Tetrachloroethane

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Tetrachloroethane*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 1413/HS/pg/459-S53
Bijlagen : 1
Datum : 18 december 2006

Mijnheer de staatssecretaris,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan tetrachloorethaan. Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige adieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over tetrachloorethaan is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport, de minister van Sociale Zaken en Werkgelegenheid en de staatssecretaris van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

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to:

the Minister of Health, Welfare and Sport

No. 2006/09OSH, The Hague, December 18, 2006

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Preferred citation:
Health Council of the Netherlands. Tetrachloroethane; Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2006; publication no. 2006/09OSH.

ISBN-10: 90-5549-625-1
ISBN-13: 978-90-5549-625-9

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Samenvatting en advieswaarde

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie WGD van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden. Deze aanbevelingen vormen de eerste stap in een drietrapsprocedure die moet leiden tot wettelijke grenswaarden, aangeduid als maximaal aanvaarde concentraties (MAC-waarden).

Het voorliggende rapport is samengesteld in samenwerking met de Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG), een adviescommissie van de Noord-Europese regeringen. Het gezamenlijke rapport over de gevolgen van beroepsmatige blootstelling aan tetrachloorethaan, gepubliceerd in Zweden in 1996 (Arbete och Hälsa 1996:28), is in zijn geheel opgenomen in deel 2 van dit advies. Deel 1 bestaat uit een kort overzicht van de onderzoeken die in deel 2 aan de orde komen, een bespreking van de onderzoeksgegevens die sinds 1996 beschikbaar zijn gekomen, en de gezondheidskundige evaluatie van beide stoffen. De conclusies in deel 1 zijn gebaseerd op wetenschappelijke publicaties die vóór januari 2006 zijn verschenen, en komen geheel voor rekening van de commissie.

Fysische en chemische eigenschappen

Tetrachloorethaan is een chemische stof die in twee isomere vormen voorkomt: 1,1,1,2-tetrachloorethaan (CAS nummer 630-20-6) en 1,1,2,2-tetrachloorethaan (CAS nummer 79-34-5). Beide isomeren zijn bij kamertemperatuur kleurloze, niet ontvlambare vloeistoffen met een grote relatieve dichtheid. Geen van beide isomeren komt van nature voor.

1,1,1,2-Tetrachloorethaan wordt niet op industriële schaal geproduceerd. Wel is de stof een veel voorkomend bijproduct van industriële chloreringsreacties met koolwaterstoffen met 2 C-atomen, waaronder de productie van 1,1,2,2-tetrachloorethaan.

1,1,2,2-Tetrachloorethaan wordt gebruikt als uitgangsstof bij de productie van andere gechloreerde koolwaterstoffen. In het verleden is 1,1,2,2-tetrachloorethaan veelvuldig gebruikt, onder meer als oplosmiddel. Vanwege zijn giftige eigenschappen wordt 1,1,2,2-tetrachloorethaan nu nog slechts op beperkte schaal geproduceerd.

Methoden voor monitoring

Verschillende organisaties, zoals het Nederlands Normalisatie-instituut, het Amerikaanse National Institute of Occupational Safety and Health (NIOSH) en het Environmental Protection Agency (EPA), en ook het Engelse Health and Safety Executive (HSE), hebben methoden beschreven om concentraties van 1,1,2,2-tetrachloorethaan in de lucht op de arbeidsplek te kunnen bepalen. Dat gebeurt achtereenvolgens door actieve monsterneming met een adsorptiebuis, thermische of vloeistofdesorptie en analyse met gaschromatografie. De geschiktheid van de methode hangt af van de herkomst van het materiaal in de adsorptiebuis. Daarnaast zijn er nog andere methoden waarbij gebruik wordt gemaakt van passieve monsterneming of draagbare chromatografische apparatuur.

Huidige grenswaarden en classificering

Voor 1,1,1,2-tetrachloorethaan zijn nationaal en internationaal geen grenswaarden vastgesteld. Voor 1,1,2,2-tetrachloorethaan geldt in de meeste landen als grenswaarde een concentratie van 7 mg/m^3 (1 ppm) als tijdgewogen gemiddelde over 8 uur. Daar is een huidnotatie aan toegevoegd. Die geeft aan dat de stof relatief gemakkelijk door de huid wordt opgenomen, wat de inwendige blootstelling substantieel kan verhogen.

Duitsland en het Amerikaanse NIOSH hebben de stof geclassificeerd als respectievelijk een ‘suspected’ en een ‘potential’ carcinogene stof. De American Conference of Governmental Industrial Hygienists (ACGIH) typeerde de stof als een A3-carcinogeen. Dat wil zeggen: het is bewezen dat de stof kankerverwekkend is in proefdieren, maar de betekenis van deze bevindingen voor de mens is niet duidelijk.

Kinetiek

1,1,1,2-tetrachloorethaan

Er zijn geen gegevens beschikbaar over de kinetiek (opname, omzetting, verdeling en uitscheiding) van 1,1,1,2-tetrachloorethaan via routes die in de arbeidssituatie van belang zijn, namelijk via inademing en contact met de huid.

Wel zijn er gegevens over opname via het maag-darmkanaal. Studies met ratten en muizen wijzen erop dat 1,1,1,2-tetrachloorethaan via die weg vrijwel volledig wordt opgenomen. Zo blijken ratten na opname onveranderd 1,1,1,2-tetrachloorethaan uit te ademen, en wel zes keer zoveel als muizen. Het metabolisme van 1,1,1,2-tetrachloorethaan verloopt vervolgens via een aantal oxidatie- en reductiereacties. De belangrijkste metabolieten zijn CO_2 in uitademingslucht en trichloorethanol en trichloorazijnzuur in urine.

Uit het onderzoek blijkt overigens ook dat de ratten 1,1,1,2-tetrachloorethaan anders metaboliseren dan het 1,1,2,2-isomeer. De hoeveelheid trichloorverbindingen in de urine van ratten die via de luchtwegen of een injectie in het buikvlies werden blootgesteld aan 1,1,1,2-tetrachloorethaan, was 20 keer zo groot als de hoeveelheid trichloorverbindingen in de urine van ratten die op dezelfde manier werden blootgesteld aan 1,1,2,2-tetrachloorethaan.

1,1,2,2-tetrachloorethaan

Gegevens uit proefdierstudies laten zien dat 1,1,2,2-tetrachloorethaan gemakkelijk wordt opgenomen via inademing en via de mond. Over opname via de huid zijn daarentegen geen experimentele gegevens beschikbaar. Er is echter berekend dat bij blootstelling aan een verzadigde oplossing van 1,1,2,2-tetrachloorethaan in water de opname door de huid 27 $\mu g/cm^2$ per uur kan bedragen.

Na opname verloopt de biotransformatie van 1,1,2,2-tetrachloorethaan mogelijk via een aantal oxidaties. Daarbij wordt chloor afgesplitst. Er zijn ook aanwijzingen voor een route waarbij de stof gereduceerd wordt. Dit reductieproces leidt

tot de vorming van een koolstofradicaal en, daaropvolgend, tot lipideperoxidatie in de lever.

Over het geheel genomen is CO_2 de belangrijkste metaboliet. In urine blijkt dichloorazijnzuur de belangrijkste metaboliet. Vergeleken met muizen ademen ratten 4 en 14 keer meer onveranderd 1,1,2,2-tetrachloorethaan uit na respectievelijk inhalatoire en orale blootstelling. De aanwezigheid van meer onveranderd tetrachloorethaan maakt ratten mogelijk gevoeliger voor effecten op het zenuwstelsel dan muizen.

Effecten op mensen

1,1,1,2-tetrachloorethaan

Er zijn geen gegevens beschikbaar uit onderzoek naar de mogelijk irriterende of sensibiliserende eigenschappen van 1,1,1,2-tetrachloorethaan bij mensen. Ook over systemische effecten zijn uit dergelijk onderzoek geen gegevens beschikbaar.

1,1,2,2-tetrachloorethaan

Over de effecten van blootstelling aan het andere isomeer, 1,1,2,2-tetrachloorethaan, zijn wel resultaten van onderzoek bij mensen beschikbaar.

Duizeligheid en slijmvliesirritatie traden op bij 2 mannelijke vrijwilligers die werden blootgesteld aan concentraties van 1000 of 1800 mg/m^3 (resp. 144 of 262 ppm). De effecten traden op binnen 10 tot 12 minuten. Bij blootstellingen tot 90 mg/m^3 (13 ppm) gedurende 10 minuten bleven ze achterwege.

Uit studies gepubliceerd vóór 1965 kwamen de lever, het maag-darmkanaal en het zenuwstelsel als meest gevoelig naar voren voor beroepsmatige blootstelling aan 1,1,2,2-tetrachloorethaan. In een van deze studies, waarin met name ook trilling van de vingers werd gerapporteerd, was naast inhalatie ook sprake van huidblootstelling, en bovendien van blootstelling aan aceton.

Wat zijn de bevindingen als het gaat om sterfte, sterfte door kanker en de incidentie van kanker? Een retrospectief cohortonderzoek liet op deze punten geen statistisch significante toename zien. Het cohort bestond uit 1099 blanke mannen die waren blootgesteld aan 1,1,2,2-tetrachloorethaan tijdens het impregneren van kleding en aan oplosmiddelen bij het chemisch reinigen van kleding.

Effecten op proefdieren

1,1,1,2-tetrachloroethaan

Een eerste mogelijk effect van het inademen van 1,1,2,2-tetrachloorethaan of contact met de vloeistof is irritatie van de huid en de slijmvliezen, en sensibilisatie. Uit dierexperimenteel onderzoek zijn daarover echter geen gegevens beschikbaar.

Met sterfte als maatstaf is 1,1,1,2-tetrachloorethaan niet erg giftig na eenmalige blootstelling. In onderzoek waarin ratten en konijnen gedurende 4 uur inhalatoir werden blootgesteld, waren de concentraties die sterfte veroorzaakten bij 50% van de blootgestelde groep (LC_{50}), respectievelijk 14 600 en 19 500 mg/m^3 (respectievelijk 2100 en 2800 ppm). Bij blootstelling via de huid was voor konijnen 20 000 mg/kg lichaamsgewicht (lg) een soortgelijke dosis (LD_{50}).

Er is geen onderzoek beschikbaar waarin proefdieren herhaaldelijk via de ademhalingswegen zijn blootgesteld. Studies waarin de testst of herhaaldelijk via een maagsonde werd toegediend, suggereren dat, behalve het centrale zenuwstelsel, bij (mannetjes)ratten de nieren en bij muizen de lever het meest gevoelig zijn voor blootstelling aan 1,1,2,2-tetrachloorethaan. Mannetjesratten die dagelijks doses van 104 en 208 mg/kg lg kregen toegediend, vertoonden echter slechts tekenen die wijzen op nierschade veroorzaakt door hyalinedruppels. Dit type nierschade bij mannetjesratten wordt in het algemeen echter beschouwd als niet relevant voor de mens.

Een ander mogelijk effect is het optreden van kanker. In een carcinogeniteitsstudie veroorzaakte dagelijkse toediening van hoeveelheden van 0, 125 en 250 mg/kg lg aan Fischerratten in geen van de blootgestelde groepen een statistisch significante toename in incidenties van tumoren. Wel was bij de vrouwtjes uit de lage doseringsgroep een statistisch significante toename te zien in de incidentie van goedaardige borsttumoren (fibroadenomen). Bij B6C3F$_1$muizen resulteerde toediening van doseringen van 0, 250 en 500 mg/kg lg in statistisch significante toenamen in de incidenties van leveradenomen bij mannetjes en vrouwtjes en van levercarcinomen bij vrouwtjes.

Is iets bekend over de invloed van blootstelling aan 1,1,1,2-tetrachloorethaan op het ontstaan en de groei van tumoren? Een tumorinitiatie/promotietest met mannelijke Osborne-Mendelratten liet geen effect zien.

Ook effecten op het erfelijk materiaal zijn onderzocht. *In vitro*-mutageniteitstesten met bacteriën en zoogdiercellen waren over het algemeen negatief; ook tests met gist en fruitvlieg waren negatief. Hetzelfde geldt voor een test om chro-

mosoomafwijkingen in een cellijn van de Chinese hamster (CHO cellen) te onderzoeken. In een andere cellijn van de Chinese hamster (longfibroblasten) veroorzaakte 1,1,1,2-tetrachloorethaan een toename van het aantal cellen met numerieke chromosoomafwijkingen (i.c. polyploïdie), maar niet van het aantal cellen met structurele chromosoomafwijkingen. Andere tests op dit gebied, gericht op het opsporen van genetische schade of primaire schade aan het DNA, en uitgevoerd met gist, schimmels, fruitvlieg en zoogdiercellen, leverden zowel positieve als negatieve resultaten op. *In vivo* werd een toename van DNA-synthese gevonden in de lever van ratten en muizen. Dit effect trad op na orale toediening. Nadat de teststof in de buikholte was geïnjecteerd, bevatte het DNA uit organen van ratten en muizen covalent gebonden 1,1,1,2-tetrachloorethaan. Een celtransformatietest in BALB/c-3T3-cellen was echter negatief.

Is tot slot uit dierexperimenteel onderzoek iets bekend over eventuele schade aan de voortplantingsorganen of aan het nageslacht? Over dat mogelijke effect van 1,1,1,2-tetrachloorethaan zijn geen gegevens beschikbaar.

1,1,2,2-tetrachloorethaan

Dierexperimenteel onderzoek geeft aan dat 1,1,1,2-tetrachloorethaan in ernstige mate irriterend is voor de huid en slijmvliezen. Er zijn geen gegevens uit dierexperimenteel onderzoek naar de sensibiliserende eigenschappen. Pas na eenmalige blootstelling aan concentraties van duizenden mg/m^3 werden er effecten gerapporteerd. Voor ratten bedroegen de LC_{50}-waarden na blootstelling gedurende 4 uur: 7000 en 8400 mg/m^3 (respectievelijk 1000 en 1200 ppm).

Anders dan bij 1,1,1,2-tetrachloorethaan zijn voor 1,1,2,2-tetrachloorethaan wel resultaten beschikbaar die afkomstig zijn uit onderzoek waarin proefdieren herhaaldelijk via de ademhalingswegen werden blootgesteld. Deze studies suggereren dat het centrale zenuwstelsel en de lever het meest gevoelig zijn. Ze hebben echter diverse tekortkomingen, zoals te kleine aantallen proefdieren, ongeschikte blootstellingscondities, zeer hoge blootstellingsconcentraties, een te beperkt aantal eindpunten en/of ontoereikende rapportage.

Behalve inhalatie-onderzoek is ook onderzoek beschikbaar over herhaalde orale toediening van 1,1,2,2-tetrachloorethaan. De lever blijkt het meest gevoelige orgaan te zijn. In onderzoek waarbij de teststof per maagsonde werd toegediend, veroorzaakte herhaalde toediening van doseringen van 200-300 mg/kg lg bij ratten zulke ernstige toxische effecten dat de experimenten binnen enkele dagen gestopt moesten worden. Bij mannetjesratten leidden doseringen van 104 mg/kg lg tot effecten in de lever: die nam toe in gewicht. Ook werd een milde tot matige vacuolisering van de levercellen geconstateerd. In tegenstelling tot wat

wordt gevonden bij orale toediening van 1,1,1,2-tetrachloorethaan, waren er geen effecten op de nieren of afwijkende urinewaarden.

Voor studies waarin 1,1,2,2-tetrachloorethaan gedurende 14 weken via het voer aan ratten en muizen werd toegediend, kon de commissie geen 'no-adverse-effect levels' (NOAELs) vaststellen. Bij mannetjesratten leidde toediening van 20 mg/kg lg, de laagste dosis die werd getest, tot een minimale tot milde vacuolisering van levercellen. Bij vrouwtjesmuizen veroorzaakte de voor hen laagste dosis, namelijk 80 mg/kg lg, bij 2 van de 10 dieren levercelhypertrofie, die minimaal tot mild van aard was.

Wat is bekend over de eventuele kankerverwekkende eigenschappen van 1,1,2,2-tetrachloorethaan? In carcinogeniteitsstudies werden mannetjes- en vrouwtjesratten (Osborne-Mendel) gedurende 78 weken blootgesteld aan doseringen van respectievelijk 62 en 108 en 43 en 76 mg/kg lg/dag, waarna een blootstellingsvrije periode volgde van 32 weken. Deze blootstelling leidde niet tot een statistisch significante toename in tumorincidenties. Muizen ($B6C3F_1$) werden gedurende 78 weken dagelijks blootgesteld aan doseringen van 142 en 284 mg/kg lg waarna een blootstellingsvrije periode van 12 weken volgde. In de hoogste doseringsgroep was er een toename in sterfte. Blootstelling resulteerde in een statistisch significante toename in de incidentie van levercarcinomen bij mannetjes en vrouwtjes.

Ontstaan en groei van tumoren na blootstelling aan 1,1,2,2-tetrachloorethaan werden onderzocht in een tumorinitiatie/promotietest in Osborne-Mendelratten. Daaruit blijken een zwak initiërend effect en een wat sterkere promoverende werking.

Om na te gaan of blootstelling tot schade aan het erfelijk materiaal kan leiden werden *in vitro*-mutageniteitstesten uitgevoerd in verschillende bacterie- en giststammen, muislymfoomcellen en fruitvliegen. De resultaten van deze testen waren overwegend negatief. Ook een test voor chromosoomafwijkingen in CHO-cellen was negatief. Andere testen, gericht op het opsporen van genetische schade of primaire schade aan het DNA en uitgevoerd met gist, schimmels, fruitvlieg en zoogdiercellen, leverden zowel positieve als negatieve resultaten op.

Bij onderzoek naar chromosoomafwijkingen dat *in vivo* werd uitgevoerd, leidde orale toediening van 1,1,2,2-tetrachloorethaan aan muizen tot een toename van het aantal erythrocyten met micronuclei. Het verschil met controlewaarden was slechts statistisch significant voor mannetjesmuizen bij toxische doseringen van 700 en 1360 mg/kg lg. Eenmalige orale toediening leidde niet tot een verhoogde DNA-herstel-synthese (UDS) of S-fase-synthese in levercellen van muizen. Na injecteren in de buikholte bevatte het DNA uit organen van ratten en muizen wel covalent gebonden 1,1,2,2-tetrachloorethaan.

