2.0-2.4 mg/liter in the 50 ppm group and to about 5.0 mg/liter in the 100 ppm group. In additional experiment in two male volunteers an oral dose of 15 mg/kg bw chloral hydrate was given (a dose known to be hypnotic) and trichloroethanol in blood was measured, showing a peak concentration of about 7 mg/liter (Ertle et al, 1972). Konietzko et al. (1975a) measured concentrations of trichloroethanol in blood of twenty male volunteers during exposure to 95 ppm trichloroethylene for 4 hours. At the end of exposure they found a mean concentration of about 3 mg/liter (estimated from graphical presentation). A much more recent study was that by Pleil et al. (1998). They exposed six volunteers (3 males, 3 females) to 100 ppm trichloroethylene for 4 hours and measured blood concentrations of the parent compound on several occasions throughout exposure and up to 20 hours afterwards. At the end of the exposure period the mean concentration blood was 1.437 ± 0.110 mg/l.

After absorption into the body trichloroethylene is metabolized but a part stays unmetabolized and is exhaled unchanged. The volunteer studies indicate that between 10 and 28% of the absorbed dose is excreted in this way. There are two important pathways in trichloroethylene biotransformation. The major route is oxidative conversion by cytochrome P-450 mixed-function oxygenases, which takes place mainly in the liver. Conjugation with glutathione, also in the liver, is the first step of an alternative, quantitatively minor biotransformation route. The oxidative pathway leads to trichloroethanol and trichloroacetic acid, both of which are excreted mainly in urine. The formation of these metabolites has been demonstrated in rodents and humans. In mice dichloroacetic acid is also formed; the evidence for dichloroacetic acid as a metabolite in rats and humans is inconclusive. If the first step of trichloroethylene oxidation indeed involves the formation of an active epoxide, as was previously thought, is uncertain. Chloral hydrate, the compound presumed to be formed from the epoxide, is an established intermediary in trichloroethylene biotransformation in both humans and rodents. But there is evidence that indicates that chloral hydrate arises through chlorine-migration in an oxygenated trichloroethylene-P450 transition state, without an active epoxide being involved. Chloral hydrate, once formed, is slowly reduced to trichloroethanol, part of which is subsequently oxidized to trichloroacetic acid. Some of the trichloroethanol is conjugated to its glucuronide. Direct conversion of chloral hydrate to trichloroacetic acid also occurs (IARC, 1995; Lash et al., 2000a).

Marked interspecies differences in oxidative biotransformation have been uncovered. Mice have a much higher oxidative capacity compared to rats. In vitro data indicate that humans have an even lower capacity for oxidative metabolism than rats (Lash et al., 2000a). Fisher et al.

(1991) examined the biotransformation rates in rats by experiment (single exposure for 4 hours) and by PBPK-modeling and concluded that in rats metabolism is saturated at 500-600 ppm. The rat PBPK-model of Boyes et al. (2000), however, indicated saturation of metabolism already at 200 ppm (single exposure, duration not indicated). Metabolism in mice is expected to be saturated only at higher exposure concentrations, but the threshold concentration is unknown. In humans no saturation of metabolism was detectable at concentrations up to 315 ppm, (single exposure for 3 hours), so the results of two volunteer studies show (Nomiyama and Nomiyama, 1977; Ikeda, 1977).

Interspecies differences also exist in the pulmonary metabolism of trichloroethylene after inhalation exposure. Mouse lung Clara cells have a much higher capacity for the formation of chloral hydrate than have rats. In vitro data indicate that humans have an even lower capacity than rats. The higher rate of chloral hydrate formation in mice leads to cytotoxicity already at very low exposure levels in this species (LOAEL 20 ppm, single exposure). These lesions are reversible (Villaschi et al, 1991; Odum et al.,1992; Green, 2000).

A quantitatively minor, but toxicologically possibly significant, metabolic pathway for trichloroethylene is the glutathione pathway. This involves formation of S-(1,2-dichlorovinyl)glutathione (DCVG) in the liver, which is subsequently converted to S-(1,2-dichlorovinyl)-L-cysteine (DCVC), a compound known to be nephrotoxic in several mammalian species. In the kidneys DCVC is either bioactivated by β-lyases (present in the epithelium of the renal tubules) or deactivated by N-acetyltranferase. The bioactivation leads to chlorothioketene and thioacyl chloride, which are reactive metabolites that may produce cytotoxicity and genotoxicity. Deactivation of DCVC leads to N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine. The occurrence of the glutathione pathway has been confirmed in rats and humans (detection of urinary N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine, DCVG in blood). As of yet no definitive answer can be given to the question if conversion of trichloroethylene through the glutathione pathway is the mechanism responsible for the kidney tumors that have been observed after exposure to trichloroethylene (WHO, 2000; Lash et al., 2000a and 2000b).

Various research groups have studied the elimination kinetics of trichloroethylene and its major metabolites, trichloroethanol and trichloroacetic acid in humans (Ertle et al., 1972; Monster et al., 1976 and 1979; Nomiyama and Nomiyama 1971, 1974 and 1977; Vesterberg et al., 1976, Sato et al., 1977). The excretion of unchanged trichloroethylene in humans was studied by Sato et al. (1977), who exposed their volunteers (4 males) to 100 ppm for 4 hours. The trichloroethylene concentration in blood at the end of the exposure was about 2 mg/L. The concentration of the compound in breath in the period after exposure was directly proportional to the concentration in blood. The elimination from tissues was best described by a three-compartment model, composed of richly perfused tissues ($t_{½}$ = 2-3 minutes), lean body mass ($t_{½}$ about 30 minutes) and fat-rich tissues ($t_{½}$ = 3.5-5 hours). Similar patterns of elimination have been observed in other volunteer studies. For the elimination of the trichloroethanol and its glucuronide from blood half lives of 8-12 hours were found after single or repeated exposure. Elimination of trichloroacetic acid from blood was considerably slower with half lives of 70-100 hours.

1

2 **Table 5: Blood values of trichloroethylene and trichloroethanol from inhalation human volunteer studies**

EXPOSURE	PARENT COMPOUND AT END OF EXPOSURE	TRICHLOROETHANOL (AT END OF EXPOSURE)	REFERENCE
Six males, 50 ppm for 4 hrs	About 1.5 mg/liter	About 3.5 mg/liter	Monster et al. (1979)
Four males, 100 ppm for 4 hrs	About 2 mg/liter	Not determined	Sato et al. (1977)
Five males, 100 ppm for 30 minutes periods, at rest	About 1.1-1.3 mg/liter	Not determined	Åstrand and Övrum (1976)
Idem, with 50 Watt workload	About 2.7-3.3 mg/liter	Not determined	Åstrand and Övrum (1976)
Idem, 200 ppm, at rest	About 1.3-2.1 mg/liter	Not determined	Åstrand and Övrum (1976)
Idem, with 50 Watt workload:	About 5.2-6.0 mg/liter	Not determined	Åstrand and Övrum (1976)
Idem, with 150 watt workload	About 9.0 mg/liter	Not determined	Åstrand and Övrum (1976)
Five males, 100 ppm for 30 minutes, at rest	Not determined	0.8 mg/liter	Vesterberg et al. (1976)
Five males, 100 ppm for 90 minutes, with 50 Watt workload	Not determined	2.2 mg/liter	Vesterberg et al. (1976)
Five males, 200 ppm for 30 minutes, at rest	Not determined	1.4 mg/liter	Vesterberg et al. (1976)
Five males, 200 ppm for 90 minutes, with 50-150 Watt workload:	Not determined	3.1 mg/liter	Vesterberg et al. (1976)
Six males, 100 ppm for 6 hours	About 1.5 mg/liter	About 4.5 mg/liter	Müller et al. (1975)
Six males, 100 ppm for 6 hours, with alcohol ingestion	About 2.7 mg/liter	About 2.0 mg/liter	Müller et al. (1975)
Five-six males, 50 ppm, 6 hours/day for 5 days	Not determined	About 1.7 mg/liter after first exposure, on later days gradually increasing to 2.0-2.4 mg/liter	Ertle et al. (1972)
Five-six males, 100 ppm, 6 hours/day for 5 days	Not determined	About 3.2 mg/liter after first exposure, on later days gradually increasing to about 5.0 mg/liter	Ertle et al. (1972)
Twenty males, 95 ppm for 4 hours	Not determined	About 3 mg/liter	Konietzko et al. (1975a)
Three males and three females, 100 ppm for 4 hours	1.437 ± 0.110 mg/l	Not determined	Pleil et al. (1998)

3

Alcohol consumption may influence trichloroethylene metabolism. Müller et al. (1975) studied the effect of simultaneous alcohol consumption on trichloroethylene biokinetics in male volunteers. The subjects were either exposed to 50 ppm 6 hours/day for 5 days or to 100 ppm for 6 hours (single exposure). When this was done simultaneously with oral ingestion of alcohol, levels of parent compounds in blood and urine were higher and those of the oxidative metabolites were lower. This indicates competitive inhibition for metabolism between alcohol and trichloroethylene. However, when the alcohol dose is given before exposure to trichloroethylene - with the intervening period being long enough -, increased metabolism of trichloroethylene occurs due to liver enzyme induction. The latter was found in rat studies (e.g. Sato et al., 1981).

Several physiologically-based pharmacokinetic (PBPK) models have been developed for the uptake, distribution and metabolism of trichloroethylene. Fisher (2000) provides a review of available models. One of the early models was that of Andersen et al. (1987) that described the blood and tissue time courses of trichloroethylene in rats. Later models also included the major metabolite trichloroacetic acid (TCA). Fisher et al. (1989, 1990) modeled the kinetics of trichloroethylene and TCA in pregnant rats and in lactating rats and nursing pups for oral and inhalation exposure. Several human models have been developed. Allen and Fisher (1993) and Fisher and Allen (1993) developed such a model for trichloroethylene and TCA and used it for estimating liver cancer risk from the mouse bioassay data. More recent models also included other metabolites. The mouse model of Abbas and Fisher (1997) and Greenberg et al (1999) includes chloral hydrate, trichloroethanol (TCOH) and its glucuronide and dichloroacetic acid. The human model of Fisher et al. (1998), for which a new volunteer study was carried out, includes TCA and TCOH. Clewell et al. (2000) present a comprehensive model for rats, mice and humans. This model includes the glutathione pathway of trichloroethylene biotransformation, making it possible to predict the target dose of reactive thiol in the kidney. Choral hydrate production in lungs is also predictable with this model. Both of these estimates (for chloral hydrate in lungs and reactive thiol in kidneys, respectively) however are highly uncertain, so the authors point out.

Risk assessment applications of PBPK-models include early predictions of human cancer risk by Koizumi (1989) and Bogen (1988) who both used a human model for trichloroethylene (parent compound) kinetics. As already mentioned above, Allen and Fisher (1993a and 1993b) modeled trichloroethylene and TCA in humans and used it for estimating liver cancer risk from the mouse bioassay data. Clewell et al. (2000) provide PBPK-derived estimates of target tissue dose for the human cancer risk for the liver, lungs and kidney target sites. This model was also used for chronic non-cancer risk assessment, using for the inhalation route liver toxicity, neurotoxicity and renal toxicity as critical effects (Barton and Clewell, 2000).

A potentially valuable model for neurotoxicological risk assessment of acute trichloroethylene exposures is that of Boyes et al. (2000) for Long-Evans rats. Comparing arterial trichloroethylene concentrations predicted with this model and the magnitude of two neurotoxic responses seen in male rats of this strain (i.e. in tests for signal detection behavior

and visual function) showed that arterial concentration of trichloroethylene at the time of testing correlated very well with the effect. Brain levels of trichloroethylene or the AUC of trichloroethylene show less correlation with the effects than arterial concentrations. This observation indicates that the parent compound blood concentration is an adequate dose metric for acute trichloroethylene neurotoxicity.

Specifically for worker exposures Simon (1997) used a PBPK-model for risk assessment with drowsiness as the endpoint of concern. This effect was assumed to be due to high levels of both trichloroethylene and trichloroethanol. A four-compartment human PBPK-model was constructed describing oxidative metabolism of trichloroethylene to trichloroethanol. Monte Carlo simulation (with probability distributions of physiological parameters statistically derived from published data) was used to deal with interindividual variability. The peak sum of the arterial blood concentration of trichloroethylene and TCOH in its volume of distribution was chosen as the dose metric. The cut-off point used in this risk assessment was geared to the volunteer study by Stewart et al. (1970). In the latter study 60% percent of subjects reported feeling sleepy after exposure to 200 ppm for 7 hours/day. So it was assumed the 60^{th} percentile of the simulated population would have the same effect at this exposure level (no effect in the remaining 40%). Therefore the 40^{th} percentile in the simulated population of the dose metric was chosen as the target dose metric, i.e. 15.42 mg/liter in males and 15.65 mg/liter in females. Next an occupational guidance value was derived in such a way that 99% of the female worker population would not exceed this target dose metric when exposed for 7 hours/day for 5 days/week (males proved less sensitive to increases of the dose metric). This led to a guidance value of 30 ppm (Simon, 1997).

4.2 Mechanism of Toxicity

The mechanism through which trichloroethylene produces neurotoxicity is not known. The observations in humans after acute inhalation of high concentrations (drowsiness, narcosis) resemble those seen for other organic solvents. According to Snyder and Andrews (1996) this suggests a purely physical interaction of these solvents with the membranes of cells in the CNS. Thus the narcotic effect of solvents would depend only on the molar concentration of the solvent in the target cell. Okamoto and Shiwaku (1994) and Kyrklund et al. (1983) both observed more specific changes in fatty acid composition of the brain (in either rats after single inhalation or gerbils exposure for 12 months). According to Feldman et al. (1970, 1992) many neurotoxic effects are due to demyelinization of axons by trichloroethylene.

A crucial question as to the possible application of PBPK-modeling for trichloroethylene neurotoxicity is which metabolite is responsible for the effect. In the series of acute rat experiments as summarized by Boyes et al (2000), the observed neurotoxicity correlated best with the concentrations of trichloroethylene in blood. As is noted by these authors the acute neurotoxicity in their experiments occurred at levels markedly in excess of the threshold for metabolic saturation (200 ppm), which also supports an important causal role for the unchanged parent compound. Other findings indicate that trichloroethanol is also important. Ertle et al. (1972) found marked increases in trichloroethanol levels in volunteers after inhalation exposure

to trichloroethylene and considered these to be responsible for the neurotoxic response. Blain et al. (1992) in their 12-week inhalation study with trichloroethylene, found this compound's effect on visual evoked potentials to correlate with blood trichloroethanol concentrations but not with blood trichloroethylene concentrations. Trichloroethanol is a known sedative, so Simon (1997) points out, and therefore he chose the peak of the sum of trichloroethanol and trichloroethylene as his dose metric in the PBPK-analysis for acute neurotoxicity by trichloroethylene after inhalation. At least in the range of exposure concentrations and the exposure conditions he studied, i.e. acute exposures up to 200 ppm, trichloroethanol even dominated the dose metric. Thus, the peak sum of [trichloroethylene] + [trichloroethanol] may be the most plausible dose metric for modeling acute trichloroethylene neurotoxicity. Additional research to provide more insight on this point would be very useful.

Mechanistic data on other noncancer toxicities (liver, kidneys, heart) are scarce. The carcinogenic effects, however, that were seen in liver, kidneys and lungs have led to a large number of mode-of-action studies. These data are summarized above in 3.5, the section on animal carcinogenicity.

4.3 Other Relevant Information

4.3.1 Species differences

See sections 3.5 and 4.1.

4.3.2 Concurrent exposures

The influence of alcohol ingestion on the neurobehavioral response to trichloroethylene was examined in several inhalation volunteer studies. Vernon and Ferguson (1970) found a clear effect of simultaneous alcohol intake at 1000 ppm with no effect at 300 ppm (exposure for 2 hours). Winneke (1982) found no effect after exposure to 350 ppm trichloroethylene for 3½ hours. A Dutch group of investigators (Windemuller & Ettema, 1978), finally, found a slight effect in volunteers exposed to 200 ppm for 2½ hours. Combination with alcohol inhibited trichloroethylene metabolism, so the increased blood concentrations in the combination group indicated.

Stewart et al. (1974b), in their series of volunteer studies, found that some subjects developed very marked skin rashes after consuming alcoholic beverages. The rashes disappeared spontaneously but they could be elicited (by drinking alcoholic beverages) up to three weeks after stopping trichloroethylene treatment (Stewart et al., 1974b). The same effect was already reported by Sbertoli and Brambilla (1962) in two workers after degreasing metal parts using trichloroethylene. This effect may be due to accumulation in the blood of acetaldehyde (a compound that is known to produce this effect) with trichloroethylene competitively inhibiting acetaldehyde dehydrogenase and thus preventing the breakdown of ethanol.

Müller et al. (1975) studied the effect of alcohol consumption on trichloroethylene biokinetics in male volunteers. The subjects were either exposed to 50 ppm 6 hours/day for 5

days or to 100 ppm for 6 hours (single exposure). When this was done simultaneously with oral ingestion of alcohol, levels of parent compounds in blood and urine were higher and those of the oxidative metabolites were lower. This indicates competitive inhibition for metabolism between alcohol and trichloroethylene. However, when the alcohol dose is given before exposure to trichloroethylene - with the intervening period being long enough -, increased metabolism of trichloroethylene occurs due to liver enzyme induction. The latter was found in rat studies (e.g. Sato et al., 1981).

Ferguson & Vernon (1970) examined the interaction with two CNS drugs, i.e. Thonzylamine hydrochloride and Meprobamate in volunteers after exposure to 300 and 1000 ppm for 2 hours. These drugs did not alter neurobehavioral performance compared to a placebo in combination with inhalation of trichloroethylene.

Bartoničěk and Teisinger (1962), in a volunteer study, showed that the drug disulfiram markedly inhibits trichloroethylene metabolism.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of human data relevant to AEGL-1

In a relatively large number of human volunteer studies the effect of trichloroethylene on neurobehavioral functioning has been examined. These studies were carried out in the 1960's and 1970's. Mild subjective symptoms were reported in some studies at levels as low as 200 ppm or even below (Stewart et al, 1970, 1974a, Nomiyama and Nomiyama, 1977, Salvini et al., 1971). In Stewart et al. (1970) subjects reported nose and throat irritation and fatigue upon exposure to 200 ppm, 7 hours/day on five subsequent days. The irritation was reported on the first exposure day only whereas fatigue was reported on day four and five only. The subjects in the study by Salvini et al. (1971) reported slight dizziness, transient eye irritation and reduced performance in neurobehavioral tests at 110 ppm for 4 hours. They also complained about the odor. Stewart et al. (1974) repeated this study with exactly the same test conditions but they were unable to reproduce the result. The published reports of these studies are limited and the reliability of these results is questionable. The effects showed poor dose-relation. Some were also reported during exposure to air only; others were reported as occurring sooner at lower dose levels than at higher dose levels, or were not reported on renewed exposure. In conclusion, the self-reported subjective symptoms in these studies are probably biased by study set-up and probably are related to the smell of the chemical.

Vernon & Ferguson (1969) observed significant effects on performance in a range of behavioral tests to measure visual-motor effects when volunteers were exposed to 1000 ppm for two hours. Symptoms of CNS-depression (dizziness, light-headedness and lethargy) were also reported at this exposure level. Simultaneous ingestion of alcohol increased these effects. At 300 ppm CNS depression was reported by 1/8 subjects only (symptoms not specified) with no effect on neurobehavioral function. Simultaneous ingestion of alcohol had no effect at this exposure level (Vernon & Ferguson, 1969). Thus 300 ppm (exposure for 2 hours) appears as an NOAEL. This NOAEL is supported by the results of the neurobehavioral study by Ettema and Zielhuis (1978), who observed marginal effects at 300 ppm in subjects exposed for 2.5 hours. In

some other studies one or more neurobehavioral parameters were affected already at concentrations lower than 300 ppm but the significance of these changes is doubtful, also because the studies are reported insufficiently. In conclusion, based on the weight of evidence from all human volunteer studies (taking into account study set-up, reliability of the results, number of volunteers involved) the level of 300 ppm for 2 hours is selected as the most appropriate starting point for AEGL-1 derivation.

5.2. Summary of animal data relevant to AEGL-1

No animal data for AEGL-1 endpoints are available.