Verder werd radioactiviteit, die via gelabeld 1,1,2,2-tetrachloorethaan aan muizen was gevoerd, deels teruggevonden in het DNA dat geïsoleerd werd uit de lever. Vermoedelijk is hier echter geen sprake van de vorming van adducten, maar van de incorporatie van koolstoffragmenten via gebruikelijke stofwisselingsprocessen. Een positief en een negatief resultaat is gemeld in celtransformatietesten met BALB/c-3T3-cellen, tenminste wanneer die werden uitgevoerd zonder toevoeging van leverenzymextracten. Twee testen die werden uitgevoerd na toevoeging van dergelijke extracten, leverden beide positieve uitslagen op.

Tot slot zijn er gegevens over eventuele schade aan de voortplantingsorganen en het nageslacht. Wanneer 1,1,2,2-tetrachloorethaan via het voer werd toegediend aan mannetjes- en vrouwtjesratten, veroorzaakten doseringen van respectievelijk 40 mg/kg lg en hoger en 170 mg/kg lg en hoger schade aan de voortplantingsorganen. De aard van de schade wijst op mogelijk verminderde vruchtbaarheid. Er zijn echter geen valide studies waarin de mogelijke effecten van blootstelling aan 1,1,2,2-tetrachloorethaan op de vruchtbaarheid of zwangerschap zijn onderzocht.

Evaluatie van de gegevens

1,1,1,2-tetrachloorethaan

Is het op basis van het beschikbare onderzoek gerechtvaardigd om een gezondheidskundige advieswaarde af te leiden?

Op basis van *in vitro*- en *in vivo*-mutageniteits- en genotoxiciteitstesten concludeert de Commissie WGD dat 1,1,1,2-tetrachloorethaan geen stochastisch genotoxisch werkingsmechanisme heeft. Op basis van de beschikbare gegevens over carcinogeniteit en genotoxiciteit concludeert de commissie dat 1,1,1,2-tetrachloorethaan weliswaar uitgebreid is onderzocht, maar dat er onvoldoende bewijs is om een classificatie te rechtvaardigen als ‘kankerverwekkend voor de mens’ of als ‘moet beschouwd worden als kankerverwekkend voor de mens’. De commissie ziet echter wel reden voor bezorgdheid. Daarom classificeert zij 1,1,1,2-tetrachloorethaan als een verdacht kankerverwekkende, niet-genotoxische, stof (te vergelijken met EU categorie (3A)).

De kwalificatie van 1,1,1,2-tetrachloorethaan als een verdacht kankerverwekkende, maar niet genotoxische verbinding maakt volgens de Commissie WGD in principe de afleiding van een gezondheidskundige advieswaarde mogelijk.

Zijn er vervolgens ook voldoende gegevens beschikbaar om een grenswaarde te kunnen adviseren? Naar het oordeel van de Commissie WGD is dat niet het geval. Er zijn namelijk geen gegevens beschikbaar uit humane of dierexperimentele inhalatiestudies die als uitgangspunt kunnen dienen voor het afleiden van een gezondheidskundige advieswaarde. Ook de orale dierexperimentele studies acht de commissie daarvoor niet geschikt. De gegevens over 1,1,2,2-tetrachloorethaan kunnen evenmin worden gebruikt. Gegevens uit proefdierstudies suggereren namelijk dat er wat betreft metabolisme en doelorganen verschillen bestaan tussen 1,1,1,2- en 1,1,2,2-tetrachloorethaan.

Het eindoordeel is dat de beschikbare toxicologische gegevens uit humaan en dierexperimenteel onderzoek onvoldoende zijn voor het afleiden van een concrete gezondheidskundige advieswaarde voor beroepsmatige blootstelling aan 1,1,1,2-tetrachloorethaan.

1,1,2,2-tetrachloorethaan

Is het op grond van het beschikbare onderzoek gerechtvaardigd om een gezondheidskundige advieswaarde af te leiden voor het tweede isomeer die hier wordt beoordeeld: 1,1,2,2-tetrachloorethaan?

Uit de resultaten van *in vitro*- en *in vivo*-mutageniteits- en genotoxiciteitsonderzoek concludeert de Commissie WGD dat 1,1,2,2-tetrachloorethaan geen stochastisch genotoxisch werkingsmechanisme heeft. Op basis van de beschikbare gegevens over carcinogeniteit en genotoxiciteit concludeert de commissie dat 1,1,2,2-tetrachloorethaan onvoldoende is onderzocht. Hoewel er onvoldoende bewijs is om een classificatie te rechtvaardigen als ‘kankerverwekkend voor de mens’ of als ‘moet beschouwd worden als kankerverwekkend voor de mens’, is er wel reden voor bezorgdheid. Daarom classificeert de commissie 1,1,2,2-tetrachloorethaan als een verdacht kankerverwekkende, niet-genotoxische, stof (te vergelijken met EU categorie (3B)).

De kwalificatie van 1,1,2,2-tetrachloorethaan als een verdacht kankerverwekkende maar niet genotoxische verbinding maakt volgens de Commissie WGD in principe de afleiding van een gezondheidskundige advieswaarde mogelijk.

Vervolgens zijn er dan gegevens nodig om een concrete grenswaarde te kunnen adviseren. In dit geval hoeft dat alleen voor blootstelling gedurende 8 uur. Een grenswaarde voor blootstelling gedurende 15 minuten is niet nodig, gezien de

beschikbare humane en dierexperimentele gegevens over irritatie en acute toxiciteit.

Voor het afleiden van een gezondheidskundige advieswaarde als tijdgewogen gemiddelde over 8 uur kunnen de beschikbare humane en dierexperimentele gegevens uit inhalatie-onderzoek, waarin effecten op het zenuwstelsel, het maagdarmkanaal en de nieren werden gevonden, naar het oordeel van de commissie niet als basis dienen.

De commissie baseert zich daarom op orale studies. Daarbij kon de commissie geen 'no-observed-adverse-effect levels' (NOAELs) vaststellen in studies waarbij ratten en muizen veertien weken lang 1,1,2,2-tetrachloorethaan via hun voer kregen toegediend. Hoewel de vacuolisering van levercellen die daarbij optrad op zich niet noodzakelijk nadelig hoeft te zijn, is de commissie van mening dat vacuolisering in dit geval een voorstadium is van ernstigere effecten, zoals hypertrofie en necrose. Op grond daarvan neemt de commissie de 'lowest-observed-adverse-effect level' (LOAEL) uit de rattenstudie van 20 mg/kg lg als uitgangspunt voor de afleiding van een gezondheidskundige advieswaarde.

De volgende stap is de toepassing van extrapolatiefactoren. In dat verband wijst de commissie op de geringe ernst van het kritische effect en de marge met de dosis die ernstiger effecten (hypertrofie/necrose) veroorzaakt. Ook moet meegewogen worden dat in het onderzoek de dieren continu toegang hadden tot het voer, zonder enige blootstellingsvrije herstelperiode, terwijl werkers doorgaans 8 uur per dag en 5 dagen per week worden blootgesteld, zodat herstel mogelijk is. Vandaar dat de commissie een factor van slechts 2 verantwoord acht als compensatie voor het ontbreken van een NOAEL. Voor het verschil in blootstellingsduur past ze geen extrapolatiefactor toe. Voor de intra- en interspeciesverschillen kent de commissie een factor 10 toe.

Uitgaande van de LOAEL van 20 mg/kg lg en een totale extrapolatiefactor van 20 beveelt de commissie voor 1,1,2,2-tetrachloorethaan een gezondheidskundige limietwaarde aan van 7 mg/m^3. De commissie neemt daarbij aan dat een werker met een lichaamsgewicht van 70 kg 10 m^3 lucht inademt gedurende een werkdag van 8 uur en dat alle ingeademde stof ook door het lichaam wordt opgenomen.

Omdat opname via de huid aanzienlijk kan bijdragen tot de lichaamsbelasting, is de commissie WGD van mening dat een huidnotatie moet worden toegekend.

Gezondheidskundige advieswaarde

De Commissie WGD van de Gezondheidsraad stelt voor 1,1,2,2-tetrachloorethaan een gezondheidskundige advieswaarde voor van 7 mg/m^3 (1 ppm), gemiddeld over een achturige werkdag (8-uur t.g.g.). Ook adviseert zij een huidnotatie.

Voor 1,1,1,2-tetrachloorethaan kan de commissie geen gezondheidskundige advieswaarde voorstellen.

Dit advies bevat een aanvullende overweging van de commissie over het gebruik van de gezondheidskundige advieswaarde van 1,1,2,2-tetrachloorethaan voor het afleiden van een grenswaarde voor 1,1,1,2-tetrachloorethaan bij beroepsmatige blootstelling.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands sets health-based recommended occupational exposure limits (HBR-OEL) for toxic substances in the workplace air. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). It constitutes the first step in a three-step procedure which leads to legally binding occupational exposure limits.

The present report on tetrachloroethane was prepared in cooperation with the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG), an advisory body of the Nordic countries. The joint report on the consequences of occupational exposure to tetrachloroethane, published in Sweden in 1996 (Arbete och Hälsa 1996:28), is included in Part II of this document. Part I consists of a summary of the data presented in Part II, presentation of data becoming available since 1996, and a discussion of the consequences of occupational exposure to the tetrachloroethane isomers. The conclusions in Part I are based on scientific publications which appeared before January 2006, and are entirely DECOS' view.

Occurrence, physical and chemical properties

Tetrachloroethane is a chemical compound occurring in two isomers: 1,1,1,2-tetrachloroethane (CAS number 630-20-6) and 1,1,2,2-tetrachloroethane (CAS number 79-34-5). At room temperature, both isomers are colourless, non-flammable, heavy liquids with a low to moderate volatility. Neither of the isomers is known to occur naturally.

1,1,1,2-Tetrachloroethane is not produced on an industrial scale. It is, however, a common by-product of many industrial chlorination reactions of C_2 hydrocarbons, among which the production of 1,1,2,2-tetrachloroethane.

1,1,2,2-Tetrachloroethane is used as an intermediate in the manufacture of other chlorinated hydrocarbons. In the past, 1,1,2,2-tetrachloroethane was rather extensively used, amongst others having many uses as a solvent. Due to its toxicity, the present production of 1,1,2,2-tetrachloroethylene may be very limited.

Monitoring

Several organisations, including the Nederlands Normalisatie-instituut, the National Institute of Occupational Safety and Health and the Environmental Protection Agency in the United States, and the Health and Safety Executive in the United Kingdom, have described methods, which can be used for analysing 1,1,2,2-tetrachlorethane in workplace air. These methods use subsequently active sampling on sorbent tubes, thermal or liquid desorption, and gas chromatographic analysis. The suitability of the method depends on the origin of the material in the sorbent tube. Further, there are other methods using passive samplers and portable chromatographs.

Current limit values and classification

For 1,1,1,2-tetrachloroethane, no limit values have been established nationally and internationally. For 1,1,2,2-tetrachloroethane, the occupational exposure limit is 7 mg/m^3 (1 ppm) in most countries. A skin notation is added. This indicates that the compound may relatively easily enter the body through the skin which may contribute significantly to the body burden.

Germany and NIOSH have classified it as a suspected and potential carcinogen, respectively; ACGIH as an A3 carcinogen (i.e., a confirmed animal carcinogen with unknown relevance to humans).

Kinetics

1,1,1,2-tetrachloroethane

No data are available on the kinetics (absorption, metabolism, distribution, and excretion) of 1,1,1,2-tetrachloroethane following the main occupational exposure routes, viz., via inhalation and skin contact.

However, there are data on absorption following oral administration. Studies in rats and mice indicate an almost complete absorption. When absorbed, rats appear to exhale unchanced 1,1,2,2-tetrachloroethane six times as much as mice. The metabolism of 1,1,1,2-tetrachloroethane proceeds through both oxidative and reductive pathways resulting in CO_2 – in exhaled air – and trichloroethanol and trichloroacetic acid – in urine – as main metabolites. The data also show that in rats, there is a difference in metabolism of 1,1,1,2- and 1,1,2,2-tetrachloroethane. The amount of trichloro compounds in the urine of rats exposed to 1,1,1,2-tetrachloroethane by inhalation or an intraperitoneal injection was 20 times as high as the amount of trichloro compounds in the urine of rats similarly exposed to the 1,1,2,2 isomer.

1,1,2,2-tetrachloroethane

Experimental animals data show that 1,1,2,2-tetrachloroethane is well absorbed following inhalation and oral administration. No experimental data were available following skin contact. However, for human skin, a steady state flux of 27 $\mu g/cm^2/h$ has been calculated based on molecular weight, octanol-water partition coefficient, and contact with a saturated aqueous solution (3 mg/mL).

Following absorption, biotransformation of 1,1,2,2-tetrachloroethane may involve a number of oxidative dechlorination pathways, and there is some evidence for a reductive pathway (leading to a carbon-centred radical and subsequent lipid peroxidation in the liver) as well. Following inhalation and oral administration, rats and mice metabolised 1,1,2,2-tetrachloroethane extensively. Generally, CO_2 was the main metabolite. In urine, dichloroacetic acid was found to be the major urinary metabolite. Compared to mice, rats exhaled 4 and 14 times more unmetabolised 1,1,2,2-tetrachloroethane following inhalation and oral administration, respectively, which might render rats more sensitive to CNS effects.

Effects in humans

1,1,1,2-tetrachloroethane

No data are available on the potential irritating or sensitising properties or on the system toxic effects of 1,1,1,2-tetrachloroethane in humans.

1,1,2,2-tetrachloroethane

There are human data available on the effects following exposure to 1,1,2,2-tetrachloroethane.

In 2 male volunteers, dizziness and mucosal irritation were observed within 10 to 12 minutes when exposed to 1000 or 1800 mg/m^3 (144, 262 ppm) 1,1,2,2-tetrachloroethane, but no such effects were seen at exposure up to 90 mg/m^3 (13 ppm) for 10 minutes.

In studies on workers occupationally exposed to 1,1,2,2-tetrachloroethane – published before 1965 –, the liver, the gastrointestinal tract, and the nervous system were the target organs. In one survey, in which especially fine finger tremor was reported, there was not only inhalation exposure but also skin contact, as well as exposure ot acetone.

No statistically significant increases in mortality, overall cancer mortality, and cancer incidences were found in a retrospective study on a cohort consisting of 1099 white men with exposure to 1,1,2,2-tetrachloroethane while using impregnating clothing machinery and some additional exposure to dry-cleaning solvents.

Effects in experimental animals

1,1,1,2-tetrachloroethane

Inhalation of 1,1,1,2-tetrachloroethane or contact with the liquid may cause irritation of the skin and mucous membranes or sensitisation. However, no such experimental animal data were available.

Taking mortality as an end point, 1,1,1,2-tetrachloroethane is not very toxic following single exposure. Four-hour LC_{50} values of 14,600 mg/m^3 (2100 ppm) were found for rats and of 19,500 mg/m^3 (2800 ppm) for rabbits; a dermal LD_{50} of 20,000 mg/kg bw was reported for rabbits.

No data were available from repeated inhalation studies. In repeated-dose gavage studies, besides the CNS, the target organs seem to be the kidney in (male) rats and the liver in mice. When male rats were given daily doses of 104 and 208 mg/kg bw, only findings indicative of hyaline droplet-induced nephropathy were observed. This type of kidney damage is generally not considered to be relevant to humans.

Another effect might be the induction of tumours. In a carcinogenicity study, daily administration of doses of 0, 125, and 250 mg/kg bw/day to Fischer rats, did not induce statistically significant increases in tumour incidences in any of the treated groups. In the females of the low-dose group, however, there was a statistically significant increase in the incidence of fibroadenomas. In $B6C3F_1$ mice, doses of 0, 250, or 500 mg/kg bw caused statistically significant increases in the incidence of hepatocellular adenomas in males and females and of hepatocellular carcinomas in females.

1,1,1,2-Tetrachlorethane was negative in a tumour-initiation/promotion assay in male Osborne-Mendel rats.

In *in vitro* mutation tests, mostly negative results were obtained in bacteria and mammalian cells; tests in yeast and fruit fly were negative as well. In a chromosome aberration assay in Chinese hamster ovary cells, 1,1,1,2-tetrachloroethane produced negative results. In Chinese hamster lung fibroblasts, the compound induced increases in the frequency of numerical chromosomal aberrations (i.c., polyploidy), but not of structural chromosomal aberrations. Other tests, indicative of genetic or primary DNA damage in yeast, fungi, fruit fly, or mammalian cells, produced both positive and negative results. *In vivo*, an increase in hepatic DNA synthesis was found in orally treated rats and mice. Following intraperitoneal injections, 1,1,1,2-tetrachloroethane was bound covalently to DNA in rat and mouse organs. A cell transformation assay in BALB/c-3T3 cells was negative.

Finally, no data were available from reproduction toxicity studies.

1,1,2,2-tetrachloroethane

Experimental animal data indicate that liquid 1,1,2,2-tetrachloroethane is strongly irritating to the skin and mucous membranes. No data were available from experimental animal sensitisation studies. Following single inhalation exposure, effects were reported only at thousands of mg/m^3. In rats, the 4-hour LC_{50} values were 7000 and 8400 mg/m^3 (1000 and 1200 ppm).

Contrary to 1,1,1,2-tetrachloroethane, there were experimental animal data on 1,1,2,2-tetrachloroethane following repeated inhalation exposure. The studies

available suggest that the CNS and the liver are the target organs. However, they suffer from several flaws such as insufficient number of animals, inappropriate exposure regimens, very high exposure concentrations, limited number of end points examined, and/or insufficient reporting.

Besides inhalation studies, studies in which 1,1,2,2-tetrachloroethane was repeatedly orally administered were available. The liver was the most sensitive organ. In gavage studies, repeated doses of 200-300 mg/kg bw induced such severe toxicity in rats that experiments were broken off within a few days. In male rats, doses of 1,1,2,2-tetrachloroethane of 104 mg/kg bw affected the liver (increased weights accompanied by mild to moderate cytoplasmic vacuolisation). Contrary to 1,1,1,2-tetrachloroethane, no effects on the kidney or urinalysis parameters were seen.

In 14-week diet studies in rats and mice, DECOS could not establish NOAELs. In male rats, administration of 20 mg/kg bw, the lowest dose tested, induced minimal to mild vacuolisation of hepatocytes. In female mice, 80 mg/kg bw, also the lowest dose, caused minimal to mild hepatocytic hypertrophy in 2/10 females.

What is known about the potential carcinogenic effects of 1,1,2,2-tetrachloroethane? In carcinogenicity studies male and female Osborne-Mendel rats were given time-weighted average doses of 62 and 108 and 43 and 76 mg/kg bw/day, respectively, for 78 weeks followed by a 32-week treatment-free period. Treatment did not cause statistically significant increases in the incidence of any tumour type in any of the groups. B6C3F_1 mice were given time-weighted average doses of 142 and 284 mg/kg bw/day for 78 weeks followed by an exposure-free period of 12 weeks. In the high-dose group, mortality was increased. Treatment caused statistically significant increases in the incidences of hepatocellular carcinomas in males and females.

1,1,2,2-Tetrachloroethane had a weakly tumour-initiating and a more strongly tumour-promoting activity in Osborne-Mendel rats in an initiation/promotion assay.

In *in vitro* mutation tests, 1,1,2,2-tetrachloroethane was negative in the majority of tests using *S. typhimurium* strains, in yeast strains, mouse lymphoma cells, and fruit flies. A test for chromosome aberrations in CHO cells was negative as well. Other tests, indicative of genetic or primary DNA damage in yeast, fungi, fruit fly, or mammalian cells, produced both positive and negative results. *In vivo*, 1,1,2,2-tetrachloroethane caused small increases in the frequency of micronuclei in erythrocytes obtained from orally treated mice which reached statistically significance only in male animals at high toxic doses of 700 and 1360 mg/kg bw/day. No UDS or S-phase synthesis was seen in hepatocytes isolated

from mice given single oral doses. Following intraperitoneal injections, 1,1,2,2-tetrachloroethane was bound covalently to DNA in rat and mouse organs. In orally dosed mice, binding of 1,1,2,2-tetrachloroethane-derived radioactivity to hepatic DNA was thought to be due to incorporation of metabolic one-carbon fragments rather than adduct formation. In cell transformation assays in BALB/c-3T3 cells, there was one positive and one negative result when tested in the absence of metabolic activation, and two positive results in the presence of metabolic activation.

Finally, there were data on the potential effects on the reproductive tissues and the offspring. 1,1,2,2-Tetrachloroethane affected the reproductive tissues of male rats at dietary doses of 40 mg/kg bw/day and higher and of female rats at 170 mg/kg bw and higher, indicating that it might compromise fertility. However, there were no data from valid studies addressing the potential effects of 1,1,2,2-tetrachloroethane on fertility or pregnancy outcome.

Evaluation

1,1,1,2-tetrachloroethane

Do the available data justify the derivation of a health-based recommended occupational exposure limit?

From *in vitro* and *in vivo* mutagenicity and genotoxicity studies, DECOS concludes that 1,1,1,2-tetrachloroethane is not a stochastic genotoxic compound. Based on the carcinogenicity and genotoxicity data, the committee concludes that 1,1,1,2-tetrachloroethane has been extensively investigated. Although there is insufficient evidence to warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern. Therefore, 1,1,1,2-tetrachloroethane is classified as a suspect (non-genotoxic) carcinogen (comparable to EU category 3(A)).

According to DECOS, the qualification of 1,1,1,2-tetrachloroethane as a suspect, non-genotoxic carcinogen, basically warrants the derivation of a health-based limit value.

Are there sufficient data available to allow recommendation of a limit value?

According to DECOS, this is not the case. The committee did not find human or experimental animal inhalation data that could be used as a starting point for deriving a health-based occupational exposure limit. DECOS also considers the oral experimental animals studies as inappropriate. Further, DECOS is of the opinion that the data on 1,1,2,2-tetrachloroethane cannot be used either. Experi-

mental animal data suggest that there are differences between 1,1,1,2- and 1,1,2,2-tetrachloroethane with respect to metabolism and target organs.

DECOS considers the toxicological database for 1,1,1,2-tetrachloroethane too poor to recommend a health-based occupational exposure limit.

1,1,2,2-tetrachloroethane

Do the available investigations justify the derivation of a health-based recommended occupational exposure limit for the other isomer evaluated: 1,1,2,2-tetrachloroethane?

From the mutagenicity and genotoxicity data, DECOS concludes that 1,1,2,2-tetrachloroethane is not a stochastic genotoxic compound. Based on the data on carcinogenicity and mutagenicity, the committee concludes that 1,1,2,2-tetrachloroethane has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern. Therefore, 1,1,2,2-tetrachloroethane is classified as a suspect (non-genotoxic) carcinogen (comparable with EU category 3(B)).

According to DECOS, the qualification of 1,1,2,2-tetrachloroethane as a suspect, non-genotoxic carcinogen, basically warrants the derivation of a health-based limit value.

Are there sufficient data available to allow recommendation of a limit value?

DECOS is of the opinion that the available human and animal experimental data on irritation and acute toxicity do not suggest the need for a short-term (15-minute) exposure limit value.

To derive a health-based recommended occupational exposure limit, DECOS is of the opinion that the available human and experimental inhalation data, which showed that the liver, the gastrointestinal tract, and the nervous system were the target organs, are inappropriate as starting points. Therefore, the committee uses oral studies. The committee could not establish NOAELs in studies in which rats and mice received 1,1,2,2-tetrachloroethane in their diets for 14 weeks. Although the hepatocellular vacuolisation observed by itself is not necessarily an adverse effect, the committee is of the opinion that in this case vacuolisation marks the first step leading to more severe effects such as hypertrophy and necrosis. Therefore, the committee takes the LOAEL of 20 mg/kg bw/day found in the rat study as the starting point for deriving a health-based occupational exposure limit (HBROEL).

To arrive at a HBROEL, extrapolation factors are used. In this respect, the committee notes the mildness of the key effect and the gap between the doses inducing the mild (vacuolisation) and more severe (hypertrophy/necrosis) effects. Further, in the experimental animal study, the animals had continuous access to the test compound without any exposure-free period to recover. On the other hand, workers are exposed 8 hours/day, 5 days/week allowing recovery. Therefore, the committee considers a factor of only 2 justified for the absence of a NOAEL and does not apply a factor for difference in 'exposure duration'. For intraspecies and interspecies variation, the committee takes a total factor of 10.

Taking the LOAEL of 20 mg/kg bw and applying the total extrapolation factor of 20, the committee recommends a health-based occupational exposure limit of 7 mg/m^3 for 1,1,2,2-tetrachloroethane, assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%.

Since dermal penetration may contribute significantly to the body burden, the committee considers a skin notation warranted.

Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards of the Health Council recommends a health-based occupational exposure limit for 1,1,2,2-tetrachloroethane of 7 mg/m^3 (1 ppm) as an 8-hour time-weighted average concentration. It also recommends a skin notation.

The committee cannot recommend a health-based occupational exposure limit for 1,1,1,2-tetrachloroethane.

This report contains an additional consideration of the committee about the use of the health-based occupational exposure limit of 1,1,2,2-tetrachloroethane for setting an occupational exposure limit for 1,1,1,2-tetrachloroethane.

Part I

Health Council of the Netherlands: Tetrachloroethane

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (Annex A). The purpose of the committee's evaluation is to set a health-based recommended occupational exposure limit for the atmospheric concentration of the substance, provided the database allows the derivation of such a value.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister on the feasibility of using the health-based value as a regulatory Occupational Exposure Limit (OEL), or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committee and method of work

This document is a co-production of DECOS and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). It is a result of an agreement between both groups to prepare jointly criteria documents which can be used by the regulatory authorities in the Netherlands and in the Nordic

countries for establishing occupational exposure limits. The members of DECOS and NEG are listed in Annex B.

The joint draft document has been prepared by Marita Luotamo, Ph.D. and Vesa Riihimäki, Ph.D. from the Finnish Institute of Occupational Health, Helsinki, Finland, and was reviewed by NEG and subsequently by DECOS, before the final document was published by the Swedish National Institute for Working Life (Arbete och Hälsa 1996:28) in 1996. The final document is included in Part II of this report. Part I consists of a summary of the data presented in Part II, presentation of data becoming available since 1996, and a discussion of the consequences of occupational exposure to the tetrachloroethane isomers. DECOS, hereafter called the committee, used data from both parts in assessing a health-based occupational exposure limit.

1.3 Data

In the sections below, a summary of the findings from the joint NEG/DECOS report on tetrachloroethane (see Part II)* is presented firstly under 'NEG data' (in 'citaat' style), while any additional information from the literature searches is subsequently included in the paragraph 'additional information'.

Additional data were obtained from searches performed in May 2005 in the on-line databases Toxline, Medline, and Chemical Abstracts, starting from 1995. The final search was performed in Medline in January 2006.

* References from Part II are referred to as 'NEGxx'.

Chapter 2

Identification, properties, and monitoring

2.1 Identification, physical and chemical properties

NEG data

Tetrachloroethane occurs in two isomeric forms: 1,1,1,2-tetrachloroethane and 1,1,2,2-tetrachloroethane.

1,1,1,2-tetrachloroethane. 1,1,1,2-Tetrachloroethane is a colourless, non-flammable, heavy liquid, miscible with ethanol, diethyl ether, acetone, benzene, and chloroform. Its melting and boiling point are -68.7 and 130.5°C, respectively. At 20°C, the vapour pressure is 0.66 kPa. 1,1,1,2-Tetrachloroethane is more stable than its 1,1,2,2-isomer.

1,1,2,2-tetrachloroethane. 1,1,2,2-Tetrachloroethane is a colourless, non-flammable, heavy liquid with a sweetish odour. It is miscible with several organic solvents. Its melting and boiling points are -42.5 and 146.5°C, respectively. At 20°C, the vapour pressure is 0.68 kPa. 1,1,2,2-Tetrachloroethane is sufficiently stable to be stored without adding stabilisers in the absence of moisture, air, and light.

Additional data

1,1,1,2-tetrachloroethane. 1,1,1,2-Tetrachloroethane is very poorly miscible with water (at 25°C: 0.11 g/100 mL).[1] The estimated partition coefficient,

log $P_{octanol/water}$ is 2.93 (http://esc.syrres.com/esc/est_kowdemo.htm; accessed September 21, 2004).

The committee did not find odour threshold data.

1,1,2,2-tetrachloroethane. 1,1,2,2-Tetrachloroethane is poorly miscible with water (at 25°C: 0.3 g/100 mL).[2] The experimental and estimated partition coefficients, log $P_{octanol/water}$, are 2.39 and 2.19, respectively (http://esc.syrres.com/esc/est_kowdemo.htm; accessed September 21, 2004). Odour threshold values of 10 (1.5 ppm)[3] and 21-35 mg/m^3 (3-5 ppm)[4] have been listed. A concentration of 1302 mg/m^3 (187 ppm) was stated to be irritating.[4]

2.2 EU classification and labelling

Additional data

1,1,1,2-tetrachloroethane. The committee did not find data on the classification and labelling of 1,1,1,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. The EU has classified and labelled 1,1,2,2-tetrachloroethane as follows:

Symbols	T	Toxic.
	N	Environmentally dangerous.
Risk phrases	R26/27	Very toxic by inhalation and in contact with skin.
	R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases	S1/2	Keep locked up and out of reach of children.
	S38	In case of insufficient ventilation, wear suitable respiratory equipment.
	S45	In case of accident or if you feel unwell, seek medical advice; immediately (show the label where possible).
	S61	Avoid release to the environment. Refer to special instructions/material safety data sheet.

2.3 Analytical methods

NEG data

1,1,1,2-tetrachloroethane. No analytical methods for the determination of 1,1,1,2-tetrachloroethane were presented.

1,1,2,2-tetrachloroethane. NIOSH has published an analytical method (method 1019) for 1,1,2,2 – tetrachloroethane (see further below). In addition, methods using passive samplers and portable chromatographs have been described.[NEG17,NEG25,NEG50]

Additional data

1,1,1,2-tetrachloroethane.

- The Nederlands Normalisatie-instituut (NEN) has published a standard (NEN 2965) which describes the determination of the concentration of vaporous chlorinated hydrocarbons in workplace air. 1,1,1,2-Tetrachloroethane is listed among the compounds for which this standard is applicable. The method uses active sorbent tube (Tenax) sampling, thermal desorption, and gas chromatography (with flame ionisation detection). The working range is 0.00017-400 mg/m^3 for an 8-hour sampling time and 0.0056->10,000 mg/m^3 for a 15-minute sampling time (sampling volume: 2.5 L). The limit of detection is 5 ng.[5]

For ambient air, NEN has published a pre-standard which describes a method (NVN 2793:2006 2^e Ontw.nl) for the determination of the concentration of vaporous chlorinated and aromatic hydrocarbons, using charcoal tube adsorption, liquid desorption, and gas chromatography. 1,1,1,2-Tetrachloroethane was listed among the compounds for which this method can be applied.[6]

1,1,2,2-tetrachloroethane. For the determination of concentrations of 1,1,2,2-tetrachloroethane in workplace air, the following methods are available:

- NVN 2948[7]/2965[5]. For the determination of 1,1,2,2-tetrachloroethane, the Dutch Occupational Hygiene Society (NvvA)[8] refers to this method developed and published by the Nederlands Normalisatie-instituut (NEN) for the determination of the concentration of vaporous chlorinated hydrocarbons. The method uses active sorbent tube (Tenax) sampling, thermal desorption, and gas chromatography (with flame ionisation detection). The working range is 0.00017-400 mg/m^3 for an 8-hour sampling time and 0.0056->10,000 mg/m^3 for a 15-minute sampling time (sampling volume: 2.5 L). The limit of detection is 5 ng.[5]
- NIOSH method 2562. NIOSH has updated the method mentioned above (i.e., method 1019), through the use of a capillary column and the incorporation of other sorbent tubes (Anasorb CMS), lowering sampling and analytical range. The working range of the new method is 0.031-4.17 ppm (0.21-28.6 mg/m^3) for a 10-L air sample. The estimated limit of detection is 0.6 µg per sample.

Range, bias, overall precision, and accuracy were not studied or determined. Evaluating method 1019, it was stated that the desorption efficiency for petroleum-based charcoal ranged from 0.83-0.87 at amounts of 160-640 µg 1,1,2,2-tetrachloroethane per sample (vs. 0.91, 0.85, 0.88, and 0.91 at 38, 76, 165, and 254 µg per sample, respectively, for Anasorb). Recoveries were lower for coconut shell charcoal, while rapid degradation into trichloroethylene was observed during storage on Pittsburgh activated carbon.[9]

- MDHS96. In 2000, the UK Health and Safety Executive (HSE) published a method for the determination of volatile organic compounds, including chlorinated hydrocarbons. This method included and replaced the existing method for chlorinated hydrocarbons (MDHS28). MDHS96 uses pumped solid sorbent tubes, solvent desorption, and gas chromatography. For 1,1,2,2-tetrachloroethane, petroleum-based charcoal and carbon disulphide are recommended as the sorbent and desorption solvent, respectively.[10]

For the determination of volatile organic compounds in ambient air, methods have been presented by NEN (NVN 2794:1985 nl)[11], NEN and the International Organization for Standardization (ISO) ((NEN-EN)-ISO 16017-2:2003)[12,13], and by the US Environmental Protection Agency (EPA) (compendium method TO-14A).[14,15] In the NEN methods, (diffusive) sampling and analysis occur by sorbet tube, liquid or thermal desorption, and (capillary) gas chromatography.[11-13] In the EPA method, whole air samples are collected in specially prepared canisters. The volatile compounds are concentrated in the laboratory with cryogen trap. They are re-volatilised, separated by on gas chromatography column, and passed to one or more detectors for identification and quantification.[14,15] These methods should be applicable for 1,1,2,2-tetrachloroethane*.

The committee notes that it is reported that in the adsorption-desorption process, degradation into trichloroethylene can occur [9,16] and that recovery and desorption can be impaired by the type of sorbent used.[9,17,18]

* The committee notes that method NVN 2794:1985 nl will be replaced by NVN 2793:2006 2e Ontw.nl. and that 1,1,2,2-tetrachloroethane is not listed among the compounds for which NVN 2793:2006 2e Ontw.nl can be applied.

Chapter 3

Sources

NEG data

1,1,1,2-tetrachloroethane. 1,1,1,2-Tetrachloroethane is a common by-product of many industrial chlorination reactions. It is not produced on an industrial scale.[NEG105]

1,1,2,2-tetrachloroethane. 1,1,2,2-Tetrachloroethane is used as an intermediate in the manufacture of other chlorinated hydrocarbons. Mostly, it is not isolated, but immediately thermally cracked; however, it can be isolated as a by-product to be used as a feedstock. Consequently, it can be present as a minor impurity in the end products.[NEG1,NEG78] In the past, 1,1,2,2-tetrachloroethane had many uses as a solvent.[NEG5,NEG75,NEG76,NEG78] Due to its toxicity and changes in manufacturing processes and in uses of chlorinated ethylenes, the production of 1,1,2,2-tetrachloroethylene may be very limited.[NEG1]

Additional data

1,1,1,2-tetrachloroethane. 1,1,1,2-Tetrachloroethane is a by-product from the production of 1,1,2,2-tetrachloroethane.[19]

If recovered from, e.g., the production of trichloroethanes, 1,1,1,2-tetrachloroethane can be used as a feedstock for the production of tri- or tetrachloroethylene.[19] Consequently, it might be present as a minor impurity in the end products.

1,1,2,2-tetrachloroethane. In the production processes of 1,1,2,2-tetrachloroethane, the 1,1,1,2-isomer can be formed as well, the amount being dependent on the process conditions.[19]

Chapter 4

Existing guidelines, standards and evaluations

4.1 Working population

Additional data

1,1,1,2-tetrachloroethane. The committee did not find occupational exposure limits in any of the countries listed below in the section on 1,1,2,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. Occupational exposure limits for 1,1,2,2-tetrachloroethane in the USA and some European countries, listed in the most recent publications available to the committee, are presented below.

country - organisation	OEL mg/m^3	ppm	time-weighted average	type of OEL	note[a]	reference[b]
the Netherlands						
- Ministry of Social Affairs and Employment	7	1	8 h	administrative		20
Germany						
- DFG MAK-Kommission	7.0	1	8 h		S,[d,e]	21
	14	2	15 min[c]			
- AGS	7	1	8 h		S	22
	14	2	15 min			
Norway	-	1			S	23
Sweden	-	-				24

Denmark	7	1	8 h			25
Finland	7	1	8 h		S	26
	21	3	15 min			
Iceland	7	1	8 h		S	27
United Kingdom						
- HSE	-	-				28
USA						
- ACGIH	-	1	8 h	TLV	S,[f]	29
- OSHA	35	5	8 h	PEL	S	30
- NIOSH	7	1	10 h	REL	S,[g]	30
European Union						
- SCOEL	-	-				31

[a] S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.