5.3 Derivation of AEGL-1

The AEGL-1 derivation is based on the NOAEL of 300 ppm from the study by Vernon and Ferguson (1969). Volunteers exposed for 2 hours to this concentration showed no significant impairment of neurobehavioral function, with marginal CNS-depression present in only 1 out of 8 volunteers. For extrapolation across durations the human PBPK model supplied by Boyes et al. (2002a, 2002b) was used. See appendix B for a detailed presentation of the modeling results. The human model applied was derived from a model for Long Evans rats developed by the same group at the US-EPA (Simmons et al., 2001). Using their human model Dr. Boyes and his co-workers first calculated the peak concentration of trichloroethylene in blood after 2 hours of exposure to 300 ppm (the NOAEL) and subsequently determined the external concentrations that would produce the same blood concentration for the other exposure durations. Thus, the dose metric in this calculation is the peak of unchanged trichloroethylene in blood. Although some data indicate that the metabolite trichloroethanol may also contribute significantly to the neurotoxic effect of trichloroethylene (and thus should be included in the dose metric), blood levels of trichloroethylene were found to correlate well with neurotoxic effects in rats after acute exposure (Boyes et al., 2000) and accordingly the latter are an acceptable dose metric. Following exposure to an external concentration of 300 ppm for 2 hours (the NOAEL), the model predicted a trichloroethylene concentration in blood of 4.78 mg /liter. This value compares reasonably well to the peak blood concentrations of trichloroethylene as seen in various volunteer studies exposed to similar concentrations (see section 4). Using the 4.78 mg/liter target value the following external concentrations were calculated for the standard AEGL durations.

Exposure Duration	Ca (target = 4.78 ± 0.02 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 4.78 ± 0.02 mg/L
8 hr	4.78 ± 0.02	232
4 hr	4.78 ± 0.02	251
1 hr	4.78 ± 0.02	392
0.5 hr (30 min)	4.78 ± 0.02	524
0.1667 hr (10 min)	4.78 ± 0.02	782

For interspecies extrapolation no uncertainty factor was needed because the NOAEL derives from a human study. For interindividual variation among humans an intraspecies factor

of 3 is used. A higher factor is not necessary because the mechanism of action (general CNS depression) does not vary more than a factor of 2-3 within the human population. This leads to the following AEGL-1 values:

TABLE 6: AEGL-1 values for Trichloroethylene [ppm]

Classification	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-1	260	180	130	84	77

6. DATA ANALYSIS FOR AEGL-2

6.1 Summary of human data relevant to AEGL-2

Human data relevant for AEGL-2 endpoints are scant. The effect of trichloroethylene on the central nervous system as known to occur in humans, involves symptoms such as dizziness, lethargy and, at sufficiently high concentrations, narcosis. These are AEGL-2 endpoints because they may impair escape. The available case reports only provide limited information on the dose response relationship for these effects. The report by Longley and Jones (1963) suggests a LOAEL for narcosis of 3000 ppm but the subjects in question had already been exposed for several hours to lower concentrations before losing consciousness immediately upon the accidental peak exposure. Even the use of trichloroethylene as anesthetic agent has not led to reliable indications of the minimum effective CxT combination for inducing narcosis. According to Langton–Hewer (1975) anesthesia requires a blood concentration of 100 mg trichloroethylene/liter for which sustained inhalation of 7000 ppm is needed (period not specified). In the controlled experiments with trichloroethylene (neurobehavioral volunteer studies) the test concentrations mostly were too low for observing escape-impairing CNS effects. In the Vernon and Ferguson (1969) study the subjects reported light-headedness, dizziness, or lethargy at 1000 ppm (exposure for 2 hours). At this exposure concentration reduced neurobehavioral performance was detected, most importantly reduced performance in the pegboard test. The experimenters noted overt motor effects (increased clumsiness) in performing the latter test. The neurobehavioral effects were exacerbated by simultaneous ingestion of alcohol. Although significant in themselves, the effects reported by Vernon and Ferguson are not escape-impairing. The neurobehavioral test systems used, are designed to detect subtle changes in neurobehavioral function. Therefore, 1000 ppm for 2h is the highest level without an AEGL-2 effect in humans.

6.2 Summary of animal data relevant to AEGL-2

Several acute neurotoxicity studies are available. In these studies effects on swimming-behavior, eye movements, shock avoidance, signal detection, visual function, T-maze alternation and ototoxicity were observed. Especially important is a series of acute rat experiments carried out at the US-EPA (Bushnell, 1997; Crofton and Zhao, 1997). With signal detection and reaction speed as endpoints and exposure durations from 0.33 to 1.0 hours, it was observed that the effect of trichloroethylene was not constant across different CxT

combinations. Concentration contributed more to the effect than did duration. For the formula $C^n xT$ empirical values for n were derived, i.e. 2.2 for signal detection and 7.1 for reaction speed. Due to the tolerance that the test animals developed during the study, the real values of n will even be somewhat higher. The NOAEL for signal detection was 800 ppm (1 hour exposure) with an LOAEL of 1200 ppm (0.67 hour exposure). The NOAEL for response time was 2000 ppm (1 hour exposure) with an LOAEL of 2400 ppm (0.67 hour exposure). For the induction of hearing-loss in rats after single exposure to trichloroethylene for six hours, a benchmark concentration for a 15 dB loss was derived of 6218 ppm. The corresponding 95% lower bound on this concentration was 5223 ppm.

6.3 Derivation of AEGL-2

The neurotoxicity parameters studied in the acute rat studies by Bushnell (1997) and Crofton and Zhao (1997) could be used for deriving an AEGL-2. However, the effects on signal detection and reaction speed as detected in these experiments, although clearly adverse in themselves, are of a relatively subtle nature and when occurring in humans, they are not expected to impair escape. The ototoxicity (hearing-loss) is a more severe effect, which, moreover, is irreversible. Based on the 95% lower bound on the Benchmark Concentration (BMDL) of 5223 ppm for this effect, PBPK-calculations were made to examine its suitability for deriving a AEGL-2. This was done by Boyes et al. (2002a), first by applying their rat model in order to extrapolate the BMDL across the required AEGL-2 durations, and subsequently by applying their human model for interspecies extrapolation (for details see appendix A). The results however were unsatisfactory in that they were in the range of concentrations known to produce narcosis in humans (calculated concentrations of 14,000 to 68,000 ppm – see appendix A). The blood target concentration in these calculations was 420 mg/liter (the model-predicted trichloroethylene concentration in rat blood at 5223 ppm for 6 hours). In the study by Kishi et al. (1993) in another strain of rats, however, marked CNS-depression was detected already at 100 mg/liter. This difference could be due to a significant contribution of an unmodeled metabolite (probably trichloroethanol) to the trichloroethylene-induced ototoxicity.

Human data relevant for AEGL-2 endpoints are scant. The effects seen at 1000 ppm by Vernon & Ferguson (1969) are relatively mild effects for an AEGL-2 level. However, because of the lack of another reliable human NOAEL, the value of 1000 ppm for 2 hours is considered the highest level without an AEGL-2 effect. At this concentration there was self-reported light-headedness, dizziness and lethargy and also a reduced performance in neurobehavioral tests (primarily the pegboard test). For extrapolation across durations (to 10 and 30 minutes, and 1, 4 and 8 hours) the human PBPK model of Boyes et al. (2002a, 2002b) was applied. Using this model Dr. Boyes and his co-workers first calculated the peak trichloroethylene concentration after 2 hours of exposure to 1000 ppm and subsequently determined the external concentrations that would produce the same blood concentration for the other exposure durations. See appendix B for a detailed presentation of the modeling results. The dose metric used is the peak of unchanged trichloroethylene in blood. The latter levels have been observed correlate with the acute neurotoxic effects of trichloroethylene. Following exposure to an external concentration of 1000 ppm for 2 hours, the model predicted a trichloroethylene concentration in blood of 18.3 mg /liter. Although no human metabolism studies are available with appropriate exposure levels

to support this calculated blood level, it should be noted that the level of 18.3 mg/liter is a factor 5 lower than the blood level needed for anesthesia (100 mg/liter). Using 18.3 mg/liter as the target value the following external concentrations were calculated for the standard AEGL durations:

Exposure Duration	Ca (target = 18.3 ± 0.2 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 18.3 ± 0.2 mg/L
8 hr	18.3 ± 0.2	719
4 hr	18.3 ± 0.2	801
1 hr	18.3 ± 0.2	1357
0.5 hr (30 min)	18.3 ± 0.2	1868
0.1667 hr (10 min)	18.3 ± 0.2	2889

For interspecies extrapolation no uncertainty factor was needed because the 1000 ppm level derives from a human study. For interindividual variation among humans an intraspecies factor of 3 is used. A higher factor is not necessary because the mechanism of action (general CNS depression) is not expected to vary more than a factor 2-3 in the human population, while, in addition the severity of the effects is considered to be less than needed for AEGL-2. This provides for the following AEGL-2 levels:

TABLE 7: AEGL-2 values for Trichloroethylene [ppm]

Classification	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-2	960	620	450	270	240

7. DATA ANALYSIS FOR AEGL-3

7.1 Summary of human data relevant to AEGL-3

Lethality data in humans are limited and do not provide any detailed information on LC-values. From the practice of use as anesthetic it is known that initially 10,000 – 20,000 ppm is used to induce narcosis. It has been reported that a blood level of 100 mg/liter is needed for narcosis, which can be reached by sustained inhalation of 7000 ppm. Pembleton (1974) exposed over 500 patients (subjected to a range of different medical treatments) initially to 10,000 ppm, which was reduced to 5000 ppm after a few minutes, and to 2000 or 1000 ppm later on. This procedure was used for various medical treatments (surgery) with probable durations up to 4 hours. It should be noted that this includes individuals with compromised health.
Cardiac arrhythmias are known to occur at high exposure levels (Barnes and Ives, 1940, Orth and Gillespie 1945, Boulton and Sweet, 1960), probably only above 10,000 ppm.
Taken together, these data are valuable as supporting information to the AEGL-3 levels, but they do not allow derivation of a specific concentration x time relationship for setting an AEGL-3. This also goes for the cardiac arrhythmias that are known to occur when trichloroethylene is used as an anesthetic. Thus, no usable human NOAEL or LOAEL for AEGL-3 derivation is available.