[d] Classified in carcinogenicity category 3B, i.e., listed among compounds for which *in vitro* or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK or BAT (biological tolerance value for working materials) value can be established provided no genotoxic effects have been detected.

[e] Listed among substances with MAK values but no pregnancy risk group classification.

[f] Classified in carcinogenicity category A3, i.e., a confirmed animal carcinogen with unknown relevance to humans: The agent is carcinogenic in experimental animals at a relatively high dose, by route(s) of administration, at site(s), of histologic type(s), or by mechanism(s) that may not be relevant to worker exposure. Available epidemiologic studies do not confirm an increased risk of cancer in exposed humans. Available evidence does not suggest that the agent is likely to cause cancer in humans except under uncommon or unlikely routes or levels of exposure.

[g] Potential occupational carcinogen, with no further categorisation.

4.2 Evaluations

NEG data

1,1,1,2-tetrachloroethane.

- IARC. The International Agency for Research on Cancer (IARC) stated that for 1,1,1,2-tetrachloroethane, there were no epidemiological data on cancer in humans available and that there was limited evidence in experimental animals for the carcinogenicity. IARC concluded that 1,1,1,2-tetrachloroethane was not classifiable as to its carcinogenicity to humans (Group 3).[NEG46]

1,1,2,2-tetrachloroethane.

- DFG. The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the Deutsche Forschungsgemeinschaft (DFG) classified 1,1,2,2-tetrachloroethane into carcinogenicity 3B ('justifiable suspected of having carcinogenic potential') and into pregnancy risk group IIC ('substances shown to be hazardous during pregnancy without further categorisation').[NEG20]

- NIOSH. In the late 1970s, the US National Institute for Occupational Health (NIOSH) recommended to handle 1,1,2,2-tetrachloroethane in the workplace as if it were a human carcinogen and to minimise exposure.[NEG75,NEG79]
- IARC. IARC stated that for 1,1,2,2-tetrachloroethane, there was inadequate evidence of the carcinogenicity in humans and limited evidence in experimental animals. IARC concluded that 1,1,2,2-tetrachloroethane was not classifiable as to its carcinogenicity to humans (Group 3).[NEG46]

Additional data

1,1,1,2-tetrachloroethane.

- IARC. In 1999, IARC re-evaluated 1,1,1,2-tetrachloroethane with respect to its carcinogenic risk to humans. It stated that there were no epidemiological data relevant to carcinogenicity available and that there was limited evidence for carcinogenicity in experimental animals. IARC concluded that 1,1,1,2-tetrachloroethane was not classifiable as to its carcinogenicity to humans (Group 3).[32]

1,1,2,2-tetrachloroethane.

- DFG. In 2005, 1,1,2,2-tetrachloroethane was (still) classified in carcinogenicity category 3B, which was reworded: this category includes ‘substances for which *in vitro* or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK or BAT (biological tolerance value for working materials) value can be established provided no genotoxic effects have been detected’. It was further listed among substances with MAK values but no pregnancy risk group classification.[21]
  In 1972, 1,1,2,2-tetrachloroethane was re-evaluated. The German committee concluded that the current MAC value of 1 ppm was merely based on rough estimates. Data from chronic experimental animal studies and from studies on humans with long-term exposure to known occupational levels were lacking.[33] 1,1,2,2-Tetrachloroethane is listed for examination of its carcinogenic potential.[21]
- ACGIH. The American Conference of Governmental Industrial Hygienists (ACGIH) classified 1,1,2,2-tetrachloroethane into carcinogenicity category A3, i.e., a confirmed animal carcinogen with unknown relevance to humans.[29] This notation was based on the production of liver tumours in mice (and possibly in rats), although the potency was very low.[34]

Based on toxic effects occurring in animals and humans at exposure concentrations around 10 ppm, ACGIH recommended an 8-hour TLV of 1 ppm. ACGIH stated that this level should be sufficient to minimise potential serious intoxication and narcosis, hepatic and gastrointestinal effects. ACGIH recommended a skin notation since systemic effects were reported to occur following dermal contact, although doses inducing acute systemic toxicity via the dermal and other routes were relatively high.[34]

- ATSDR. In 1996, the Agency for Toxic Substances and Disease Registry (ATSDR) derived an intermediate-duration inhalation MRL (Minimal Risk Level)* of 0.4 ppm. The MRL was based on a LOAEL of 130 ppm for hepatic effects found in an inhalation study in rats exposed 5 hours/day, 5 days/week, for 15 weeks. Further, ATSDR derived an intermediate duration oral MRL of 0.6 mg/kg bw/day, based on a NOAEL of 56 mg/kg bw/day for body weight gain in rats (LOAEL: 100 mg/kg bw), and a chronic-duration MRL of 0.04 mg/kg bw/day, based on a LOAEL of 43 mg/kg bw/day for respiratory effects in female rats exposed by gavage for 78 weeks.[35]
- IARC. In 1999, IARC re-evaluated 1,1,2,2-tetrachloroethane with respect to its carcinogenic risk to humans. It stated that there was inadequate evidence in humans and limited evidence in experimental animals for carcinogenicity. IARC concluded that 1,1,1,2-tetrachloroethane was not classifiable as to its carcinogenicity to humans (Group 3).[36]
- OECD. 1,1,2,2-Tetrachloroethane has been evaluated within the framework of the High Production Volume (HPV) Chemicals Programme of the Organisation for Economic Co-operation and Development (OECD). In 2002, a final draft of the SIDS** Initial Assessment Report (SIAR) and the summary conclusions of the SIAR have been published. Based on past human experience, 1,1,2,2-tetrachloroethane was considered as very toxic to humans exposed acutely. The substance was concluded to be irritating to the skin and eyes. Repeated-dose experimental animal and human case studies showed that the liver and the kidneys are the target organs but the nervous system and

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* MRLs are derived for acute (1-14 days), intermediate (15-364), and chronic (365 days and longer) duration for the oral and inhalation exposure routes. They are derived when reliable and sufficient data exist to identify the target organ of effect or the most sensitive health effect(s) for a specific duration and exposure route. MRLs are generally based on the most sensitive chemical-induced end point considered to be relevant to humans. Serious health effects (such as irreparable damage to the liver and the kidneys, or birth defects) are not used as a basis. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. They are intended to serve as screening levels to be used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

** SIDS: Screening Information Data Set.

the haematological system can be affected as well. The studies available did not allow drawing conclusions on its reproduction and developmental toxicity. *In vitro* and *in vivo* genotoxicity studies indicated that 1,1,2,2-tetrachloroethane might have some genotoxic potential. In an oral long-term bioassay, the substance induced hepatocellular carcinomas in mice, but no carcinogenic effects in rats.

From past experience, the threshold chronic toxicity by inhalation in humans was estimated to be around 70 mg/m^3. From limited experimental animal studies, LOAELs in rats were located around 14 mg/m^3 with an exposure by inhalation during 9 months and possibly around 3 mg/kg bw in a gavage study over 27 weeks.[37]

- WHO. Under the joint sponsorship of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO), and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC), a Concise International Chemical Assessment Document (CICAD) on 1,1,2,2-tetrachloroethane was published in 1998. The data included in the document were obtained from a document prepared by the Environmental Health Directorate of Health Canada, from a review by ATSDR, and from a comprehensive literature search performed in August 1995. Based on animal experimental data, it was concluded that the acute toxicity of 1,1,2,2-tetrachloroethane was slight to moderate and that the compound might induce skin, eye, and mucosal irritation. Results from principally limited short-term and subchronic studies indicated that the liver is the target organ. Although most of the available studies were thought to be inadequate to allow a confident determination of a NO(A)EL or LO(A)EL for hepatic or other effects, minimal effects on the liver (increase in lipid content) and other end points (increase in adrenocorticotropic hormone; reversible alterations in haematological parameters) were observed in rats exposed to 13.3 mg/m^3 for up to 9 months. In limited, primarily range-finding studies and early investigations, reproductive and developmental effects were seen only at doses causing decreased maternal body weights. Oral exposure of rats and mice for up to 78 weeks resulted in a significantly increased incidence of hepatocellular carcinomas in both male and female mice, while in rats, there was only a non-statistically significant increase at the highest dose (which was lower, on a time-weighted average base, that the lowest dose tested in mice). The chemical was a potent promoter but did not act as an initiator, in an initiation/promotion assay. Based on *in vitro* and *in vivo* data, 1,1,2,2-tetrachloroethane was concluded to have no, or at most a weak, genotoxic potential. Although the

available data were incomplete, the liver tumours might have been induced by mechanisms that might not be relevant to humans, for which humans are less susceptible, or for which there might be a threshold. However, based on the data available, not any firm conclusion could be drawn with respect to the potential carcinogenicity of 1,1,2,2-tetrachloroethane in humans. Based on the incidence of hepatocellular carcinomas in mice exposed for 78 weeks, the potency, expressed as the dose associated with a 5% increased in tumours ($TD_{0.05}$) was calculated to range from 5.8 to 28 mg/kg bw/day. From this, air guidance values of 3.4-16 and 0.34-1.6 µg/m^3 associated with cancer risks of 10^{-5} and 10^{-6}, respectively, were presented.[38]

Chapter 5

Kinetics

NEG data

1,1,1,2-tetrachloroethane. Studies with rodents suggested that metabolism of 1,1,1,2-tetrachloroethane proceeds via both oxidative and reductive pathways.[NEG10] Apart from CO_2, trichloroethanol and trichloroacetic acid were the main (urinary) metabolites.[NEG42,NEG66] After exposure by inhalation and intraperitoneal injection, rats given 1,1,1,2-tetrachloroethane excreted about 20 times as much trichloro compounds in the 48-hour urine when compared with rats given 1,1,2,2-tetrachloroethane.[NEG42] Following oral exposure, approximately 65 and 84% of the administered dose were metabolised in rats and mice, respectively; rats and mice exhaled approximately 34 and 6%, respectively, of the dose as parent compound.[NEG66]

1,1,2,2-tetrachloroethane. Following one single - 20-seconds lasting - inhalation by a volunteer, 97% of the inhaled 1,1,2,2-tetrachloroethane were retained.[NEG67] For human skin, a steady state flux of 27 µg/cm^2/hour has been calculated based on molecular weight, octanol-water partition coefficient, and contact with a saturated aqueous solution (3 mg/mL).[NEG106]

One minute to 240 minutes after a single intravenous injection into mice, highest concentrations of irreversibly bound metabolites were found in the respiratory and gastrointestinal tract, the liver, the gallbladder contents, the adrenal cortex, and the testes.[NEG23]

Biotransformation of 1,1,2,2-tetrachloroethane may involve a number of oxidative dechlorination pathways, and there is some evidence for a reductive pathway (leading to a carbon-centred radical

and subsequent lipid peroxidation in the liver) as well (see Figure 1, page 7 of Part II).[NEG1,NEG36,NEG78,NEG113] Following oral administration, approximately 80 and 70% were metabolised in rats and mice, respectively.[NEG66] In mice injected intraperitoneally with radiolabelled compound, (exhaled) CO_2 was the main metabolite (accounting for 50% of the label recovered during 3 days). After 3 days, 28% were excreted in the urine (main metabolites: dichloroacetic acid, trichloroethanol, oxalic acid, and trichloroacetic acid accounting for 27, 10, 7, and 4% in the 24-hour urine, respectively), 4% were exhaled as parent compound, and 16% were retained in the animal. In rats exposed by inhalation or intraperitoneal injection, only relatively small amounts of trichloroacetic acid and trichloroethanol were recovered from 48-hour urine.[NEG113]

Additional data

1,1,1,2-tetrachloroethane. The committee did not find additional data on the kinetics of 1,1,1,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. Hanley *et al*. investigated the disposition and macromolecular interactions (hepatic protein and DNA binding, cellular injury, histopathology; see also Section 5.2.3 and 5.2.4) of 1,1,2,2-tetrachloroethane in rats and mice in order to try to understand the different sensitivity towards the tumorigenic potential of 1,1,2,2-tetrachloroethane between these species. The fate of [1,2-^{14}C]-tetrachloroethane was followed for 72 hours in male Osborne-Mendel rats and male B6C3F_1 mice after single oral (gavage in corn oil) doses of 150 mg/kg bw or 6-hour exposure to 10 ppm (69 mg/m^3) (see Table 1).

Table 1 Distribution of radioactivity following 6-hour inhalation (10 ppm) or oral exposure (150 mg/kg bw) of [1,2-^{14}C]-tetrachloroethane in rats and mice.

	inhalation		oral	
	rat	mouse	rat	mouse
expired air:				
tetrachloroethane	7.6%[a]	1.8%	9.4%	0.7%
CO_2	25.1%	32.2%	32.2%	50.1%
urine	18.9%	25.7%	23.1%	21.9%
faeces	5.1%	5.8%	4.1%	5.7%
carcass	30.3%	25.1%	23.0%	17.4%
skin	11.9%	3.6%	7.1%	2.9%
(cage wash	1.1%	5.8%	1.0%	1.5%)

[a] Percentage of total radioactivity recovered for 72 hours.

Tetrachloroethane was metabolised extensively in both species following both routes. Following inhalation, ca. 8 and 2% of the radioactivity recovered were exhaled as parent compound by rats and mice, respectively. The radiolabel was excreted mainly as expired CO_2 (25-32%) or via the urine (16-29%), while ca. 5% were recovered from the faeces. The carcass contained 25-30%. On a body weight basis, the mouse achieved a 3.2-fold greater body burden of tetrachloroethane and a 3.5-fold greater body burden of metabolised tetrachloroethane compared to rats. Following oral administration, rats expired 14 times more parent compound than mice did (ca. 9 vs. 0.7%). The majority of the radioactivity was excreted as CO_2 (32 and 50%) or in the urine (ca. 23%). In separate experiments, in which rats (n=8) and mice (n=16) were exposed to concentrations of radiolabelled tetrachloroethane of 10 ppm for 6 hours, the irreversible binding of radioactivity to hepatic macromolecules was investigated in livers prepared from rats and mice sacrificed immediately after exposure and from mice sacrificed 24 and 48 hours post-exposure. For both rats and mice, the majority of radiolabel irreversibly associated with hepatic macromolecules was found to be non acid-hydrolysable, suggesting extensive incorporation of one-carbon fragments similar to that seen in concomitant experiments with ^{14}C-formate. Mice had ca. 1.9-fold greater extent of irreversible associated radioactivity at the end of the 6-hour exposure compared to rats. At 24 and 48 hours, the extent of radiolabel irreversibly associated with the TCA-precipitable hepatic macromolecules in mice had decreased compared with values obtained immediately after exposure. When a single oral dose of 500 mg/kg bw was given to mice, hepatic glutathione levels were depressed by a maximum of 14% below control values one hour post-administration. Thereafter, glutathione levels were comparable to control values or slightly higher (maximum 1.3-fold at t=6 h), indicating that no significant depletion had occurred. On a body weight basis, mice and rats had similar body burdens, but mice metabolised slightly more test substance (mouse/rat ratio: 1.12).[39]

Chapter 6

Effects

6.1 Observations in humans

NEG data

1,1,1,2-tetrachloroethane. No data on effects in humans following exposure to 1,1,1,2-tetrachloroethane were presented.

1,1,2,2-tetrachloroethane. In two male volunteers, dizziness and mucosal irritation were observed within 10 to 12 minutes when exposed to 1000 or 1800 mg/m^3 (144, 262 ppm) 1,1,2,2-tetrachloroethane, but no such effects were seen at exposure up to 90 mg/m^3 (13 ppm) for 10 minutes.[NEG57] Data from surveys on workers occupationally exposed to 1,1,2,2-tetrachloroethane, showed that the liver, the gastrointestinal tract, and the nervous system are the target organs.[NEG38,NEG48,NEG59] In a survey on 380 workers of 23 Indian, bangle-manufacturing, cottage industries with exposure levels ranging from 63-685 mg/m^3 (9-98 ppm), about 50% of the most severely exposed workers exhibited fine finger tremor and about a third complained of headache and vertigo. There seemed to be a dose-related increase in the incidence of the tremors, being 14, 33, 41, and 50% in workers of 4 different factories with exposure levels of 63-119 (9-17 ppm), 280-518 (40-74 ppm), 350-427 (50-61 ppm), and 455-685 mg/m^3 (65-98 ppm), respectively. Besides inhalation exposure to tetrachloroethane, there was exposure to acetone and diacetone alcohol (minor) as well as direct dermal contact with the liquid. Liver effects were not found. However, the observations made were limited to recording symptomatology and some signs, as the workers were reluctant to submit blood for examinations. Further, Lobo-Mendonça stated that the study could not disclose the full picture of the effects of 1,1,2,2-tetra-

chloroethane because labour turnover was high and workers who had become ill were not included.[NEG59] No statistically significant increases in mortality, overall cancer mortality, and cancer incidences were found in a retrospective study on a cohort consisting of 1099 white men with exposure to 1,1,2,2-tetrachloroethane while using impregnating clothing machinery and some additional exposure to dry-cleaning solvents.[NEG76]

Additional data

1,1,1,2-tetrachloroethane. The committee did not find additional data on the effects in humans following exposure to 1,1,1,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. Heffter (see above) investigated the illnesses of workers of an aircraft factory in Germany who regularly used varnishes containing 30-50% 1,1,2,2-tetrachloroethane. 1,1,2,2-Tetrachloroethane was concluded to be the aetiological agent, but Heffter did not present exposure data (see [40]).

Jeney *et al*. reported (in Hungarian) the findings from a 3-year study of the effects of 1,1,2,2-tetrachloroethane on workers of a penicillin plant in former Czechoslovakia. They presented exposure data. However, during the course of the study, ventilation systems were changed several times, (part of the) processes were moved to a new plant, work shifts were shortened from 8 to 6 hours, and the workers started to wear overalls that were periodically cleared. During the study, signs and symptoms noticed at the screening examinations definitely decreased. However, the practice of transferring workers to other areas with considerably less tetrachloroethane exposure as soon as they showed initial signs of liver dysfunction might have been a major factor. Further, because the period under each ventilation system did not coincide with the yearly intervals for which signs and symptoms were reported, it was not possible to accurately correlate the results of the screening examinations with exposure levels. Nevertheless, despite improvements in exposure conditions, levels in the new plant still ranged between 1.5 and 36.4 ppm (ca. 10 and 250 mg/m^3), with exposures of 15 ppm (ca. 100 mg/m^3) for most of each work shift and up to 36.4 ppm (ca. 250 mg/m^3) during cleaning operations, and workers still showed indications of liver dysfunction. The results of the examinations revealed no neurological disorders, such as paraesthesia (see [40]).

Lobo-Mendonça[41] presented data on number of workers in job categories and incidences of signs and symptoms (see Table 2). A limited number of measurements performed in the breathing zone of workers in (only) 7 factories indicated concentration ranges of 17-98 ppm (119-685 mg/m^3) for cylinder making and turning (7 measurements in 5 factories), of 20-61 ppm (140-427 mg/m^3) for pol-

Table 2 Effects on 380 bangle-factory workers in 23 factories[41] (adopted from [13]).

job category	cylinder making, turning	polishing, separating	cylinder cutting	heating	packing	other	total
no. of workers	85	107	52	50	42	44	383
effects	incidences (%) of symptoms						
tremors	54 (63)	39 (36)	14 (27)	3 (6)	14 (33)	9 (20)	133 (35)
anaemia	33 (39)	45 (42)	12 (23)	9 (18)	15 (36)	14 (32)	128 (34)
vertigo	33 (39)	43 (40)	6 (12)	22 (44)	7 (17)	5 (11)	116 (31)
headache	22 (26)	36 (34)	8 (15)	18 (36)	11 (26)	6 (14)	101 (27)
abdominal pain	14 (16)	36 (34)	11 (21)	16 (32)	6 (14)	7 (16)	90 (24)
anorexia	22 (26)	28 (26)	14 (27)	11 (22)	8 (19)	3 (7)	86 (23)
flatus	17 (20)	11 (10)	7 (13)	1 (2)	4 (10)	5 (11)	45 (12)
vomiting	9 (11)	13 (12)	2 (4)	7 (14)	2 (5)	2 (5)	35 (9)
fatigue	13 (15)	10 (11)	4 (8)	1 (2)	2 (5)	3 (7)	33 (9)
nervousness	6 (7)	14 (13)	0 (0)	8 (16)	0 (0)	1 (2)	29 (8)
constipation	5 (6)	7 (7)	3 (6)	5 (10)	3 (7)	4 (9)	27 (7)
nausea	1 (1)	10 (11)	1 (2)	11 (22)	0 (0)	1 (2)	24 (6)
sweating	0 (0)	13 (12)	0 (0)	7 (14)	0 (0)	1 (2)	21 (6)
numbness	2 (2)	5 (5)	1 (1)	2 (4)	0 (0)	0 (0)	10 (3)
weight loss	0 (0)	2 (2)	4 (8)	0 (0)	0 (0)	2 (5)	8 (2)

ishing and separating (4 measurements in 1 factory), 14 ppm (97 mg/m^3) for cylinder cutting (1 measurement), 11 ppm (77 mg/m^3) for heating (1 measurement), 9 ppm (63 mg/m^3) for packing (1 measurement).

Obviously, no systematic determination of concentrations in air was performed. Breathing zone air samples were taken in only some of the 23 factories, and no information was presented on the number of persons sampled, number of samples taken, or duration of sampling. Lobo-Mendonça did not give details on the dose-related increased incidences of tremors in workers of the 4 factories (see NEG data summary above), such as the actual number of workers involved. The data suggest that the workers in these factories were exposed to concentrations ranges. However, for 3 of these factories, they are the results of only 2 measurements in each of the factories. The level of 9 ppm concerned a workers involved in packing bangles (at a site near cylinder making), the other levels were related to cylinder making. No data were given on the fourth factory with exposure levels of 50-61 ppm. Although anaemia was seen in about one-third of the workers, Lobo-Mendonça considered this not related to treatment but to the socio-economic status of the workers who generally lived in rural unhygienic surroundings. To what extent this contributed to other findings could not be assessed since an unexposed control group was not included. Although some activities were usually done by women or young people, there were no data on sex and age. The

polishers/separators were exposed to undiluted tetrachloroethane and the cylinder makers/turners to a 50:50 mixture of tetrachloroethane and acetone, which has a much higher vapour pressure (ca. 35 times that of tetrachloroethane), but the exposure to acetone was not given any attention. For both groups of workers, there was considerable dermal exposure and exposure to air levels that was said to vary within and between factories.

In conclusion, exposure to concentrations of 1,1,2,2-tetrachloroethane of 90 mg/m^3 (13 ppm) for 10 minutes did not induce mucosal irritation or dizziness in 2 male volunteers. Other studies showed that occupational exposure to 1,1,2,2-tetrachloroethane induced effects on the liver, the gastrointestinal tract, and the nervous system. However, the committee is of the opinion that these studies have too many drawbacks to assess a clear dose-response relationship that can be used for deriving 15-minute or 8-hour health-based occupational exposure limits.

6.2 Animal experiments

6.2.1 Irritation and sensitisation

NEG data

1,1,1,2-tetrachloroethane. No data on the irritating or sensitising properties of 1,1,1,2-tetrachloroethane were presented.

1,1,2,2-tetrachloroethane. Animal data on 1,1,2,2-tetrachloroethane showed that the liquid is strongly irritating to the skin and the mucous membranes.[NEG93] No data from sensitisation studies were presented.

Additional data

DECOS did not find additional data from irritation and sensitisation studies on 1,1,1,2- or 1,1,2,2-tetrachloroethane.

6.2.2 *Toxicity due to single exposure*

NEG data

1,1,1,2-tetrachloroethane. Four-hour LC_{50} values were 14,600 and 19,500 mg/m^3 (2100 and 2800 ppm), in rats and rabbits, respectively. The oral LD_{50} was 670 mg/kg bw in rats and 1500 mg/kg bw in mice. In rabbits, a dermal LD_{50} of 20,000 mg/kg bw was established.[NEG80]

1,1,2,2-tetrachloroethane. The 2-hour LC_{50} value in mice was 4500 mg/m^3 (650 ppm); the oral LD_{50} in rats 250 mg/kg bw.[NEG80] Mice exposed to tetrachloroethane (not specified, but very likely to be the 1,1,2,2-isomer) showed a lateral position and loss of reflexes at 7460-10,000 and 9,996-14,990 mg/m^3 (1091-1455 ppm and 1450-2180 ppm), respectively.[NEG55,NEG77] Dose-dependent prostration to deep narcosis was seen in cats exposed to 4880-41,900 mg/m^3 (710-6100 ppm).[NEG56]

Additional data

For 1,1,2,2-tetrachloroethane, additional lethal toxicity data included LC_{50} values of 1000 and 1200 ppm (6960 and 8350 mg/m^3; 4 hours) for rats and of 650 ppm (4524 mg/m^3; 8 hours) for mice, and an oral LD_{50} of 570 mg/kg bw for rats.[33,37]

6.2.3 *Toxicity due to repeated exposure*

NEG data

1,1,1,2-tetrachloroethane. In an oral (gavage) carcinogenicity study in which F344/N rats were given daily doses of 125 or 250 mg/kg bw, 5 days/week, for 103 weeks (sacrifice: at week 104), signs of CNS toxicity were seen in the high-dose group from week 44 onward. The only tumorigenic effect found was a statistically significant increase in the incidence of fibroadenomas of the mammary gland in the females of the low-dose group (high dose: 7/46, low dose: 15/49, controls: 6/49). When $B6C3F_1$ mice were given doses of 250 or 500 mg/kg bw/day according to a similar regimen, signs of CNS toxicity (weakness, inactivity, loss of coordination) were observed in the high-dose group from week 51 onward. All animals of this group died or were killed moribund by week 65. A statistically significant increase in the incidence of hepatocellular adenomas in males (low dose: 14/46, high dose: 21/50, controls: 6/48) and females (low: 8/46, high: 24/48, control: 4/49) and of hepatocellular carcinomas in females (low: 5/46, high: 6/48, control: 1/49) was observed.[NEG72]

1,1,1,2-Tetrachloroethane was not active in an oral (diet) initiation/promotion assay (initiator: diethylnitrosamine; end point: increase in γ-glutamyl-transferase positive foci) in partially hepatectomised male Osborne-Mendel rats.[NEG64,NEG92]

1,1,2,2-tetrachloroethane. Cats and rabbits exposed to 800-1100 mg/m^3 (116-160 ppm), 8-9 hours/day, for 4 weeks, showed initial stage of prostration but no remarkable changes in body weight, behaviour, body temperature, or haematology.[NEG56] Exposure of female rats to 3900 mg/m^3 (560 ppm), 5 or 6 hours/day, 5 days/week, for 15 weeks, induced effects on the liver (increased relative liver weight, hyperplasia, granulation, and vacuolisation) and in a slightly decreased haematocrit.[NEG103] Only very slight effects (decreased body weight at 4 months; increased liver lipids at 7 months) were found in a separate experiment using male rats exposed to 13.3 mg/m^3 (1.9 ppm), 4 hours/day, for up to 9 months.[NEG87]

Following daily oral (gavage) administration of 8 mg/kg bw for 60 or 150 days or 20 mg/kg bw for 60 days to male rats, mainly effects on the liver as well as some effects on the kidneys, testes, and thyroid were observed. No effects were seen when 3.2 mg/kg bw was given for 150 days.[NEG33]

In an oral (gavage) carcinogenicity study, no statistically significant increase in the incidence of any type of tumour was found in Osborne-Mendel rats given time-weighted average doses of up to 108 mg/kg bw/day, 5 days/week, for 78 weeks* and killed after another, treatment-free 32 weeks. In the males of the high-dose group, hepatocellular carcinomas and neoplastic liver nodules were observed in 2/49 and 1/49 animals, respectively, compared with none in 20 vehicle controls. The number and kind of non-neoplastic, inflammatory, degenerative, and proliferative lesions were similar among dosed and control rats. In B6C3F$_1$ mice, time-weighted average doses of 142 or 284 mg/kg bw/day, for 78 weeks (sacrifice: after another, treatment-free 12 weeks), caused an increase in the incidence of hepatocellular carcinomas (males: 13/50 and 44/49 vs. 2/19 and 1/18 in untreated and vehicle controls, respectively; females: 30/48 and 43/47 vs. 0/19 and 0/20 in untreated and vehicle controls, respectively). A large number of high-dose mice died at weeks 69 and 70, apparently from acute toxic nephrosis, leaving only one high-dose male mouse by week 90 (vs. 34% of females).[NEG71] In an initiation/promotion assay in male Osborne-Mendel rats (see 1,1,1,2-tetrachloroethane), 1,1,2,2-tetrachloroethane had a weak initiating and a stronger promoting activity.[NEG64,NEG92]

Additional data

1,1,1,2-tetrachloroethane. Preceding the 2-year NTP carcinogenicity study (see above under 'NEG data'), 14-day and 13-week range-finding experiments were performed. When given rats (n=5/sex/group) daily oral (gavage) doses of 0, 10, 50, 100, 500, or 1000 mg/kg bw for 14 days, mortality occurred in 1/5 females at

* During the last 45 weeks of the treatment period, the high dose was administered with a pattern of 1 treatment-free week followed by 4 weeks (5 days/week) of treatment.

500 mg/kg bw and in 3/5 males and 1/5 females at 1000 mg/kg bw. In the latter group, final body weights were decreased by 9 and 6% in males and females, respectively. At necropsy, there were no compound-related findings. In the 13-week experiment, rats (n=10/sex/group) were treated with daily doses of 0, 5, 10, 50, 100, or 500 mg/kg bw. Mortality occurred in the control group (1 male) and the groups receiving 100 and 500 mg/kg bw (1 female and 1 female and 1 male, respectively). Final body weights were decreased by 7% in male animals given 500 mg/kg bw; in female animals, they were lower in all exposed groups by 3 to 8%. In the females receiving 500 mg/kg bw, signs of CNS toxicity (loss of equilibrium) were seen. There were no compound-related histological changes. In the 2-year study with daily doses of 125 and 250 mg/kg bw, survival was decreased in low-dose (not significant) and high-dose (p=0.001) males, but not in females, when compared to control groups. Body weights were not affected. Non-neoplastic effects observed included increases in the incidence of renal mineralisation, characterised by multifocal deposits of basophilic material and crystals in the tubules of the papilla, in male rats (high dose: 26/48; low dose: 19/50; controls: 12/48), of hepatic clear-cell changes in males (high dose: 2/48; low dose: 6/49; controls: 0/49) and females (high dose: 9/44; low dose: 3/49; controls: 0/48), of hepatic fatty metamorphosis in males (high dose: 10/48; low dose: 6/49; controls: 5/49) and females (high dose: 7/44; low dose: 1/49; controls: 3/48), and of lung alveolar emphysema in males (high dose: 6/46; low dose: 11/50; controls: 2/49) and females (high dose: 12/46; low dose: 9/47; controls: 5/49). According to the NTP, the emphysema was frequently associated with pulmonary haemorrhage and focal granulomatous inflammation suggesting a mechanical, probably intubation-induced lesion. The clear-cell changes could have been foci or cellular alterations and, therefore, relevant to hepatic carcinogenesis. However, since they were not discussed or addressed to by the NTP, the committee considers them to be a more generalised effect (such as related to glycogen accumulation).[42] Based on the survival and histology findings, the committee concludes that 125 mg/kg bw is a NOAEL for non-neoplastic effects in this oral rat carcinogenicity study.

In similar pre-chronic studies in mice, no body weight, macroscopic (14-day study) or microscopic (13-week study) effects were seen. Mortality occurred in 1/5 males and 2/5 females receiving 1000 mg/kg bw/day for 14 days and in 1/10 males receiving 500 mg/kg bw/day for 13 weeks. In the 2-year study, survival in the females given 250 mg/kg bw, the low-dose group, was significantly lower when compared to that of controls (p=0.039). There was no increase in the incidence of non-neoplastic lesions in the low-dose animals. At 500 mg/kg bw, the high dose, animals showed much higher incidences of non-neoplastic liver

lesions (inflammation, fatty metamorphosis, necrosis, hepatocytomegaly) when compared to controls.[42] Based on a decreased survival in female mice at 250 mg/kg bw/day, the lowest dose tested, the committee cannot establish a NOAEL for non-neoplastic effects in this oral mouse carcinogenicity study.

The committee notices that the aforementioned carcinogenicity studies did not include haematology, clinical chemistry, or urinalysis evaluations.

Results from 2-year toxicity and carcinogenicity studies with halogenated ethanes performed previously by the US National Cancer Institute (NCI) and the National Toxicology Program (NTP) urged NTP to perform a series of experiments with, amongst others, 1,1,1,2-tetrachloroethane in an attempt to determine some of the structure-activity relationships involved in hyaline droplet-induced nephropathy. All compounds investigated were given to male F344/N rats (n=5/group) by gavage at doses of 0.62 and 1.24 mmol/kg bw/day, for 21 days. After sacrifice, all animals were examined grossly. Right kidneys, livers, and right testes were weighted. Microscopic evaluation included the right kidney, the left liver lobe, and any lesion observed grossly. Further, urinalysis (creatinine, glucose, total protein, aspartate aminotransferase, γ-glutamyl transpeptidase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity) was performed. Treatment with 1,1,1,2-tetrachloroethane did not induce clinical signs of toxicity, effect on body weights, or mortality. Effects observed were limited to the kidneys. In the high-dose group receiving 1.24 mmol or 208 mg/kg bw, animals had statistically significantly increased absolute and relative kidney weights when compared to controls. Evaluation of urinalysis parameters showed increased urine protein output and *N*-acetyl-β-D-glucosaminidase activity and decreased γ-glutamyl transpeptidase activity. Microscopically, hyaline droplet accumulation – described as 'one severity grade above controls' – and increased incidences of tubular regeneration (in 5/5 animals; severity – on a scale of 1-5: 1.6) and granular casts (in 5/5; severity: 1.4) were observed. Further, the mean renal proliferative cell nuclear antigen (PCNA) labelling index was significantly - 2.2-fold – higher when compared to controls, indicating replicative DNA synthesis. In the low-dose group (given 104 mg/kg bw), kidney effects included hyaline droplet accumulation (described as 'one severity grade above controls') and an increased incidence of tubular regeneration (in 3/5; severity: 1.0).[43] Bucher did not identify immunologically the protein droplets observed as accumulations of α_{2u}-globulin. However, the committee assumes that the renal findings described are suggestive of male rat-specific α_{2u}-globulin-induced nephropathy, and, therefore, of questionable relevance to human risk assessment.

1,1,2,2-tetrachloroethane. In an experiment to examine the disposition and macromolecular interactions in the liver, Hanley *et al*. administered oral (gavage) doses of 1,1,2,2-tetrachloroethane of 25-300 mg/kg bw/day to male rats (Osborne-Mendel; n=6/group) and mice ($B6C3F_1$; n=6/group), for 4 days. In rats given 300 mg/kg bw, substantial CNS depression and debilitation was observed and the experiment was terminated after 3 doses. One rat died. No such effects were reported for any of the other treatment groups.[39]

Preceding the NCI carcinogenicity study (see above under 'NEG data'), a range-finding experiment was performed by giving rats (n=5/sex/group) daily oral (gavage) doses of 56, 100, 178, and 316 mg/kg bw/day for 6 weeks, followed by a 2-week observation period to detect any delayed toxicity. Mortality occurred in 5/5 female rats at 316 mg/kg bw and 1/5 male rats at 100 mg/kg bw. Body weight decreases by 3, 9, and 38% in males and by 9, 24, and 41% in females were seen at doses of 56, 100, and 178 mg/kg bw/day, respectively. In mice given doses of 32 to 316 mg/kg bw according to a similar schedule, there were no effects on body weight or mortality in any of the exposed groups. No more data were presented. In the final chronic study in rats, with time-weighted average doses of 62 and 108 mg/kg bw for males and of 43 and 76 mg/kg bw for females, body weights were dose-relatedly decreased for both male and female rats throughout the treatment period and tended to converge to those of controls during the observation period. During the first 5 weeks of the study, 10 high-dose females died: 8 with pneumonia, 2 with no reported lesions. In males, there was no statistically significant association between increased dose and decreased survival rates. During (parts of) the treatment period, signs of toxicity, such as hunched appearance, squinted or reddened eyes, abdominal urine stains, respiratory signs, were observed at higher frequency in the exposed rats when compared to controls. In mice receiving time-weighted average doses of 142 and 284 mg/kg bw/day, treatment did not induce appreciable body weight effects. In both males and females, there was a significant decrease in survival in the high-dose group. In males, this was mainly due to the death of 33 animals in weeks 69 and 70. In females, 50% survived more than 82 weeks and 34% more than 90 weeks. Generally, patterns of behaviour, physical appearance, and the number and kind of non-neoplastic lesions were similar among dosed and control mice.[44] Based on signs of toxicity observed in male and female rats at 62 and 43 mg/kg bw/day, respectively, the lowest doses tested, the committee cannot establish a NOAEL for non-neoplastic effects in this oral rat carcinogenicity study. For mice, the NOAEL for non-neoplastic effects is set at 142 mg/kg bw, based on decreased survival in animals given 284 mg/kg bw/day.