7.2 Summary of animal data relevant to AEGL-3

The acute lethality of trichloroethylene was examined in rats and mice. The main symptom of intoxication was depression of the nervous system (unsteady gait, stupor, narcosis). Post mortem results were not reported in most studies. The 4-hour LC_{50}-value in rats is 12500 ppm and in mice 8450 ppm. Six-hour LC_{50}-values (rats, mice) were 5918 and 5857 ppm, respectively. The 1-hour LC_{50} in rats was about 26000 ppm. Adams et al. (1951) determined no-effect-times for mortality in rats. In their broadest time series, that with a test concentration of 20,000 ppm, there were no deaths after 0.3 hours of exposure whereas after 0.4 hours there were 2/20 deaths. Similarly, at 9600 ppm there were no deaths after 0.8 hours (no-effect) and 3/20 deaths after 1.0 hours (lowest effect). From the study by Friberg et al. (1953) a mouse NOAEL for mortality of 4600 ppm for a 4-hour exposure, can be derived (LOAEL 5350 ppm).

Using the model Doseresp. (version 2.00) for probit analysis, LC_{05} and LC_{01} values for the different AEGL durations were calculated, based on the data from Adams et al. (1951). The 95% lower confidence limits of these values (the Benchmark Concentrations, BMC), were also derived. For a detailed presentation of all LC-values calculated with Doseresp. 2.00, see appendix C. The LC_{05}- and LC_{01}-values and their lower 95% confidence levels were as follows:

Time	LC_{01}	LC_{01} 95% lower confidence limit	LC_{05}	LC_{05} 95% lower confidence limit
10 minutes	19,240	11,170	29,540	18,450
30 minutes	9297	5720	14,270	9959
1 hour	5875	3576	6472	3977
4 hour	2347	1243	3603	2318
8 hour	1483	704	2277	1308

7.3 Derivation of AEGL-3

The lower 95% confidence levels of the LC_{05}-values (the BMC for the 5% response) derived from the rat study by Adams et al. (1951), may be considered the most appropriate basis for deriving the AEGL-3 (Fowles et al., 1999). The calculations with the PBPK-model by Boyes et al. (2002) show consistently that compared to rats, humans need much higher external concentrations for reaching a certain concentration in blood. For this reason no interspecies uncertainty factor is needed for a toxicokinetic difference between rat and humans. Although a toxicodynamic difference may still exist between rat and humans, it is expected that this variability is small compared to the clear difference in uptake between rats and humans. Hence, an interspecies uncertainty factor is considered not necessary for the derivation of the AEGL-3. For interindividual variation among humans an intraspecies factor of 3 is used. A higher factor is not necessary because the mechanism of action is not expected to vary greatly between individuals. Using this approach, AEGL-3 values of 6150, 3300, 1325, 770, and 435 ppm were calculated for the 10 min, 30 min, 1h, 4h, and 8h period respectively. Although this procedure is valid, the obtained values are considered to be too low compared to the available human evidence. Therefore, an alternative approach was developed. This starts with the NOAEL for mortality observed in mice: 4600 ppm for 4 hours (Friberg et al., 1953). Although this

concentration is probably nominal, it has been shown in many studies that actual concentrations are close to nominal levels. Therefore, the level of 4600 ppm can be used. A value of n of 1.511 is derived from the study of Adams et al. (1951) by probit analysis. As explained above an interspecies extrapolation factor is not considered necessary. As also explained above, for interindividual variation among humans an intraspecies factor of 3 is appropriate. A higher factor is not necessary because the mechanism of action is not expected to vary greatly between individuals. Finally, in AEGL-3 derivation it is also taken into account that cardiac arrhythmias may occur in humans at levels of 10,000 ppm and above and that 10,000 ppm will quickly result in complete narcosis. This level should not be exceeded. In addition, general anesthesia may be associated with vomiting, another risk factor especially in the absence of medical assistance. Therefore, the 10 min AEGL-3 is set at the same value as the 30 minute value (6100 ppm) instead of the calculated value of 12600 ppm. This leads to the following values for AEGL-3:

TABLE 8: AEGL-3 Values for Trichloroethylene [ppm]

Classification	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-3	6100	6100	3800	1500	970

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

TABLE 9: Summary of AEGL Values for Trichloroethylene (ppm)

Classification	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-1 (Nondisabling)	260	180	130	84	77
AEGL-2 (Disabling)	960	620	450	270	240
AEGL-3 (Lethal)	6100	6100	3800	1500	970

8.2 Comparison with other standards and guidelines

NIOSH Immediately Dangerous to Life and Health (IDLH) is defined by the NIOSH/OSHA Standard Completions Program only for the purpose of respirator selection and represents a maximum concentration from which, in the event of respiratory failure, one could escape within 30 minutes without experiencing any escape-impairing or irreversible health effects.

TABLE 10. Extant Standards and Guidelines for trichloroethylene

Guideline	Exposure Duration				
	10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
AEGL-1	260 ppm [1400 mg/m^3]	180 ppm [970 mg/m^3]	130 ppm [700 mg/m^3]	84 ppm [450 mg/m^3]	77 ppm [410 mg/m^3]
AEGL-2	960 ppm [5200 mg/m^3]	620 ppm [3300 mg/m^3]	450 ppm [2400 mg/m^3]	270 ppm [1400 mg/m^3]	240 ppm [1300 mg/m^3]
AEGL-3	6100 ppm [33000 mg/m^3]	6100 ppm [33000 mg/m^3]	3800 ppm [20000 mg/m^3]	1500 ppm [8100 mg/m^3]	970 ppm [5200 mg/m^3]
ERPG-1 (AIHA)[a]			100 ppm		
ERPG-2 (AIHA)			500 ppm		
ERPG-3 (AIHA)			5000 ppm		
EEGL (NRC)[b]	None established				
SMAC[c]	None established				
PEL-TWA (OSHA)[d]					50 ppm
PEL-STEL (OSHA)e					200 ppm
IDLH (NIOSH)[f]	1000 ppm				
REL-TWA (NIOSH)[g]					25 ppm
REL-STEL (NIOSH)[h]	None established				
TLV-TWA (ACGIH)[i]					50 ppm

TLV-STEL (ACGIH)[j]					200 ppm

[a]**ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association**
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual=s ability to take protection action.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

[b] **EEGL (Emergency Exposure Guidance Levels, National Research Council**
The EEGL is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury.

[C] **SMAC (Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants)** provide guidance on chemical exposures during normal operations of spacecraft as well as emergency situations. The one-hour SMAC is a concentration of airborne substance that will not compromise the performance of specific tasks by astronauts during emergency conditions or cause serious or permanent toxic effects. Such exposure may cause reversible effects such as skin or eye irritation, but they are not expected to impair judgment or interfere with proper responses to emergencies. SMACs are derived for exposure periods of 1 hour, 24 hours, 7 days and 180 days.

[d] **OSHA PEL-TWA (Occupational Safety and Health Administration, Permissible Exposure Limits - Time Weighted Average)** is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[e] **OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** is defined analogously to the ACGIH-TLV-STEL.

[f] **IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[g] **NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** is defined analogously to the ACGIH-TLV-TWA.

[h] **NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** is defined analogous to the ACGIH TLV-STEL.

[i] **ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[h] **ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

8.3 Data quality and research needs

Confidence in the AEGL-1 is high because there is a relatively large data base of volunteer studies, all with test concentrations in the range relevant for AEGL-1. For guideline derivation from the human NOAEL only one uncertainty factor was required. In addition, cross-duration extrapolation was done with a relevant human PBPK model.

The confidence in the AEGL-2 is medium. The human data base for AEGL-2 was limited. Highly relevant animal data were available but they could not be reliably extrapolated from rat to humans with the relevant PBPK-models in their current form. For guideline derivation from the human NOAEL only one uncertainty factor was required. In addition, cross-duration extrapolation was done with a relevant human PBPK model.

The confidence in the AEGL-3 is medium. The human data base for AEGL-3 was very limited. Animal data were of an early date. Nevertheless the rat study by Adams et al. (1951) represents an elaborate time series and thus provides a good picture of the dose response for mortality in this species. Similarly the mouse study by Friberg et al. (1953) had a large number of dose levels and its NOAEL of 4600 ppm therefore represents a relatively exact measure of the highest non-lethal concentration in this species. Based on this NOAEL only one uncertainty factor was needed for guideline derivation.

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APPENDIX A
PBPK modeling

U. S Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC 27711

February 14, 2002

To: Mr. Paul Janssen
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From: Dr. William K. Boyes, NTD/NHEERL/ORD/U.S. EPA
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Dr. Marina Evans, ETD/NHEERL/ORD/U.S. EPA

Subject: Physiologically-Based Pharmacokinetic Modeling of Trichloroethylene

We are pleased to respond positively to your request (attachment 1) for physiologically-based pharmacokinetic modeling (PBPK) of trichloroethylene (TCE). In the course of these simulations we have used both a rodent and a human model. For all simulations, the simulation modeling program was Simusolv® (Version 3.0, 1993).

Rodent Modeling. The rodent model we used was that of Simmons et al. (2002, attachment 2). This model used the basic model structure provided by Ramsey and Andersen (1984) as a starting point. The Simmons et al. (2002) model used parameters specific for the Long-Evans rat to the extent possible. The PBPK model consisted of 5 compartments: fat; slowly perfused tissue; rapidly perfused viscera; liver; and, brain. The model used Long-Evans specific parameters for Vmaxc (the maximum rate of metabolism), partition coefficients and the volumes of the brain, liver and fat compartments. Because blood flow values for Long Evans rats were not available, values from F-344 rats were used as discussed in Simmons et al. (2002). The PBPK model input parameters used in the rodent model are shown in Appendix 1.

Rodent Step 1. We used the rodent PBPK model for TCE to estimate the arterial concentration of TCE (Ca) following exposure to an external concentration of 5223 ppm TCE for 6 hr. Ca was taken at the 6 hr time point. This point of departure is that requested by Dr. Janssen in his e-mail of December 17, 2001 (attachment 1). We simulated this point of departure Ca under two conditions.

First, we used the model to estimate Ca under the assumption that the external concentration of TCE was zero at the beginning of the 6 hr exposure, This is meant to simulate the condition of an accident where the ambient air concentration of TCE is very low (at background levels) and then rapidly rises as the result of a sudden large release of TCE to the atmosphere. We used a rise time of 8 minutes, such that at the end of 8 minutes the external air concentration was 95% of the target concentration, in this case 5223 ppm TCE. This resulted in a Ca of 419 mg TCE/liter blood.

Second, we used the model to estimate Ca under the assumption that the concentration of TCE was the same at the beginning and the end of the 6 hr exposure period. This is meant to simulate the condition where TCE has been released to the ambient air and a person travels into the area of TCE and remains there for 6 hr. In other words, the external concentration of TCE was 5223 ppm at the beginning of the exposure and held there for 6 hr. This resulted in a Ca of 420 mg TCE/ liter blood.

Rodent Step 2. We took the point-of-departure Ca's estimated under Rodent Step 1 and used the PBPK model to estimate the external concentration of TCE required to produce this Ca at the exposure durations specified by Dr. Janssen in the December 17, 2001 e-mail. These exposure durations were 8 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). We then used a 'boot-strap' modeling approach, based on consultation by Mr. Chris Eklund with Dr. Mel Andersen (Colorado State University). In this method, the exposure concentration of TCE was varied until the desired Ca was achieved. In this type of modeling, the simulations are repeated until Ca reaches or is very close to the target value.

As in Rodent Step 1, we considered the case where the initial external concentration of TCE was zero and then rose over the time-course of the exposure (with C_{95} achieved at 8 minutes of exposure). These simulation results are shown in Table 1.

Table 1. The external exposure concentration required to achieve a Ca of 419 mg TCE/L blood in rats at varied exposure durations, when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 419 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 419 mg/L
8 hr	419.6	4770
4 hr	419.0	6050
1 hr	419.1	9950
0.5 hr (30 min)	419.1	13,100
0.1667 hr (10 min)	419.0	22,450

As can be seen in Table 1, the boot-strapping procedure was successful in all cases, in that the exposure concentration of TCE was varied until Ca was either 419.0 mg TCE/L or within ±1 mg of this target value. As expected, as the exposure duration decreased, the exposure concentration of TCE needed to achieve the target Ca increased.

Then, we considered the case where the initial and final exposure concentrations of TCE were equal. These simulation results are shown in Table 2.

From Table 2, it may be seen that at all exposure durations we were able to successfully vary the exposure concentration of TCE to achieve the target Ca of 420 mg TCE/L. As in Table 1, the exposure concentration of TCE required to achieve the target Ca increased as the exposure duration decreased. Comparing the results shown in Tables 1 and 2, the impact of the value used for the initial concentration of TCE is evident at the shorter exposure durations. When the initial concentration of TCE was zero, higher concentrations of TCE were needed at 0.5 and 0.1667 hr to achieve the target Ca (Table 1) than when the exposure concentration of TCE was the same at the beginning and the end of the exposure period (Table 2).

Table 2. The external exposure concentration required to achieve a Ca of 420 mg TCE/L blood in rats at varied exposure durations, when the external concentration of TCE was the same at the beginning and the end of the exposure period.

Exposure Duration (hr)	Ca (target = 420 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 419 mg/L
8 hr	420.1	4775
4 hr	420.0	6050
1 hr	420.0	9865
0.5 hr (30 min)	420.0	12,780
0.1667 hr (10 min)	420.0	20,515

Human Modeling. The human model we used was based on the rodent model of Simmons et al. (2002, attachment 2). As for the rodent model, the human PBPK model consisted of 5 compartments: fat; slowly perfused tissue; rapidly perfused viscera; liver; and, brain. The human PBPK model input parameters are shown in Appendix 2.

Human Step 1. We took the point of departure Ca's (419 and 420 mg TCE/L blood) estimated under Rodent Step 1 and used the human PBPK model to estimate the external concentrations of TCE required to produce these Ca's at 8 hr, 6 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). Similar to the rodent modeling, a 'bootstrap' approach was used in which the exposure concentration of TCE was varied until the desired Ca was achieved.

First, we considered the case where the initial external concentration of TCE was zero and then rose over the time-course of the exposure (with C_{95} achieved at 8 minutes of exposure). These simulation results are shown in Table 3.

As expected, the exposure concentration of TCE necessary to achieve the target Ca increased as the exposure duration decreased. Comparing Tables 1 (the rodent) and 3 (the human), it can be readily seen that the exposure concentrations required to achieve the point of departure Ca are much greater for humans than for rodents. The exposure concentration of TCE required to achieve Ca in humans at 0.1667 is approaching the concentration of TCE in air at saturation (75,100 ppm at 20°C) (Arlien-Söborg, 1992).

Then, we considered the case where the initial and final exposure concentrations of TCE were equal. These simulation results are shown in Table 4.

Table 3. The external exposure concentration required to achieve a Ca of 419 mg TCE/L blood in humans at varied exposure durations, when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 419 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 419 mg/L
8 hr	419 (± 1)	14,485
6 hr	419 (± 1)	15,050
4 hr	419 (± 1)	16,400
1 hr	419 (± 1)	29,200
0.5 hr (30 min)	419 (± 1)	40,940
0.1667 hr (10 min)	419 (± 1)	64,600

Table 4. The external exposure concentration required to achieve a Ca of 420 mg TCE/L blood in humans at varied exposure durations, when the external concentration of TCE was the same at the beginning and the end of the exposure period.

Exposure Duration (hr)	Ca (target = 420 mg TCE/L blood	TCE concentration (ppm) necessary to achieve Ca of 420 mg/L
8 hr	420 (± 1)	14,515
6 hr	420 (± 1)	15,075
4 hr	420 (± 1)	16,400
1 hr	420 (± 1)	28,830
0.5 hr (30 min)	420 (± 1)	39,900
0.1667 hr (10 min)	420 (± 1)	60,500

The human simulations are similar to those for the rodent, in that at the shorter exposure durations, lower concentrations of TCE were needed to achieve the target Ca when the exposure concentration of TCE was same at the beginning and end of the exposure period (Table 4) than when the exposure concentration of TCE was zero at the beginning of the exposure period (Table 3).

Human Step 2. We considered the effect in humans of a TCE-induced decrease in alveolar ventilation on the concentrations of TCE required to achieve the target Ca. In the Simmons et al. (2002) rodent model used in this report, alveolar ventilation was decreased 20% from the baseline value. This decrease was based on the findings of Dallas et al. (1991) that in rodents exposure to 500 ppm TCE resulted in a 20% decrease in alveolar ventilation relative to exposure to 50 ppm TCE. To consider the influence of decreased alveolar ventilation in humans, the simulations reported in Tables 3 and 4 (where alveolar ventilation was set to the baseline value reported in Appendix) were repeated with alveolar ventilation decreased by 20% relative to the baseline value reported in Appendix 2. Cardiac output was adjusted to maintain a constant relationship between cardiac output and alveolar ventilation (alveolar ventilation = 0.8 x cardiac output, Appendix 2).

As above, we first considered the case where the initial external concentration of TCE was zero and then rose over the time-course of the exposure (with C_{95} achieved at 8 minutes of exposure). These simulation results are shown in Table 5.

Comparing Tables 3 and 5, it can be seen that a decrease in the rate of ventilation resulted in an increase in the exposure concentration of TCE required to achieve Ca. These results are consistent with the expectation that decreased alveolar ventilation, coupled with a corresponding decrease in cardiac output would result in an increase in the external concentration of TCE required to achieve the target Ca.

Table 5. The external exposure concentration required to achieve a Ca of 419 mg TCE/L blood in humans at varied exposure durations under decreased ventilation (20%), when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 419 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 419 mg/L
8 hr	419 (± 1)	14,920
6 hr	419 (± 1)	15,710
4 hr	419 (± 1)	17,540
1 hr	419 (± 1)	32,500
0.5 hr (30 min)	419 (± 1)	45,000
0.1667 hr (10 min)	419 (± 1)	68,850

Following this, we considered the case where alveolar ventilation was decreased by 20% in humans the initial and final exposure concentrations of TCE were equal. These simulation results are shown in Table 6.

Table 6. The external exposure concentration required to achieve a Ca of 420 mg TCE/L blood in humans at varied exposure durations under decreased ventilation (20%), when the external concentration of TCE was the same at the beginning and the end of the exposure period.

Exposure Duration (hr)	Ca (target = 420 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 420 mg/L
8 hr	420 (± 1)	14,950
6 hr	420 (± 1)	15,730
4 hr	420 (± 1)	17,535
1 hr	420 (± 1)	32,035
0.5 hr (30 min)	420 (± 1)	43,840
0.1667 hr (10 min)	420 (± 1)	64,750

As noted previously in the report, it may also be seen here that the initial exposure concentration influences the exposure concentration needed to achieve a set Ca at shorter exposure durations.

In his e-mail of December 17, 2001, Dr. Janssen requested that we consider the uncertainty in these model estimates. In the work reported here, we have explored uncertainty in the model outcome by taking into consideration of the influence of the initial concentration of TCE on the model outcome. Additionally, in the human model we have addressed uncertainty by examination of the influence of a possible TCE-induced effect on ventilation.

With regard to the request in the December 17, 2001 e-mail to consider trichloroethanol, we are excited about the possibility of doing so. Although time constraints prevented us from adding the appropriate equations to account for trichloroethanol in our model, we anticipate that we would be able to do so, if, after consideration of the present modeling results, this is still of interest to Dr. Janssen's committee.

Conclusions:

While we believe that the above analysis complies with your request, we have reservations about use of these values for setting AEGL-2 for human exposures. The concentrations predicted to result in an irreversible hearing loss in humans from acute exposures to TCE are very high, and exceed by a factor of ~10 the likely anesthetic concentrations (3000 ppm for 1 hr; Longley and Jones 1963, as reported in ATSDR, 1996). Another reservation is that the current analysis assumes that the amount of hearing loss is related to peak blood concentration of TCE, as opposed to some other dose metric such as area under the curve, an assumption for which we do not have data to support or refute at this time. Finally, we have evidence that for toluene, a solvent with similar behavioral and ototoxic effects to TCE, humans may be more sensitive to behavioral deficits than are rats on an equal blood concentration basis (Benignus et al., 1998).

We believe, however, that the approach used here of selecting a dose that is associated with an adverse effect as a departure point, and then using a PBPK model to predict concentration/duration combinations expected to also result in the same internal dose, is conceptually sound and preferable to other procedures for exposure duration adjustments.