The committee notices that the carcinogenicity studies mentioned above did not include haematology, clinical chemistry, or urinalysis evaluations.

NTP tested 1,1,2,2-tetrachloroethane as part of the investigation on structure-activity relationships involved in hyaline droplet-induced nephropathy (see above). In the high-dose group receiving 208 mg/kg bw/day by gavage, all animals died or were killed moribund before the end of the study (i.e., on day 13 and 14). Animals were thin and lethargic, having diarrhoea and abnormal breathing and ruffled fur. In the low-dose group (104 mg/kg bw), treatment did not cause changes in any of the kidney and urinalysis parameters. However, there were increases in absolute and relative liver weights that were accompanied by mild to moderate hepatic cytoplasmic vacuolisation.[43]

In addition, NTP conducted 15-day and 14-week studies in which groups of male and female F344/N rats and B6C3F$_1$ mice were administered 1,1,2,2-tetrachloroethane (purity: ≥99%) in microcapsules in the feed. Microcapsules loaded with neat test compound and placebos (empty microcapsules for controls) were prepared using food-grade, modified corn starch and reagent-grade sucrose (80:20) to produce dry microspheres; the outer surfaces of the microcapsules were dusted with food-grade, hydrophobic, modified corn starch. In the 15-day studies, animals (n=5/sex/group/species) received diets containing 0, 3325, 6650, 13,300, 26,600, or 53,200 ppm tetrachloroethane. All rats exposed to 26,600 or 53,200 ppm were killed moribund on day 11*. All other rats survived to the end of the study, showing decreased mean final body weights and body weight gains. Males and females receiving 6650 or 13,300 ppm (i.e., 400 and 500 mg/kg bw) and females receiving 3325 ppm (i.e., 300 mg/kg bw) lost weight during the study. Feed consumption decreased with increasing doses. Clinical signs observed were thinness and ruffled fur in males at doses ≥13,300 ppm and in females at doses ≥6650 ppm and lethargy in males and females at doses of 53,200 ppm. Relative weights of the thymus (decrease at ≥6625 ppm), kidneys (increase in males at ≥3325 ppm, in females at ≥6625 ppm), and the liver (at 13,300 ppm decrease in males, increase in females) had changed. Macroscopic and microscopic post-mortem examinations showed thin carcasses at doses ≥13,300 ppm, alopecia in 4 females at 13,300 ppm and in 2 males and all females at 53,200 ppm, with minimal to moderate acanthosis (in females only), and hepatodiaphragmatic nodules, accompanied with mild to moderate centrilobular degeneration, in one control female, one female at 6625 ppm, one male and one female at 13,300 ppm, and one female and 2 males at 26,600 ppm. In mice, all

* Because of 100% mortality in these two dose groups, no final body weigts or weight changes or average daily doses expressed as mg/kg bw were calculated.

males and females exposed to 53,200 ppm, all males exposed to 26,600 ppm, and 2 males exposed to 13,300 ppm died or were killed moribund before the end of the study. All animal groups with survivors showed decreased final mean body weights and weight gains; all males and females in these groups (except females exposed to 3325 ppm) lost weight during the study. Clinical signs included hyperactivity at doses ≥3325 ppm, lethargy in males at 26,600 and 53,200 ppm and in females at 26,600 ppm, and thinness and ruffled fur in males at ≥6650 ppm and in females at 26,600 and 53,200 ppm. Relative weights of the liver (decrease in males at ≥3325 ppm, in females at 13,300 and 26,600 ppm) and thymus (decrease in females at ≥3325 ppm) had changed. Post-mortem examinations showed thin carcasses in males at 6650 and 13,300 ppm and in females at 13,300 and 26,600 ppm, and, in all treated groups, pale and mottled livers with hepatocellular degeneration characterised by hepatocellular swelling, cytoplasmic rarefaction, single paranuclear vacuoles, hepatocellular necrosis with occasional pooling of sinusoidal erythrocytes, and infrequent mild mononuclear infiltrates.[45]

Subsequently, groups of 10 male and female rats were fed diets containing microencapsulated 1,1,2,2-tetrachloroethane at doses of 268, 589, 1180, 2230, or 4600 ppm, resulting in daily amounts of 20, 40, 80, 170, and 320 mg/kg bw, respectively, for 14 weeks. Additionally, untreated and vehicle-treated groups (n=10/sex/group) were included. Groups of 10 male and 10 female 'special study' rats designated for haematology and clinical chemistry analyses on study days 5 and 21 received the same doses as the 'core study' rats. Clinical findings were recorded and animals weighted initially, weekly, and at the end of the studies. During weeks 4 and 13, functional observation batteries (covering only home cage and open field end points) were performed on 'core study' rats of the untreated, vehicle-treated, and 3 lowest dose groups. At the end of the study, reproductive tissue evaluations were performed on 'core study' animals of both control groups and the groups given 40, 80, and 170 mg/kg bw/day (see Section 5.2.5). Necropsy was performed on all animals. Organs weighted included heart, right kidney, liver, lung, right testis, and thymus. Animals of the 'core study' control rats and animals exposed to 320 mg/kg bw/day were scheduled for complete histological examinations. All animals survived treatment. Final mean body weights and body weight gains were statistically significantly decreased in male and female animals at doses ≥80 mg/kg bw while mean body weight gain was also decreased in females of the 40-mg/kg bw group. Clinical signs including thinness and pallor were seen in all rats given 170 and 320 mg/kg bw/day, while there were no treatment-related findings in any of the groups at the cage observations during week 4 and 13. Clinical chemistry evaluation showed changes, such

Table 3 Incidences of selected non-neoplastic lesions in male F344/N rats exposed to 1,1,2,2-tetrachloroethane for 14 weeks.

mg/kg bw/day	0[a]	20	40	80	170	320
ppm	0[a]	268	589	1180	2300	4600
liver						
hepatocyte, cytoplasmic vacuolisation	0	7[b, c] (1.3)[d]	9[c] (2.0)	10[c] (1.9)	8[c] (1.4)	0
hepatocyte, hypertrophy	0	0	0	1 (1.0)	9[c] (1.3)	10[c] (3.2)
hepatocyte, necrosis	0	0	0	0	8[c] (1.0)	10[c] (1.6)
pigmentation	0	0	0	0	7[c] (1.0)	10[c] (1.9)
bile duct, hyperplasia	0	0	0	0	0	10[c] (1.7)
hepatocyte, mitotic alteration	0	0	0	0	0	6[c] (2.0)
mixed cell focus	0	0	0	0	3	5[e]
eosinophilic focus	0	0	0	0	1	1
clear-cell focus	0	0	0	0	1	1
basophilic focus	0	0	0	0	0	3
spleen						
pigmentation	0	0	1 (1.0)	9[c] (1.0)	9[c] (1.0)	9[c] (1.6)
red pulp, atrophy	0	0	0	0	5[e] (1.0)	9[c] (1.4)
lymphoid follicle, atrophy	0	0	0	0	0	5[e] (1.0)
bone						
metaphysic, atrophy	0	0	0/9	0	0	10[c] (2.1)
bone marrow						
atrophy	0	0	0/9	0	3 (1.0)	10[c] (1.5)
prostate gland						
atrophy	0	0	0	0	0	9[c] (2.0)
preputial gland						
atrophy	0	0/5	0/5	0/6	0	10[c] (1.4)
seminal vesicle						
atrophy	0	0	0/9	0	0	10[c] (2.7)
testes						
germinal epithelium, atrophy	0	0/9	2/9 (1.5)	0	0	10[c] (2.2)

[a] Vehicle controls.
[b] Number of animals with lesions; 10 animals examined unless otherwise noted.
[c] $p \leq 0.01$ (Fisher exact test).
[d] Average severity grade of lesions; 1=minimal, 2=mild, 3=moderate, 4=severe.
[e] $p \leq 0.05$ (Fisher exact test).

as increased activities of serum alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, and 5'-nucleotidase and increased total bile acid concentrations seen at doses of 80 mg/kg bw and/or greater, indicative of liver damage. Changes in haematology values were indicative of an effect on the circulating erythroid mass, characterised by a minimal to mild microcytic non-responsive anaemia, in animals fed doses ≥40 mg/kg bw. At post-mortem examinations, there were thin carcasses, pale livers, and liver foci in exposed animals. Relative organ weight changes included increases for the liver in animals fed

doses of 40 mg/kg bw and greater (by 12-73%), for the kidney in males at doses ≥80 mg/kg bw (by 10-75%) and in females at ≥170 mg/kg bw (by 32 and 69%), for the heart in animals fed doses of 170 mg/kg bw and greater (by 8-35%), and for the lungs in animals fed 320 mg/kg bw (by 25 and 53%), and decreases for the thymus at 320 mg/kg bw (by 25%). Upon microscopic examination, the liver, spleen, bone, bone marrow, and reproductive organs (see Section 5.2.5) were affected (see Table 3 and 4). The liver was the most sensitive organ showing a statistically significant increase in the incidence of minimal to mild cytoplasmic vacuolisation of hepatocytes in male rats fed 20 mg/kg bw/day.

Table 4 Incidences of selected non-neoplastic lesions in female F344/N rats (n=10) exposed to 1,1,2,2-tetrachloroethane for 14 weeks.

mg/kg bw/day	0[a]	20	40	80	170	320
ppm	0[a]	268	589	1180	2300	4600
liver						
hepatocyte, cytoplasmic vacuolisation	0	0	10[b, c] (1.7)[d]	10[c] (2.2)	4[e] (1.3)	0
hepatocyte, hypertrophy	0	0	0	4[e] (1.0)	10[c] (1.7)	10[c] (2.8)
hepatocyte, necrosis	0	0	0	1 (1.0)	7[c] (1.0)	10[c] (1.1)
pigmentation	0	0	0	0	10[c] (1.3)	10[c] (2.0)
bile duct, hyperplasia	0	0	0	0	5[e] (1.0)	10[c] (1.9)
hepatocyte, mitotic alteration	0	0	0	0	3 (2.0)	10[c] (1.9)
mixed cell focus	0	0	0	0	8[c]	1
eosinophilic focus	0	0	0	0	4[e]	2
clear-cell focus	0	0	0	0	2	3
basophilic focus	0	0	0	0	1	0
spleen						
pigmentation	1 (1.0)	0	0	4 (1.0)	8[c] (1.1)	8[c] (1.3)
red pulp, atrophy	0	0	0	0	0	9[c] (1.6)
lymphoid follicle, atrophy	0	0	0	0	0	3 (1.0)
bone						
metaphysic, atrophy	0	0	0	0	9[c] (1.8)	9[c] (2.9)
bone marrow						
atrophy	0	0	0	0	4[e] (1.0)	7[c] (1.7)
uterus						
atrophy	0	0	0	0	7[c] (1.4)	9[c] (2.2)
ovary						
interstitial cell, cytoplasmic alteration	0	0	0	0	3 (1.0)	10[c] (2.0)
clitoral gland						
atrophy	0/9	0/7	0/7	0	1 (1.0)	7[c] (1.4)

[a] Vehicle controls.
[b] Number of animals with lesions; 10 animals examined unless otherwise noted.
[c] p≤0.01 (Fisher exact test).
[d] Average severity grade of lesions; 1=minimal, 2=mild, 3=moderate, 4=severe.
[e] p≤0.05 (Fisher exact test).

Similarly, groups of 10 male and female mice were fed doses of 589, 1120, 2300, 4550, or 9100 ppm, resulting in average daily amounts of 100, 200, 370, 700, and 1360 mg/kg bw for males and of 80, 160, 300, 600, and 1400 mg/kg bw for females. Untreated and vehicle-treated control groups (n=10/sex/group) were included. Clinical findings were recorded and animals weighted initially, weekly, and at the end of the studies. During weeks 4 and 13, functional observation batteries (covering only home cage and open field end points) were performed on mice of the untreated, vehicle-treated, and 3 mid-dose groups. At the end of the study, reproductive tissue evaluations were performed on core study animals of both controls and the three mid-dose groups (see Section 5.2.5). Necropsy was performed on all animals. Organs weighted included heart, right kidney, liver, lung, right testis, and thymus. Animals of both control groups and the highest dose group were scheduled for complete histological examinations. All mice survived treatment. Final mean body weights and body weight gains were statistically significantly decreased in male animals at doses $\geq$370 mg/kg bw and in female animals at doses of 600 and 1360 mg/kg bw while mean body weight gain was also decreased in females of the 300-mg/kg bw group. Clinical signs including thinness were seen in all rats given 1300 or 1400 mg/kg bw/day, in 2 females and 9 males at 600/700 mg/kg bw, and in one female and 3 males at 300/370 mg/kg bw, while there were no treatment-related findings in any of the groups at the cage observations during week 4 and 13. Similar to rats, changes in clinical chemistry values indicated dose-related hepatic effects in female and male mice fed doses of 160/200 mg/kg bw. At post-mortem examinations, there were thin carcasses in the animals of the two highest dose groups, pale livers in males at doses of 370 mg/kg bw and greater and in all treated female groups, and pale kidneys in one male of the 700- and 1360-mg/kg bw group. Relative organ weight changes included increases for the liver in males at doses $\geq$200 mg/kg bw (by 18-40%) and in females at doses $\geq$80 mg/kg bw (by 6-42%) and decreases for the kidney in males at doses of 370, 700, and 1360 mg/kg bw (by 11,14, and 7%, respectively) and in females at 1400 mg/kg bw (by 10%). Upon microscopic evaluation, the liver and the preputial gland (see Section 5.2.5) were affected (see Table 5 and 6). There were minimal to mild hypertrophy of hepatocytes in 2/10 females at 80 mg/kg bw and minimal hepatocytic hypertrophy in 7/10 males and 9/10 females, mild hepatocytic necrosis in 1/10 males, and minimal focal pigmentation in 2/10 females at the next greater dose of 200/160 mg/kg bw/day.

Table 5 Incidences of selected non-neoplastic lesions in male B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane for 14 weeks.

mg/kg bw/day	0[a]	100	200	370	700	1360
ppm	0[a]	589	1130	2300	4550	9100
liver						
hepatocyte, hypertrophy	0	0	7[b, c] (1.0)[d]	10[c] (2.2)	10[c] (2.8)	10[c] (3.1)
hepatocyte, necrosis	0	0	1 (2.0)	8[c] (1.1)	8[c] (1.0)	9[c] (1.0)
pigmentation, focal	0	0	0	10[c] (1.2)	10[c] (1.4)	8[c] (1.3)
bile duct, hyperplasia	0	0	0	7[c] (1.4)	9[c] (1.3)	10[c] (2.0)
preputial gland						
atrophy	0	4[e] (1.0)	2 (1.0)	0/8	4[e] (2.5)	5[e]/9 (2.2)

[a] Vehicle controls.
[b] Number of animals with lesions; 10 animals examined, unless otherwise noted.
[c] $p \leq 0.01$ (Fisher exact test).
[d] Average severity grade of lesions; 1=minimal, 2=mild, 3=moderate, 4=severe.
[e] $p \leq 0.05$ (Fisher exact test).

Table 6 Incidences of selected non-neoplastic lesions in female B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane for 14 weeks.

ppm	0[a]	80	160	300	600	1400
mg/kg bw/day	0[a]	589	1130	2300	4550	9100
liver						
hepatocyte, hypertrophy	0	2[b] (1.5)[c]	9[d] (1.0)	10[d] (1.9)	10[d] (2.5)	10[d] (3.0)
hepatocyte, necrosis	0	0	0	3 (1.0)	7[d] (1.0)	4[e] (1.0)
pigmentation, focal	0	0	2 (1.0)	9[d] (1.0)	8[d] (1.0)	7[d] (1.1)
bile duct, hyperplasia	0	0	0	8[d] (1.0)	10[d] (1.4)	10[d] (2.0)

[a] Vehicle controls.
[b] Number of animals with lesions; 10 animals examined.
[c] Average severity grade of lesions; 1=minimal, 2=mild, 3=moderate, 4=severe.
[d] $p \leq 0.01$ (Fisher exact test).
[e] $p \leq 0.05$ (Fisher exact test).

The committee could not identify a NOAEL in these 14-week oral studies since effects were observed in male rats and female mice at the lowest doses tested: in male rats given daily doses of 20 mg/kg bw, there was an increase in the incidence of cytoplasmic vacuolisation of hepatocytes of minimal to mild severity; in female mice given 80 mg/kg bw, 2/10 females showed minimal to mild hepatocytic hypertrophy.