We suggest that you consider using the PBPK model described here to conduct a similar analysis, but with a different adverse effect as the departure point. A possibility would be the anesthetic concentration reported above (i.e. 3000 ppm for 1 hour), or other adverse outcomes could also be considered that you feel are consistent with the definition of AEGL-2. As we now have the functioning PBPK models for rats and humans and have conducted the analyses described above, running a comparable analysis using different blood concentrations as the departure point could be accomplished simply. We are willing to help you in this further effort.

Attachments:

1) e-mail correspondence from Dr. Paul Janssen, December 17, 2001.
2) Simmons et al. (2002)

Appendix 1. Input Parameters for the Rodent PBPK model for Trichloroethylene.

Parameter	Value	Source
Body Weight (kg)[a]	0.4	
Alveolar Ventilation Rate (QPC) (L/hr/kg)[b]	10.5	Simmons et al. (2002)
Cardiac Output – (QCC) (L/hr/kg)[c]	11.2	Simmons et al. (2002)
Organ Volume (%)		
Fat (VFC)[d,e]	$y = -3.9889x10^{-6}(BW)^3 + 0.0062(BW)^2 - 2.7643(BW) + 407.61$	Simmons et al. (2002)
Liver (VLC)[d,f]	$y = 0.0286(BW) + 4.0216$	Simmons et al. (2002)
Brain (VBC)[d,g]	$y = -0.00083(BW) + 0.8257$	Simmons et al. (2002)
Slow (VSP)[d,h]	70%	Simmons et al., (2002)
Rapid (VRP)[d,i]	91% - VFC – VLC – VBC - VSP	Gargas et al. (1986)
Blood Flow (%)[j]		
Fat (QFC)	8.2%	Delp et al. (1998)
Liver (QLC)	24.2%	Delp et al. (1998)
Brain (QBC)	2.7%	Delp et al. (1998)
Slow (QSC)	25.7%	Delp et al. (1998)
Rapid (QRC)	100 – QFC – QLC – QBC - QSC	
Partition Coefficients		
Blood/air[d]	20.69	Simmons et al. (2002)
Brain/air[d]	14.58	Simmons et al. (2002)
Liver/air[d]	21.34	Simmons et al. (2002)
Fat/air[d]	470.00	Simmons et al. (2002)
Rapid/air[d,k]	21.34	Simmons et al. (2002)
Slow/air[d]	12.36	Simmons et al. (2002)
VmaxC (mg/hr/kg)[d,l]	8.68	Simmons et al. (2002)
KM (mg /L)	0.25	Simmons et al. (2002)

[a] Based on the weight range used in Simmons et al. (2002), 0.4 kg was selected as the body weight for all rodent modeling in this report.

[b] Set at 0.8 of the baseline value (15.4 L/hr/kg) based on Dallas et al. (1991). Allometrically scaled based on BW(kg), $QP = QPC \times BW(kg)^{0.74}$.

c QCC was calculated based on QPC = 0.94 QCC from Gargas et al. (1986). Allometrically scaled for each rat based on BW (kg), $QC = QCC \times BW(kg)^{0.74}$.

[d] Long-Evans specific.

[e] BW = body weight in gm. Fat weight (gm) calculated based on BW. Fat volume percentage calculated as (fat weight/body weight) x 100.

[f] BW = body weight in gm. Liver weight (gm) calculated based on BW. Liver volume percentage calculated as (liver weight/body weight) x 100.
[g] BW = body weight in gm. Brain volume percentage was calculated based on BW.
[h] Adjusted from the 74% (Paustenbach et al., 1988) and 75% (Gargas et al., 1986) values that have typically been used in our laboratory to maintain a non-negative rapidly perfused volume fraction.
[i] VRP has partial specificity for the Long-Evans rat as VFC, VLC and VBC are Long-Evans specific. VRP equation based on Gargas et al. (1986).
[j] Values for the F-344 rat were used as Long-Evans blood flow values are not available.
[k] Set equal to the liver/air partition coefficient.
[l] Vmax = Vmaxc x $BW(kg)^{0.74}$.

Appendix 2. Input Parameters for the Human PBPK model for Trichloroethylene.

Parameter	Value	Source
Body Weight (kg)	70	
Cardiac Output (QCC) (L/hr/kg)[a]	16.1	Brown et al. (1997)
Alveolar Ventilation Rate (QPC) (L/hr/kg)[b]	QCC*0.8	Brown et al. (1997)
Organ Volume (%)		
Fat (VFC)	21.42	Brown et al. (1997)
Liver (VLC)	2.6	Brown et al. (1997)
Brain (VBC)	2.00	Brown et al. (1997)
Slow (VSP)[c]	43.71	Brown et al. (1997)
Rapid (VRP)[d]	89.9% – VFC – VLC – VBC – VSP	
Blood Flow (%)		
Fat (QFC)	5.2	Brown et al. (1997)
Liver (QLC)	22.7	Brown et al. (1997)
Brain (QBC)	11.4	Brown et al. (1997)
Slow (QSC)[c]	24.9	Brown et al. (1997)
Rapid (QRC)	100 – QFC – QLC – QBC – QSC	
Partition Coefficients		
Blood:Air[e]	8.11	Gargas et al. (1989)
Brain/Air[f]	14.58	Simmons et al. (2002)
Liver/Air[f]	21.34	Simmons et al. (2002)
Fat/Air[f]	470.00	Simmons et al. (2002)
Rapid/Air[f]	21.34	Simmons et al. (2002)
Slow/Air[f]	12.36	Simmons et al. (2002)
VmaxC (mg/hr/kg)[g,h]	6.85	Lipscomb et al. (1998)
KM (mg /L)[i]	1.43	Lipscomb et al. (1998)

a Allometrically scaled based on BW(kg), QP = QPC x BW $(kg)^{0.74}$.

[b] Allometrically scaled based on BW (kg), QC = QCC x $BW(kg)^{0.74}$.

[c] Sum of muscle + skin.

[d] VRP equation based on Brown et al. (1997). Perfused tissue volume figured by subtraction of % bone weight from 100%. Marrow weight was subtracted from total bone mass, i.e. skeleton-red marrow-yellow marrow (14.3%-2.1%-2.1%= 10.1%), resulting in a perfused tissue volume of 89.9.

[e] This is the human blood/air partition coefficient reported by Gargas et al. (1989).

[f] As the human tissue/air partition coefficient was not available, the rodent tissue/air partition coefficient reported by Simmons et al. (2002) was used.

[g] Vmax = Vmaxc x $BW(kg)^{0.74}$.

[h] the mean of 6 human subjects from Lipscomb et al. (1998).

[i] the mean of 6 human subjects was converted from the original value of 266 ppm from Lipscomb et al. (1998). mg/m^3=(ppm * MW)/24.45. Divided by 1000 to convert to mg/L, i.e. 1000 L=1 m^3. MW for TCE is 131.39.

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APPENDIX B

U. S Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC 27711

March 16, 2002

To: Mr. Paul Janssen
Centre of Substances and Risk Assessment
National Institute of Public Health and the Environment
Bilthoven, The Netherlands

From: Dr. William K. Boyes, NTD/NHEERL/ORD/U.S. EPA
Dr. Jane Ellen Simmons, ETD/NHEERL/ORD/U.S. EPA
Mr. Chris Eklund, ETD/NHEERL/ORD/U.S. EPA

Subject: Physiologically-Based Pharmacokinetic Modeling of Trichloroethylene

We are pleased to respond positively to your request of Feb. 20, 2002 (attachment 1) for additional physiologically-based pharmacokinetic modeling (PBPK) of trichloroethylene (TCE). In the course of these simulations we used the human model we described in our memo of February 14, 2002. This human model we used was based on the rodent model of Simmons et al. (**2002, previously provided**). The model consisted of 5 compartments: fat; slowly perfused tissue; rapidly perfused viscera; liver; and, brain. The human PBPK model input parameters are shown in Appendix 1. For all simulations, the simulation modeling program was Simusolv® (Version 3.0, 1993).

In each case reported below, the point of departure was one requested by Mr. Janssen in his e-mail of February 20, 2002 (attachment 1). We simulated this point of departure for arterial concentration (Ca) by estimating Ca under the assumption that the external concentration of TCE was zero at the beginning of the exposure period. This is meant to simulate the condition of an accident where the ambient air concentration of TCE is very low (at background levels) and then rapidly rises as the result of a sudden large release of TCE to the atmosphere. We used a rise time of 8 minutes, such that at the end of 8 minutes the external air concentration was 95% of the target concentration. The Ca at the specified time point was taken as the point of departure Ca. We then used the PBPK model to estimate the external concentration of TCE required to produce this Ca at exposure durations of 8 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). This was done by the same 'boot-strap' modeling approach we used in our earlier work for you. In this method, the exposure concentration of TCE was varied until the desired Ca was achieved. In this type of modeling, the simulations are repeated until Ca reaches or is very close to the target value.

AEGL-1 modeling.

AEGL-1 modeling Step 1. Human NOAEL of 300 ppm for 2 hr. We used the human

PBPK model for TCE to estimate the arterial concentration of TCE (Ca) following exposure to an external concentration of 300 ppm TCE for 2 hr. Ca was taken at the 2 hr time point. This resulted in a Ca of ~ 4.78 mg TCE/liter blood. We took this point of departure Ca and used the PBPK model to estimate the external concentration of TCE required to produce this Ca at exposure durations of 8 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). These results are shown in Table 1.

Table 1. The external exposure concentration required to achieve a Ca of ~ 4.78 mg TCE/L blood in humans at varied exposure durations, when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 4.78 ± 0.02 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 4.78 ± 0.02 mg/L
8 hr	4.78 ± 0.02	232
4 hr	4.78 ± 0.02	251
1 hr	4.78 ± 0.02	392
0.5 hr (30 min)	4.78 ± 0.02	524
0.1667 hr (10 min)	4.78 ± 0.02	782

As can be seen in Table 1, the boot-strapping procedure was successful in all cases, in that the exposure concentration of TCE was varied until Ca was either 4.78 mg TCE/L or within ± 0.02 mg of this target value. Although other external exposure concentrations close to those reported met this criterion, the reported number represents that concentration, among those we tried, that most closely matched Ca. As expected, as the exposure duration decreased, the exposure concentration of TCE needed to achieve the target Ca increased.

AEGL-1 modeling Step 2. Human L0AEL of 200 ppm for 7 hr. We used the human PBPK model for TCE to estimate the arterial concentration of TCE (Ca) following exposure to an external concentration of 200 ppm TCE for 7 hr. Ca was taken at the 7 hr time point. This resulted in a Ca of ~ 3.93 mg TCE/liter blood. We took this point of departure Ca and "bootstrapped" the PBPK model to estimate the external concentration of TCE required to produce this Ca at exposure durations of 8 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). These results are shown in Table 2.

As can be seen in Table 2, the boot-strapping procedure was successful in all cases, in that the exposure concentration of TCE was varied until Ca was either 3.93 mg TCE/L or within ± 0.02 mg of this target value. Although other external exposure concentrations close to those reported met this criterion, the reported number represents that concentration, among those we tried, that most closely matched Ca. As expected, as the exposure duration decreased, the exposure concentration of TCE needed to achieve the target Ca increased.

Table 2. The external exposure concentration required to achieve a Ca of ~3.93 mg TCE/L blood in humans at varied exposure durations, when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 3.93 ± 0.02 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 3.93 ± 0.02 mg/L
8 hr	3.93 ± 0.02	198
4 hr	3.93 ± 0.02	213
1 hr	3.93 ± 0.02	328
0.5 hr (30 min)	3.93 ± 0.02	437
0.1667 hr (10 min)	3.93 ± 0.02	647

AEGL-2 modeling.

AEGL-2 modeling Step 1. Human L0AEL of 1000 ppm for 2 hr. We used the human PBPK model for TCE to estimate the arterial concentration of TCE (Ca) following exposure to an external concentration of 1000 ppm TCE for 2 hr. Ca was taken at the 2 hr time point. This resulted in a Ca of ~ 18.3 mg TCE/liter blood. We took this point of departure Ca and "bootstrapped" the PBPK model to estimate the external concentration of TCE required to produce this Ca at exposure durations of 8 hr, 4 hr, 1 hr, 0.5 hr (30 min) and 0.1667 hr (10 min). These results are shown in Table 3.

Table 3. The external exposure concentration required to achieve a Ca of ~18.3 mg TCE/L blood in humans at varied exposure durations, when the external concentration of TCE was zero at the beginning of the exposure.

Exposure Duration (hr)	Ca (target = 18.3 ± 0.2 mg TCE/L blood)	TCE concentration (ppm) necessary to achieve Ca of 18.3 ± 0.2 mg/L
8 hr	18.3 ± 0.2	719
4 hr	18.3 ± 0.2	801
1 hr	18.3 ± 0.2	1357
0.5 hr (30 min)	18.3 ± 0.2	1868
0.1667 hr (10 min)	18.3 ± 0.2	2889

As can be seen in Table 3, the boot-strapping procedure was successful in all cases, in that the exposure concentration of TCE was varied until Ca was either 18.3 mg TCE/L or within ± 0.2 mg of this target value. Although other external exposure concentrations close to the reported concentration met this criterion, the reported number represents the concentration, among those we tried, that most closely matched Ca. As expected, as the exposure duration decreased, the exposure concentration of TCE needed to achieve the target Ca increased. This represents that portion of the modeling requested in your February 20, 2002 e-mail we were able to accomplish before leaving tomorrow for the Society of Toxicology (SOT) meeting. Knowing that you are under time constraints, we wanted to send you this preliminary report. If your time frame permits, we will resume work on this when our group returns from SOT. If sufficient time remains, we will complete the requested modeling. If that is not possible due to your

deadlines, we will, at the least, review the modeling results provided here and provide them to you in final form.

Appendix 1. Input Parameters for the Human PBPK model for Trichloroethylene.

Parameter	Value	Source
Body Weight (kg)	70	
Cardiac Output (QCC) (L/hr/kg)[a]	16.1	Brown et al. (1997)
Alveolar Ventilation Rate (QPC) (L/hr/kg)[b]	QCC*0.8	Brown et al. (1997)
Organ Volume (%)		
Fat (VFC)	21.42	Brown et al. (1997)
Liver (VLC)	2.6	Brown et al. (1997)
Brain (VBC)	2.00	Brown et al. (1997)
Slow (VSP)[c]	43.71	Brown et al. (1997)
Rapid (VRP)[d]	89.9% – VFC – VLC – VBC – VSP	
Blood Flow (%)		
Fat (QFC)	5.2	Brown et al. (1997)
Liver (QLC)	22.7	Brown et al. (1997)
Brain (QBC)	11.4	Brown et al. (1997)
Slow (QSC)[c]	24.9	Brown et al. (1997)
Rapid (QRC)	100 – QFC – QLC – QBC – QSC	
Partition Coefficients		
Blood:Air[e]	8.11	Gargas et al. (1989)
Brain/Air[f]	14.58	Simmons et al. (2002)
Liver/Air[f]	21.34	Simmons et al. (2002)
Fat/Air[f]	470.00	Simmons et al. (2002)
Rapid/Air[f]	21.34	Simmons et al. (2002)
Slow/Air[f]	12.36	Simmons et al. (2002)
VmaxC (mg/hr/kg)[g,h]	6.85	Lipscomb et al. (1998)
KM (mg /L)[i]	1.43	Lipscomb et al. (1998)

a Allometrically scaled based on BW(kg), QP = QPC x BW $(kg)^{0.74}$.

[b] Allometrically scaled based on BW (kg), QC = QCC x $BW(kg)^{0.74}$.

[c] Sum of muscle + skin.

[d] VRP equation based on Brown et al. (1997). Perfused tissue volume figured by subtraction of % bone weight from 100%. Marrow weight was subtracted from total bone mass, i.e. skeleton-red marrow-yellow marrow (14.3%-2.1%-2.1%= 10.1%), resulting in a perfused tissue volume of 89.9.

[e] This is the human blood/air partition coefficient reported by Gargas et al. (1989).

[f] As the human tissue/air partition coefficient was not available, the rodent tissue/air partition coefficient reported by

Simmons et al. (2002) was used.
[g] Vmax = Vmaxc x $BW(kg)^{0.74}$.
[h] the mean of 6 human subjects from Lipscomb et al. (1998).
[i] the mean of 6 human subjects was converted from the original value of 266 ppm from Lipscomb et al. (1998). mg/m^3=(ppm * MW)/24.45. Divided by 1000 to convert to mg/L, i.e. 1000 L=1 m^3. MW for TCE is 131.39.

References

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APPENDIX C

LC-values derived from Adams et al (1951) using Doseresp. Version 2.00 (Probit analysis)

Time (min)	LC50	LC50 95% lower confidence limit	LC50 95% upper confidence limit
10	83.170	50.890	177.800
30	40.190	28.660	68.690
60	25.400	19.730	38.120
120	16.050	13.270	21.640
240	10.150	8.472	12.950
480	6.412	5.026	8.341

Time (min)	LC05	LC05 95% lower confidence limits	LC05 95% upper confidence limits
10	29.540	18.450	47.910
30	14.270	9.959	19.310
60	9.022	6.472	11.350
120	5.702	3.977	7.056
240	3.603	2.318	4.625
480	2.277	1.308	3.130

Time (min)	LC01	LC01 95% lower confidence limits	LC01 95% upper confidence limits
10	19.240	11.170	30.180
30	9.297	5.720	12.830
60	5.875	3.576	7.836
120	3.713	2.144	4.993
240	2.347	1.243	3.291
480	1.483	704	2.219

APPENDIX D

Quantitative Cancer Risk Assessment for Trichloroethylene

A large number of unit risk derivations (slope factors) are available (see the animal carcinogenicity section in the main document). WHO (2000) presents estimates derived using the linearized multistage model, the model formerly used by the US-EPA. The Leydig cell tumors in rats were the most sensitive endpoint with an extra cancer risk of 4.3×10^{-7} per microgram/m^3 of lifetime exposure. New preliminary estimates – as part of the trichloroethylene cancer risk assessment that is in progress at the US-EPA - are presented by Rhomberg (2000). The latter show a wide variation. For the renal tumors as observed in rats after inhalation (the study by Maltoni et al., 1986), the estimated extra cancer risks varied from 2.2×10^{-10} to 2.8×10^{-8} per microgram/m^3 of lifetime exposure. For these tumors a genotoxic mode of action cannot be ruled out, based on the available evidence. For the mouse tumors in lungs and liver, however, mechanistic data indicate a special mode of action for which a threshold exists. This is in line with the results seen in genotoxicity assays which indicate that trichloroethylene essentially lacks genotoxic activity. Given that, it is plausible that the Leydig cell tumors seen in some rat studies, also arise through a non-genotoxic mechanism. In view of all this, linear extrapolation using the WHO cancer risk estimate provides a conservative assessment of the possible cancer risk posed by trichloroethylene.

Basis for the calculation is the unit risk as presented by WHO (2000) based on Leydig cell tumors (the most sensitive endpoint). Using this unit risk will protect for possible renal cancers because the risks values for the latter are considerably lower.

To convert the unit risk to a level of trichloroethylene that would cause a theoretical excess cancer risk of 10^{-4}:

Risk of $1 \times 10^{-4} = (1 \times 10^{-4}) / 4.3 \times 10^{-7} = 0.232$ mg/m^3 (virtually safe dose)

To convert a 70-years exposure to an 8-hours exposure:

8-h exposure = virtually safe dose x 25,600 days x (24h/8h)
= (0.232 mg/m^3) x 25,600 x (24h/8h)
= 17800 mg/m^3 (rounded value)

To account for the uncertainty regarding the variability in the stage of the cancer process at which trichloroethylene or its metabolites may act, a multistage factor of 6 is applied according to the procedure described in the AEGL Standing Operating Procedures.