6.2.4 *Genotoxicity*

NEG data

1,1,1,2-tetrachloroethane. In *in vitro* studies, no mutagenic activity was found in the majority of the bacterial test systems (*S. typhimurium*) applied[NEG37,NEG63,NEG64,NEG82,NEG93,NEG108], while positive results were obtained in a mouse lymphoma assay in the presence of a metabolic activation system (without metabolic activation: negative).[NEG96,NEG97]

Tested in CHO cells, 1,1,1,2-tetrachloroethane did not induced an increase in the incidence of chromosome aberrations.[NEG30]

In yeast (*S. cerevisiae*), a negative result is reported (end point: chromosome loss)[NEG109]; in fungi (*A. nidulans*), chromosome malsegregation was induced.[NEG14] 1,1,1,2-Tetrachloroethane did not induce mitotic recombinations in *D. melanogaster* (Vogel *et al*., 1993). In CHO cells, 1,1,1,2-tetrachloroethane induced an increase in the incidence of sister chromatid exchanges in the absence of a metabolic activation system (with metabolic activation: negative).[NEG30] DNA repair tests in rat hepatocytes were negative.[NEG64,NEG110]

Following single intraperitoneal injections, 1,1,1,2-tetrachloroethane was bound covalently to DNA in rat and mouse lung, liver, kidney, and stomach.[NEG10]

A cell transformation assay in BALB/c-3T3 cells was negative.[NEG64]

1,1,2,2-tetrachloroethane. In vitro testing in bacteria for mutations showed conflicting, but mostly negative, results in a variety of *S. typhimurium* strains.[NEG3,NEG37,NEG57,NEG63,NEG64,NEG82,NEG93,NEG108]

It did not induce mutations in yeast strains (*S. cerevisiae*)[NEG74] or in *D. melanogaster* (sex-linked recessive lethal mutations).[NEG112]

A chromosome aberration test in CHO cells was negative.[NEG30]

In *E. coli*, it caused DNA damage (DNA repair without but not with metabolic activation; induction of prophage lambda with but not without metabolic activation).[NEG3,NEG19] In yeast (*S. cerevisiae*), 1,1,2,2-tetrachloroethane induced gene conversions and mitotic recombinations at high concentrations[NEG7] and, in fungi (*A. nidulans*), chromosome malsegregation[NEG14]. 1,1,2,2-Tetrachloroethane did not induce mitotic recombinations in *D. melanogaster*.[NEG107] SCE tests in CHO[NEG30] or BALB/c-3T3 cells[NEG13] gave positive results (both with and without metabolic activation). DNA repair tests in rat and mouse hepatocytes were negative.[NEG64,NEG110]

In vivo, no UDS was observed in hepatocytes isolated from mice 2 or 12 hours after given single oral doses of 50-1000 mg/kg bw.[NEG65] A test on S-phase synthesis, indicative of cell proliferation and non-genotoxic mechanisms involved in rodent carcinogenesis, in hepatocytes isolated from mice 24 or 48 hours after single doses of 200-700 mg/kg bw, was negative and equivocal in males and females, respectively.[NEG65] Following intraperitoneal injection, 1,1,2,2-tetrachloroethane was bound covalently to DNA in rat and mice liver, lung, kidney, and stomach.[NEG11]

Cell transformation assays in BALB/c-3T3 cells were positive when tested with metabolic activation, and conflicting (one positive, one negative) without metabolic activation.[NEG13,NEG12,NEG64]

Additional data

1,1,1,2-tetrachloroethane

- *In vitro* tests:
  - Gene mutation assays. 1,1,1,2-Tetrachloroethane did not induce reverse point mutations (ilv locus) in the yeast diploid D7 strain of *S. cerevisiae* when tested in the absence and presence of a metabolic activation system, probably obtained from induced livers of Swiss albino CD1 mice. The concentrations tested were 5, 7.5, and 10 mM (i.e., 840, 1260, and 1680 µg/mL), resulting in survival percentages of 95, 74, and 25, respectively, without S9 and of 100, 67, and 43, respectively, with S9).[46]
    Myhr and Caspary re-tested some of the compounds, among which 1,1,1,2-tetrachloroethane, that were evaluated earlier in the mouse lymphoma L5178Y $tk^{+/-}$ assay by the National Toxicology Program (see [47,48] and Table 4 in Part II). 1,1,1,2-Tetrachloroethane was found negative when tested in the absence and presence of a liver S9 mix prepared from livers of Arochlor-induced male F344/N rats. Six to 7 concentrations (solvent: ethanol) were tested in mostly triplicate cultures in 2 trials without S9 (concentration ranges: 12.5-500 and 50-300 nL/mL; i.e., 20-780 and 80-470 µg/mL) and in 2 trials with S9 (ranges: both 12.5-300 nL/mL; i.e., 20-470 µg/mL). The highest concentrations were lethal to the lymphoma cells. Reviewing the earlier positive NTP data, Myhr and Caspary concluded that a convincing response was obtained only at one single concentration of 300 µg/mL in 3 experiments (one with and 2 without metabolic activation) in which highly toxic treatments (giving 5-6% relative total growth) were achieved.[49] 1,1,1,2-Tetrachloroethane was one of the compounds tested in a Japanese collaborative study of the mouse lymphoma assay in order to clarify the performance of this test for the detection of *in vitro* clastogens and spindle poisons. Testing in 2 laboratories resulted in negative results in the absence of metabolic activation (concentration ranges: 125-400 and 125-500 µg/mL; solvent not reported). In the presence of an S9 mix, probably obtained from livers of phenobarbital- or 5,6-benzoflavone-induced Sprague-Dawley rats (see [50]), results were negative in one laboratory (range: 100-400 µg/mL), while in the other laboratory, an abrupt increase in the mutation frequency at only one concentration, i.e., the highest non-cytotoxic concentration tested, viz., 200 µg/mL,

occurred.[51] In a follow-up series of experiments, tetrachloroethane was found negative when tested with (in 2 laboratories) and without (in one laboratory) metabolic activation. Concentrations (in DMSO) were not reported but the highest concentration might have been 500 µg/mL (see [52]).[50] Prolongation of incubation times from 3 (as was applied in the assays mentioned afore) to 24 hours did not change results when testing concentrations up to 450 µg/mL (contrary to other compounds tested in these experiments).[52]

The compound did not induce sex-linked recessive lethal mutations in *D. melanogaster* when administered by feeding (1000 µg/mL) and injection (1500 µg/mL).[53]

- Cytogenicity assays. The ability of 1,1,1,2-tetrachloroethane to induce chromosomal aberrations was tested in Chinese hamster lung fibroblasts in the absence and presence of a metabolic activation system derived from livers of phenobarbital- or 5,6-benzoflavone-induced male Sprague-Dawley rats at concentrations (solvent: DMSO) of 100, 200, 400, and 800 µg/mL. One hundred cells (metaphases) for each dose group were analysed after continuous treatment for 24 or 48 hours until harvest without a metabolic activation system or treatment for 6 hours in the absence and presence of S9 mix after which cells were cultured in fresh medium for another 18 hours. Precipitates were observed at doses of 400 and 600 µg/mL; one dose of 600 µg/mL and all doses of 800 µg/mL were toxic. Under the conditions of this test, 1,1,1,2-tetrachloroethane did not cause increases in the frequency of structural chromosomal aberrations. However, 24- and 48-hour continuous treatment without S9 mix and 6-hour treatment with S9 mix increased the frequency of polyploid cells.[54]
- Other tests. 1,1,1,2-Tetrachloroethane did not induce mitotic gene conversions in *S. cerevisae* strain D7 cells harvested from stationary growth phase with and without S9 (concentrations: 5, 7.5, and 10 mM; i.e., 840, 1260, and 1680 µg/mL). A dose-dependent increase in convertant frequencies was observed in cells harvested from logarithmic growth phase, contained a high level of cytochrome P-450 (concentrations: 1, 2.5, 5 mM; i.e., 170, 420, 840 µg/mL; 7.5 mM was toxic).[46]

- *In vivo* tests:

  1,1,1,2-Tetrachloroethane was found positive in a replicative DNA synthesis test in hepatocytes prepared from male $B6C3F_1$ mice (n=4 or 5/group) 48 hours after oral administration of a single dose of 1000 mg/kg bw. This dose was considered the maximum tolerated dose, set at about half of the LD_{50} that was determined in a preceding experiment with 4 to 5 animals. Results

were negative when hepatocytes were prepared 24 and 39 hours after treatment, and at a single dose of 500 mg/kg bw and similar preparation times.[55]

1,1,2,2-tetrachloroethane

- *In vitro* tests:
  - Gene mutation assays. 1,1,2,2-Tetrachloroethane was negative in the mouse lymphoma L5178Y $tk^{+/-}$ assay in the absence and presence of a liver S9 mix prepared from livers of Arochlor-induced or uninduced male F344/N rats. Six to 7 concentrations (solvent: ethanol) were tested in mostly triplicate cultures in 2 trials without S9 (concentration ranges: 60-200 and 25-300 nL/mL; i.e., 100-320 and 40-480 mg/L) and in 3 trials with S9 (ranges: 50-300, 50-500, and 50-300 nL/mL; i.e., 80-480, 80-800, and 80-480 mg/L). The highest concentrations were lethal to the lymphoma cells.[45]
    1,1,2,2-Tetrachlorethane did not cause increases in the frequency of sex-linked recessive mutations in *D. melanogaster* exposed to atmospheres of 5 ppm (35 mg/m^3) for 7 hours or 50 ppm (350 mg/m^3) for 40 minutes. Exposures produced sedation but did not affect survival.[56]
  - Other tests. 1,1,2,2-Tetrachloroethane was negative in an unscheduled DNA synthesis (UDS) test in human diploid fibroblasts with exposures of 3 hours and concentrations up to 250 mg/L in the presence of an S9 mix obtained from induced male rat livers and up to 15,869 mg/L in the absence of metabolic activation.[56]
- *In vivo* tests:
  In a dominant lethal assay, 1,1,2,2-tetrachloroethane did not affect pregnancy frequency, numbers of corpora lutea or implantations, or the frequency of early deaths. In this test, male CD rats (n=10/group) were exposed to 0, 5, or 50 ppm (0, 35, 350 mg/m^3), 7 hours/day, for 5 consecutive days, after which each male was mated each week with 2 virgin untreated females for 9 consecutive weeks. Treatment did not induce effects on body weights or clinical signs of toxicity.[56]
  The induction of chromosomal aberrations was examined in bone marrow cells obtained from male and female CD rats (n=5/group) 6, 12, and 24 hours after a single 7-hour exposure to 0, 5, and 50 ppm (0, 35, 350 mg/m^3) and 6 hours after exposure to 0, 5, and 50 ppm, 7 hours/day, for 5 consecutive day. Only in bone marrow cells obtained from females 6 hours after a single exposure to 50 ppm, a small increase in the frequency of chromosomal aberrations other than gaps was found. Treatment did not induce overt signs of toxicity. There were no data on cytotoxicity (mitotic index).[56]

At the end of a 14-week toxicity study, peripheral blood samples were obtained from male and female mice B6C3F$_1$ mice orally (microcapsules in feed) treated with average daily doses of 1,1,2,2-tetrachloroethane of 80-1360 and 100-1400 mg/kg bw, respectively. Smears were immediately prepared and fixed. The frequency of micronuclei was determined in 2000 normochromatic erythrocytes in each of 5 animals per sex per exposure group. A dose-related increase in the frequency of micronuclei in normochromatic erythrocytes was found (see Table 7).

Table 7 Frequency of micronuclei in peripheral blood erythrocytes of mice orally (feed) treated with 1,1,2,2-tetrachlorethane for 14 weeks.[45]

males			females		
dose (mg/kg bw)	micronucleated NCEs[a]/1000 NCEs	p value[b]	dose (mg/kg bw)	micronucleated NCEs/1000 NCEs	p value
0	2.10±0.29		0	2.20±0.25	
100	2.30±0.25	0.38	80	2.50±0.27	0.33
200	3.00±0.16	0.10	160	2.60±0.19	0.28
370	3.30±0.12	0.05	300	2.90±0.29	0.16
700	4.50±0.27	0.002	1000	3.40±0.10	0.05
1360	5.10±0.43	0.0002	1400	3.80±0.34	0.02
	$p<0.001$[c]			$p=0.008$	

[a] NCE = normochromatic erythrocytes.
[b] Pairwise comparison with the vehicle controls, significant at $p\leq0.005$.
[c] Significance of micronucleated NCEs/1000 NCEs tested by the one-tailed Cochran-Armitage trend test, significant at $p\leq0.025$.

Trend analyses on the frequencies of micronucleated erythrocytes were positive (males: $p\leq0.001$; females: $p=0.008$), but the increases in frequencies were only statistically significant from vehicle control values (by pairwise comparison) for the 2 highest male dose groups, i.e., at 700 and 1360 mg/kg bw.[45] The committee notes that the numbers (ca. 3-5) of micronucleated erythrocytes induced in this study at clearly toxic doses (see Section 5.2.3) are relatively low and comparable to background/control levels that can be found in bone marrow. Therefore, the committee considers that 1,1,2,2-tetrachloroethane is at most marginally genotoxic in this study.
1,1,2,2-Tetrachloroethane was found positive in a replicative DNA synthesis test in hepatocytes prepared from male B6C3F$_1$ mice (n=4 or 5/group) 24 or 48 hours after oral administration of a single dose of 200 mg/kg bw (negative at 39 hours) and in hepatocytes prepared 24, 39, or 48 hours after treatment with a dose of 400 mg/kg bw.[55] Hanley *et al*. did not find an increase in DNA synthesis, expressed as dpm of ^{3}H-thymidine incorporated per µg of DNA, in

the livers of Osborne-Mendel rats and B6C3F$_1$mice removed 28 hours after a single oral (gavage) dose of 150 mg/kg bw. When given daily doses of 25-300 mg/kg bw for 4 days, 2.8- and 4.8-fold increases in of ^{3}H-thymidine incorporation were induced in rats at doses of 75 and 150 mg/kg bw, respectively. At 300 mg/kg bw, the increase was 2.5-fold (only), which was attributed to the poor health status of the animals (see Section 5.2.3). In mice, DNA synthesis was increased 1.7- and 4.4-fold at doses of 150 and 300 mg/kg bw, respectively.[39]

The potential to alkylate hepatic DNA was evaluated in B6C3F$_1$ mice. After a single oral (gavage) dose of 150 mg/kg bw, there was irreversible binding of ^{14}C-activity to purified hepatic DNA. Further HPLC analysis and comparison with chromatographic profiles obtained from mice treated with ^{14}C-formate revealed that the radioactivity was associated with parent DNA purine bases, suggesting incorporation of one-carbon fragments via normal anabolic pathways rather than DNA adduct formation.[39]

6.2.5 *Reproduction toxicity*

NEG data

1,1,1,2-tetrachloroethane. No reproduction toxicity was observed in rats exposed by inhalation or orally to 1,1,1,2-tetrachloroethane, although neonates born to exposed females died within 2 days of birth.[NEG104]

1,1,2,2-tetrachloroethane. Exposure of male rats to 13.3 mg/m^3 (1.9 ppm), 4 hours/day, for up to 9 months did not affect reproductive parameters upon mating with untreated female rats.[NEG87] Intraperitoneal injection of 300 or 400 mg/kg bw during organogenesis showed some indication of an embryotoxic effect in one strain of mice but not in another.[NEG88]

Additional data

1,1,1,2-Tetrachloroethane. The committee did not find additional data on the reproduction toxicity of 1,1,1,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. Sperm were obtained from B6C3F$_1$ mice (n=10/group) sacrificed 5 weeks after they received the last of 5 consecutive exposures to 0, 5, or 50 ppm (0, 35, or 350 mg/m^3), 7 hours/day. Sperm were scored and placed in one of the following categories: normal; abnormal; hook upturned or elongated; banana-shaped head; amorphous head; abnormal tail (sharp, 180^o

angle or tight coiling only); miscellaneous (could include multiple tails, double heads, twisted neck, filamentous mid-piece, enlarged mid-piece, plier type). Apart from a statistically significant increase in the frequency of hook upturned or elongated sperm in the high-concentration animals, no abnormalities were found. Since this increase was small (2.4 times higher than that in concurrent controls) and within the range normally expected of a control group, McGregor considered this finding of doubtful significance. Exposure did not induce clinical signs of toxicity or effects on body weights.[56]

As part of a 14-week toxicity study in which 1,1,2,2-tetrachloroethane was administered to F344/N rats and $B6C3F_1$ mice encapsulated in the feed (see Section 5.2.3), reproductive tissue evaluations were performed on animals of control and mid-dose groups. In male rats (n=10/group) orally (microcapsules in feed) exposed to daily doses of 20-320 mg/kg bw for 14 weeks, there were statistically significant decreases in epididymal spermatozoal motility values at doses of 40 mg/kg bw or greater, in left epididymis weights at 80 mg/kg bw and greater, and in left cauda epididymis weights at 170 mg/kg bw. In the 320-mg/kg bw dose group, generally mild atrophy of the prostate gland, preputial gland, seminal vesicles, and testicular germinal epithelium were observed (see Table 3). In similarly treated females (n=10/group), females exposed to 170 mg/kg bw differed significantly from controls in relative length of time spent in the oestrus stages. At doses of 170 and 320 mg/kg bw, minimal to mild atrophy of the uterus and clitoral gland and cytoplasmic alteration of the ovarian interstitial cells were observed histologically. The incidences of these lesions in the 320-mg/kg bw group and of the uterine atrophy in the 170-mg/kg bw group were significantly higher than those in the vehicle controls (see Table 4).[45]

In $B6C3F_1$ mice (n=10/sex/group), given oral (microcapsules in feed) doses of 100-1360 (males) or 80-1400 (females) mg/kg bw for 14 weeks, weights of the left cauda epididymis, left epididymis, and left testis at 1360 mg/kg bw, and of the left testis at 700 mg/kg bw were significantly decreased. In males given 1360 mg/kg bw, a decreased epididymal spermatozoal motility was observed. In females, a prolonged oestrus cycle was seen at 1400 mg/kg bw.[45]

NTP performed pilot, range-finding gavage and dosed-feed developmental toxicity studies. In the gavage studies, for which no abstracts or data were available, groups of female rats and rabbits were given doses of 1,1,2,2-tetrachloroethane of 100-250 and 10-200 mg/kg bw/day, respectively.[42] In the dosed-feed studies, groups of 8-9 pregnant CD Sprague-Dawley rats were given doses of ca. 0.05-0.6% resulting in amounts estimated to be 34, 98, 180, 278, and 330 mg/kg bw/

day on gestational days 6-16. A control group (n=9) was included. During the study, body weights were recorded at gestational days 4, 6, 9, 11, 14, and 16 and feed consumption at days 6-11 and 11-16. Animals were observed twice daily for overt signs of toxicity or mortality. At Caesarean section, scheduled at gestational day 20, terminal body and gravid uterine weights and the number of implantation sites, resorptions, dead and life fetuses were recorded. All animals survived treatment; clinical signs, especially rough hair coat, were noted at 278 and 330 mg/kg bw/day. Except for the lowest dose group, there were statistically significant decreases in maternal body weights (starting from gestational day 9) and weight gains, when compared to controls. In the animals given 34 mg/kg bw, body weights were lower at gestational day 16. Feed intake was reduced in all tetrachloroethane-treated groups. Decreased average fetal weights were seen at 90 mg/kg bw and greater. There was complete resorption of litters in 1/9 and 4/9 animals of the groups given 98 and 330 mg/kg bw, respectively (vs. 0/9 in controls). In the latter treatment group, this caused a decreased gravid uterine weight.[57] Based on decreases in maternal and fetal body weights, the committee concludes that in this rat study, the NOAELs for maternal toxicity and fetotoxicity are <34 mg/kg bw and 34 mg/kg bw, respectively.