(17800 mg/m^3)/6 = 2967 mg/m^3 (534 ppm)

Therefore, based on the potential carcinogenicity of trichloroethylene, a conservative acceptable 8-hours exposure would be 2967 mg/m^3 (534 ppm). For shorter exposures the acceptable exposures increase proportionally in a linear fashion:

8-h exposure : 2967 mg/m^3 (534 ppm)
4-h : 5934 mg/m^3 (1068 ppm)
1-h : 23700 mg/m^3 (4270 ppm)
0.5 h : 47400 mg/m^3 (8530 ppm)

For deriving the corresponding 10^{-5} and 10^{-6} cancer risks the above figures must be divided by 10 and 100 respectively. This gives the following set of cancer risk values:

Exposure (hours)	**10^{-4} cancer risk**	**10^{-5} cancer risk**	**10^{-6} cancer risk**
8	2967 mg/m^3 (534 ppm)	297 mg/m^3 (53 ppm)	30 mg/m^3 (5 ppm)
4	5934 mg/m^3 (1068 ppm)	593 mg/m^3 (107 ppm)	59 mg/m^3 (11 ppm)
1	23700 mg/m^3 (4270 ppm)	2370 mg/m^3 (427 ppm)	237 mg/m^3 (43 ppm)
0.5	47400 mg/m^3 (8530 ppm)	4740 mg/m^3 (853 ppm)	474 mg/m^3 (85 ppm)

Comparing these values with the AEGL-2 and AEGL-3 values shows that all the calculated acceptable exposures that would result in a 1:10^{-4} cancer risk are above the AEGL-2 and –3 values.

APPENDIX E

Derivation of level of distinct odor awareness

Several sources report odor thresholds for trichloroethylene. These values are provided below.

100 ppm	ATSDR toxicological profile (with reference to HSDB 1994)
100 ppm	Environmental Health Criteria (with reference to Torkelson and Rowe, 1982)
47 ppm	AIHA 1989
28 ppm	EPA Health Assessment document (with reference to J.E. Amoore and E. Hautala. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. *Journal of Applied Toxicology*, 3(6):272-290. 1983).
82 ppm	Mean value reported by AIHA website

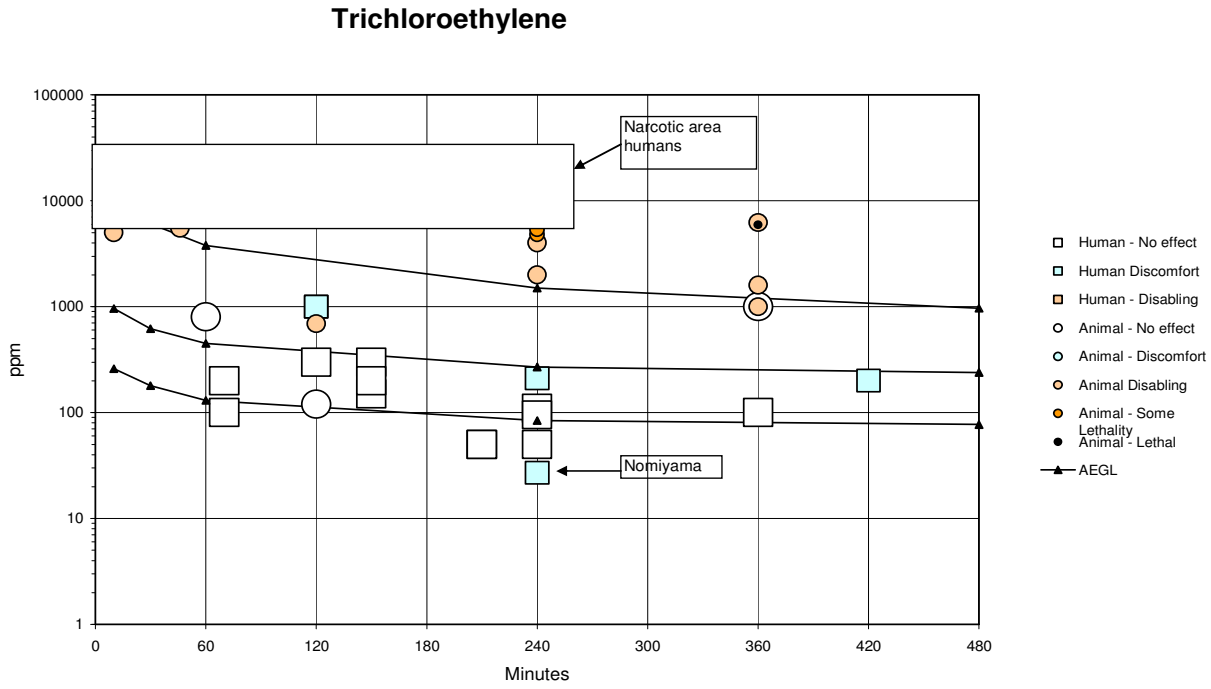
If no information is available on the quality of these data and the measurement of n-butanol, the guidance document on odor annoyance states that the lowest value should be used.

The level of distinct odor awareness can be calculated to be:

$LOA = C_{0,\ stand} \times 10^{2.52\ /\ 2.33} = 28\ ppm \times 10^{2.52\ /\ 2.33} = 337\ ppm.$

APPENDIX F

Category plot for trichloroethylene



APPENDIX G

Derivation Summary for trichloroethylene AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR TRICHLOROETHYLENE (CAS Reg. No. 79-01-6) DERIVATION SUMMARY

AEGL-1 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
260 ppm [1400 mg/m^3]	180 ppm [970 mg/m^3]	130 ppm [700 mg/m^3]	84 ppm [450 mg/m^3]	77 ppm [410 mg/m^3]

Key Reference: Vernon and Ferguson (1969)

Test Species/Strain/Number: Groups of 8 male volunteers.

Exposure Route/Concentrations/Durations: Inhalation exposure to 100, 300 or 1000 ppm for 2 hours.

Effects:
100 ppm: no effects.
300 ppm: marginal CNS-depression without significant impairment of neurobehavioral functions in 1 out of 8 volunteers.
1000 ppm: self-reported light-headedness, dizziness and lethargy and reduced performance in neurobehavioral tests.

Endpoint/Concentration/Rationale: The 300 ppm exposure for 2 hours was concluded to be a NOAEL for AEGL-1 effects. The corresponding peak concentration of trichloroethylene in blood (4.78 mg/L) was calculated with aid of a PBPK-model and used as point of departure to calculate the air trichloroethylene concentrations for the respective exposure durations that would result in a peak concentration of 4.78 mg/L.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1
Intraspecies: 3
An intraspecies factor of 3 is considered sufficient since the mechanism of action (CNS-depression) is not expected to vary more than a factor 2-3 within the human population.

Modifying Factor: None

Animal to Human Dosimetric Adjustment: not applicable; human data

Time Scaling: PBPK-model was used to calculate the air concentrations of trichloroethylene for the respective exposure durations that would lead to a peak concentration of trichloroethylene in blood of 4.78 mg/L.

Data Adequacy: Sufficient.

AEGL-2 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
960 ppm [5200 mg/m^3]	620 ppm [3300 mg/m^3]	450 ppm [2400 mg/m^3]	270 ppm [1400 mg/m^3]	240 ppm [1300 mg/m^3]

Key Reference: Vernon and Ferguson (1969)

Test Species/Strain/Number: Groups of 8 male volunteers.

Exposure Route/Concentrations/Durations: Inhalation exposure to 100, 300 or 1000 ppm for 2 hours.

Effects:

100 ppm: no effects.

300 ppm: marginal CNS-depression without significant impairment of neurobehavioral functions in 1 out of 8 volunteers.

1000 ppm: self-reported light-headedness, dizziness and lethargy and reduced performance in neurobehavioral tests.

Endpoint/Concentration/Rationale: The 1000 ppm exposure for 2 hours was concluded to be the highest exposure level without AEGL-2 effects. The corresponding peak concentration of trichloroethylene in blood (18.3 mg/L) was calculated with aid of a PBPK-model and used as point of departure to calculate the air trichloroethylene concentrations for the respective exposure durations that would result in a peak concentration of 18.3 mg/L.

Uncertainty Factors/Rationale:

Total uncertainty factor: 3

Interspecies: 1

Intraspecies: 3

An intraspecies factor of 3 is considered sufficient since the mechanism of action (CNS-depression) is not expected to vary more than a factor 2-3 within the human population.

Modifying Factor: None

Animal to Human Dosimetric Adjustment: not applicable; human data

Time Scaling: PBPK-model was used to calculate the air concentrations of trichloroethylene for the respective exposure durations that would lead to a peak concentration of trichloroethylene in blood of 18.3 mg/L.

Data Adequacy: The human data base for AEGL-2 was limited and the confidence in the AEGL-2 is therefore medium.

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AEGL-3 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
6100 ppm [33000 mg/m^3]	6100 ppm [33000 mg/m^3]	3800 ppm [20000 mg/m^3]	1500 ppm [8100 mg/m^3]	970 ppm [5200 mg/m^3]

Key Reference: Friberg et al. (1953); (Adams et al. (1951) for derivation of n)

Test Species/Strain/Number: Groups of 8 mice (sex and strain not reported).

Exposure Route/Concentrations/Durations:

Respiratory exposure to nominal concentrations of 3750, 4600, 5350, 7600, 8600, 9300, 11,500, 12,000, 14,100, 14,750 ppm for 4 hours.

Mortality:

3750 ppm: 0/8
4600 ppm: 0/8
5350 ppm: 2/8
7600 ppm: 2/8
8600 ppm: 6/8
9300 ppm: 7/8
11,500 ppm: 5/8
12,000 ppm: 6/8
14,100 ppm: 5/8
14,750 ppm: 8/8

Endpoint/Concentration/Rationale:

The 4600 ppm exposure concentration was chosen as point of departure as the highest nonlethal concentration.

Uncertainty Factors/Rationale:

Total uncertainty factor: 3
Interspecies: 1
Intraspecies: 3

The calculations with the PBPK-model show consistently that compared to rats, humans need much higher external concentrations for reaching a certain concentration in blood. Although a toxicodynamic difference may still exist between rat and humans, it is expected that this variability will be small compared to the clear difference in uptake between rats and humans. A factor of 3 for intraspecies extrapolation is considered sufficient because the mechanism of action is not expected to vary greatly between individuals.

Modifying Factor: NA

Animal to Human Dosimetric Adjustment: NA

Time Scaling:

Time-scaling is performed using n=1.511 derived by probit analyses of the rat data from the Adams *et al*. (1951) study. Because cardiac arrhythmias may occur in humans at concentrations higher than 10,000 ppm and because 10,000 ppm will quickly result in complete narcosis AEGL-3 levels should not exceed 10,000 ppm. Therefore, the 10-min AEGL-3 value is set equal to the 30-min value instead of using the calculated value of 12,6000 ppm.

Data Adequacy:

The database is limited but sufficient.

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