Similar experiments were performed using Swiss CD-1 mice. Feeding doses estimated to be 24-587 mg/kg bw (ca. 0.01-0.3%) during gestational days 6-16 did not induce significant maternal or developmental toxicity. However, only 37% of the animals appeared to be pregnant resulting in groups of 3-6 animals.[58] In the repeat experiment, groups of mice initially received doses of 4.0, 7.5, and 10.0%, mistakenly because of formulation errors. All animals (n=13-14/group) died or were killed moribund by gestational day 13. In a second repeat, groups of 5-11 pregnant animals were given dietary doses of 0, 0.5, 1.0, 1.5, 2.0, and 3.0% which resulted in calculated amounts of 0, 987, 2120, 2216, and 4575 mg/kg bw, respectively; for the group receiving 3%, no calculation was made because all 9 animals died or were killed moribund by gestational day 12. Before scheduled sacrifice at gestational day 17, 2/10, 4/5, and 5/7 pregnant mice of the groups given 1.0, 1.5, and 2.0%, respectively, died or were killed moribund. No mortality or abnormal clinical signs were observed at 0.5%. At doses of 1% and greater, there were decreased maternal body weights and weight gains accompanied by decreased feed consumption. At necropsy, abnormal livers were noted. No effects were seen at 0.5%. At scheduled necropsy of the surviving animals, complete resorption of litters was observed in 2/8, 1/1, and 1/2 animals of the 1.0, 1.5, and 2.0% group, respectively (vs.1/11 in controls). No developmental effects were reported for the 0.5% group.[59] Although high mortality and low pregnancy

rates hamper the validity of this study, the data suggest that 987 mg/kg bw might be a NOAEL for maternal toxicity and fetotoxicity in mice.

6.3 Summary and evaluation

6.3.1 Human data

1,1,1,2-tetrachloroethane. The committee did not find data on effects in humans following exposure to 1,1,1,2-tetrachloroethane.

1,1,2,2-tetrachloroethane. In 2 male volunteers, exposure to concentrations of 1,1,2,2-tetrachloroethane of 90 mg/m^3 (13 ppm) for 10 minutes did not induce mucosal irritation or dizziness, while mucosal irritation and dizziness were reported at levels of 1000 mg/m^3 (144 ppm) and higher. The committee did not find human data on skin irritation and sensitisation.

In studies on workers occupationally exposed to 1,1,2,2-tetrachloroethane – published before 1965 – , the liver, the gastrointestinal tract, and the nervous system were the target organs. In one of these surveys, involving 23 Indian factories and 380 workers of which about one half was in direct contact (both dermal and by inhalation) with 1,1,2,2-tetrachloroethane at exposure levels of approximately 70 to 700 mg/m^3 (10-100 ppm) as well as to unknown levels of acetone, symptoms and signs concerning the gastrointestinal tract and the nervous system, especially tremors, were reported. However, due to drawbacks in design, the committee is of the opinion that these studies are inappropriate to assess clear dose-response relationships from which an 8-hour health-based occupational exposure limit might be derived.

6.3.2 Animal data

1,1,1,2-tetrachloroethane. The committee did not find experimental animal data on the irritating and sensitising potential of 1,1,1,2-tetrachloroethane. Acute lethality data included 4-hour LC_{50} values of 14,600 mg/m^3 (2100 ppm) for rats and of 19,500 mg/m^3 (2800 ppm) for rabbits, oral LD_{50} values of 670 mg/kg bw for rats and of 1500 mg/kg bw for mice, and a dermal LD_{50} of 20,000 mg/kg bw for rabbits.

The committee did not find data from repeated inhalation studies. Repeated-dose studies available were only gavage studies with a limited scope: a carcinogenicity study in rats and mice, a tumour-initiation/promotion assay in male rats, and a 21-day study in male rats exploring structure-activity relationships

involved in hyaline droplet-induced nephropathy. Besides the CNS, the target organs seem to be the kidney in (male) rats and the liver in mice.

In the 21-day experiment in which male rats were given daily doses of 104 and 208 mg/kg bw, there were findings indicative of hyaline droplet-induced nephropathy. Treatment did not cause clinical signs of toxicity, effects on body, liver, and testes weights (only organs weighted), mortality, microscopic hepatic lesions, or gross organ lesions. This was in contrast to animals treated with similar doses of the 1,1,2,2-isomer which did not affect the kidneys but showed affected livers at 104 mg/kg bw and severe toxicity, including mortality, at 208 mg/kg bw.

In the carcinogenicity study in Fischer rats, the only tumorigenic effect observed was a statistically significant increase in the incidence of fibroadenomas in the female animals of the low-dose group, receiving 125 mg/kg bw/day (incidence: 15/46 vs. 7/46 and 6/49 in the high-dose, receiving 250 mg/kg bw/day, and control group, respectively). Non-neoplastic effects included CNS effects in the animals of the high-dose group from week 44 and kidney effects (mineralisation) in 19/50 (38%) and 26/48 (54%) male rats at the low and high dose, respectively (vs. 12/48 - 25% - in controls). In $B6C3F_1$ mice treated with doses of 0, 250, or 500 mg/kg bw according to a regimen similar to that in the rats, a statistically significant increase in the incidence of hepatocellular adenomas in males (low dose: 14/46, high dose: 21/50, controls: 6/48) and females (low: 8/46, high: 24/48, control: 4/49) and of hepatocellular carcinomas in females (low: 5/46, high: 6/48, control: 1/49) was observed. In the high-dose group, there were effects on the CNS at week 51 and all animals died or were killed by week 65. At necropsy, there were high incidences of non-neoplastic liver lesions in the high dose group. The committee concludes that under the conditions of this study, 1,1,1,2-tetrachloroethane is carcinogenic to mice but not to rats. For rats, a NOAEL of 125 mg/kg bw is established.

1,1,1,2-Tetrachlorethane was negative in the tumour-initiation/promotion assay (initiator: diethyl-nitrosamine; end point: increase in γ-glutamyl-transferase positive foci) in male Osborne-Mendel rats.

In *in vitro* mutation tests, mostly negative results were obtained in bacteria (*S. typhimurium)* and mammalian cells (mouse lymphoma cells) and negative results in yeast (*S. cerevisiae*) and in *D. melanogaster* (sex-linked recessive lethal mutations). In cytogenicity assays, in Chinese hamster ovary cells, 1,1,1,2-tetrachloroethane produced positive results in the absence, but not in the presence of a metabolic activation system in an SCE test and negative results in a chromosome aberration assay. In Chinese hamster lung fibroblasts, the compound induced polyploidy, but no increases in the frequency of structural chro-

mosomal aberrations. Other test results included a negative test on mitotic chromosome loss in yeast (*S. cerevisiae*), a negative test on mitotic gene conversions in cells from the stationary phase but positive when cells were from the logarithmic growth phase (which contained a high level of cytochrome P-450) in yeast (*S. cerevisiae*), a positive test on induction of chromosome malsegregation in fungi (*A. nidulans*), and a negative test on mitotic recombination in *D. melanogaster*. DNA repair tests in rat hepatocytes were negative. *In vivo*, an increase in hepatic DNA synthesis was found in orally treated rats and mice. Following intraperitoneal injections, 1,1,1,2-tetrachloroethane was bound covalently to DNA in rat and mouse organs. A cell transformation assay in BALB/c-3T3 cells was negative. From these data, the committee concludes that 1,1,1,2-tetrachloroethane is not a mutagenic compound but might have some weak genotoxic potential.

The committee did not find data from reproduction toxicity studies.

1,1,2,2-tetrachloroethane. Experimental animal data indicate that liquid 1,1,2,2-tetrachloroethane is strongly irritating to the skin and mucous membranes. The committee did not find experimental animal sensitisation studies. Following single inhalation exposure, effects were reported only at thousands of mg/m^3. In rats, the 4-hour LC_{50} values were 7000 and 8400 mg/m^3 (1000 and 1200 ppm), oral LD_{50}s 250 and 650 mg/kg bw. For mice, a 2-hour LC_{50} of 4500 mg/m^3 (650 ppm) was reported.

Following repeated exposure by inhalation, the CNS and the liver seem to be the target organs. However, the studies available suffer from several flaws such as insufficient number of animals, inappropriate exposure regimens, very high exposure concentrations, limited number of end points examined, and/or insufficient reporting. Therefore, the committee considers them not suitable to serve as a basis for the establishment of a health-based recommended exposure limit.

In repeated-dose oral studies, the liver was the most sensitive organ. In gavage studies, repeated doses of 200-300 mg/kg bw induced such severe toxicity in rats that experiments were broken off untimely within a few days. In male rats, doses of 1,1,2,2-tetrachloroethane of 104 mg/kg bw affected the liver (increased weights accompanied by mild to moderate cytoplasmic vacuolisation) but no effects on the kidney or urinalysis parameters were seen. In another study in which male rats were exposed to 8 mg/kg bw/day for 60 or 150 days or to 20 mg/kg bw/day for 60 days, effects on the liver as well as on the kidneys, testes, and thyroid were observed, but not at 3.2 mg/kg bw given for 150 days. However, this study could not be evaluated because of lacking quantitative data on dose-response or dose-effect. In carcinogenicity studies in which examinations

were limited to observation of signs of toxicity, body weight recordings, and (histo)pathology (after a 32-week treatment-free period), non-neoplastic findings included signs of toxicity in male and female rats at time-weighted average doses of 62 and 43 mg/kg bw, respectively, and decreased survival in mice at 284 mg/kg bw. The number and kind of non-neoplastic histological lesions were similar among dosed and control groups. In 14-week diet studies in rats and mice, the committee could not establish NOAELs, since effects were observed in male rats and female mice at the lowest doses tested. In male rats given daily doses of 20 mg/kg bw, there was an increase in the incidence of cytoplasmic vacuolisation of hepatocytes of minimal to mild severity; in female mice given 80 mg/kg bw, 2/10 females showed minimal to mild hepatocytic hypertrophy.

1,1,2,2-Tetrachloroethane had a weakly tumour-initiating and a more strongly tumour-promoting activity in Osborne-Mendel rats in an initiation/promotion assay (initiator: diethylnitrosamine; end point: increase in γ-glutamyltransferase positive foci). However, in an oral carcinogenicity study, it did not cause statistically significant increases in the incidence of any tumour type in male or female Osborne-Mendel rats at time-weighted average levels of up to 108 mg/kg bw/day, for 78 weeks (sacrifice after 110 weeks); in the males of the high-dose group, receiving 108 mg/kg bw/day, there was a not statistically significant increase in the incidence of hepatocellular carcinomas (2/49 vs. 0/20 in vehicle controls) and neoplastic liver nodules (1/49 vs. 0/20). In $B6C3F_1$ mice, time-weighted average oral (gavage) doses of 142 or 284 mg/kg bw/day, for 78 weeks (sacrifice after 90 weeks), caused increased incidences of hepatocellular carcinomas (males: 13/50, 44/49 vs. 2/19 and 1/18 in untreated and vehicle controls, respectively; females: 30/48, 43/47 vs. 0/19 and 0/20 in untreated and vehicle controls, respectively). The committee concludes that under the conditions of this study, 1,1,2,2-tetrachloroethane was carcinogenic to mice but not to rats.

In *in vitro* mutation tests, 1,1,2,2-tetrachloroethane was negative in the majority of tests using *S. typhimurium* strains, in yeast strains, mouse lymphoma cells, and *D. melanogaster* (sex-linked recessive lethal mutations). In CHO cells, it caused increases in the frequency of SCEs but not in the incidence of chromosomal aberrations. In yeast (*S. cerevisiae*), gene conversions and mitotic recombinations were found at high concentrations; in fungi (*A. nidulans*), it caused chromosome malsegregation. 1,1,2,2-Tetrachloroethane did not induce mitotic recombinations in *D. melanogaster*. It caused DNA damage in *E. coli* (induction of prophage lambda with but not without metabolic activation; DNA repair without but not with metabolic activation). DNA repair tests in rat and mice hepatocytes were negative. *In vivo*, 1,1,2,2-tetrachloroethane caused small increases in the frequency of micronuclei in erythrocytes obtained from orally treated mice

which reached statistically significance only in male animals at high toxic doses of 700 and 1360 mg/kg bw/day. No UDS of S-phase synthesis was seen in hepatocytes isolated from mice given single oral doses. Following intraperitoneal injections, 1,1,2,2-tetrachloroethane was bound covalently to DNA in rat and mouse organs. In orally dosed mice, binding of 1,1,2,2-tetrachloroethane-derived radioactivity to hepatic DNA was thought to be due to incorporation of normal metabolic one-carbon fragments rather than adduct formation. In cell transformation assays in BALB/c-3T3 cells, there was one positive and one negative result when tested in the absence of metabolic activation, and two positive results in the presence of metabolic activation. From these data, the committee concludes that 1,1,2,2-tetrachloroethane is not a mutagenic compound but might have some weak genotoxic potential.

No effects on reproductive parameters were seen when male rats were exposed to 13.3 mg/m^3 (1.9 ppm), 4 hours/day, for up to 9 months and mated with untreated females. In male rats giving diets containing daily doses of 20-320 mg/kg bw, for 14 weeks, effects on reproductive tissues were observed such as decreased epididymal spermatozoal motility at doses of 40 mg/kg bw and higher, decreased epididymis weights at 80 mg/kg bw and higher, and atrophy of reproductive tissues at 320 mg/kg bw. In similarly treated females, changes in relative length of time spend in oestrus stages and atrophy of reproductive tissues were seen at 170 mg/kg bw and higher. In mice, decreases in reproductive organ weights and spermatozoal motility were seen at doses of 700 and 1360 and of 1360 mg/kg bw, while oestrus cycle was prolonged in females at 1400 mg/kg bw. In pilot developmental toxicity studies in which 1,1,2,2-tetrachloroethane was administered in the diet of rats and mice during gestation, there were decreases in maternal body weights at doses of 34 mg/kg bw, the lowest dose tested, and higher and in fetal body weights at $\geq$90 mg/kg bw. High mortality and low pregnancy rates hamper the validity of the mouse study, but 987 mg/kg bw might be a NOAEL for maternal toxicity and fetotoxicity. Visceral and skeletal variations were not addressed in these studies. Intraperitoneal injection of 300 or 400 mg/kg bw during organogenesis showed some indication of an embryotoxic effect in AB-Jena but not in DBA mice. The data indicate that 1,1,2,2-tetrachlorethane might compromise fertility. However, the committee did not find valid studies addressing the effects of 1,1,2,2-tetrachloroethane on fertility or pregnancy outcome.

Chapter 7

Hazard assessment

7.1 Assessment of health hazard

7.1.1 1,1,1,2-Tetrachloroethane

The committee did not find data on effects in humans following exposure to 1,1,1,2-tetrachloroethane.

The committee did not find experimental animal data on the irritating or sensitising properties of 1,1,1,2-tetrachloroethane. Four-hour LC_{50} values of 14,600 mg/m^3 (2100 ppm) for rats and of 19,500 mg/m^3 (2800 ppm) for rabbits were reported. The committee did not find other acute inhalation data.

The committee did not find repeated inhalation studies. Repeated-dose studies available were only oral studies with a limited scope: a carcinogenicity study in rats and mice, a tumour-initiation/promotion assay in male rats, and a 21-day study in male rats exploring structure-activity relationships involved in hyaline droplet-induced nephropathy. Besides the CNS, the target organs seem to be the kidney in (male) rats and the liver in mice.

In the 21-day experiment in which male rats were given daily doses of 104 and 208 mg/kg bw, there were findings indicative of hyaline droplet-induced nephropathy, which has, in the committtee's opinion, no relevance to human risk assessment. Treatment did not cause clinical signs of toxicity, effects on body, liver, and testes weights (only organs weighted), mortality, microscopic hepatic lesions, or gross organ lesions. This was in contrast to animals treated with simi-

lar doses of the 1,1,2,2-isomer which showed affected livers at 104 mg/kg bw and severe toxicity, including mortality, at 208 mg/kg bw.

In carcinogenicity studies, non-neoplastic effects included CNS effects in Fischer rats given 250 mg/kg bw/day from week 44 and kidney effects (mineralisation) in male rats at 125 and 250 mg/kg bw/day. In $B6C3F_1$ mice treated with doses of 250 and 500 mg/kg bw/day, the high-dose animals showed effects on the CNS at week 51 and they all died or were killed by week 65. 1,1,1,2-Tetrachloroethane was carcinogenic to mice causing statistically significant increases in the incidence of hepatocellular adenomas in males and females and of hepatocellular carcinomas in females at doses of 250 or 500 mg/kg bw, but not to rats. 1,1,1,2-Tetrachlorethane was negative in the tumour-initiation/promotion assay in male Osborne-Mendel rats.

From the mutagenicity and genotoxicity data, the committee concludes that 1,1,1,2-tetrachloroethane is not a stochastic genotoxic compound.

Based on the carcinogenicity and genotoxicity data, the committee concludes that 1,1,1,2-tetrachloroethane has been extensively investigated. Although there is insufficient evidence to warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern. Therefore, 1,1,1,2-tetrachloroethane is classified as a suspect (non-genotoxic) carcinogen (comparable to EU category 3(A)).

Since the committee considers 1,1,1,2-tetrachloroethane as a suspect, non-genotoxic carcinogen, a threshold approach is warranted. However, the committee did not find human or experimental animal inhalation data that could be used as starting point for deriving a health-based occupational exposure limit.

The committee considers the oral carcinogenicity studies in rats and mice (NTP[60]) as inappropriate for use as a starting point. Haematology, clinical chemistry, or urinalysis evaluations were not made. Administration of 1,1,1,2-tetrachloroethane in the diet could have resulted in toxicity at lower doses, as suggested by the results of studies with 1,1,2,2-tetrachlorethane.

Further, the committee is of the opinion that the data on 1,1,2,2-tetrachloroethane cannot be used. Experimental animal data suggest that there are differences between 1,1,1,2- and 1,1,2,2-tetrachloroethane with respect to metabolism (kind of metabolites; percentage metabolised) and target organs (kidney vs. liver) (Bucher[43]).

The committee is of the opinion that the data on the toxicity of 1,1,1,2-tetrachloroethane are insufficient to allow the recommendation of a health-based occupational exposure limit.

7.1.2 *1,1,2,2-Tetrachloroethane*

The committee did not find data on the toxicokinetics in humans exposed to 1,1,2,2-tetrachloroethane. Based on molecular weight and the octanol-water partition coefficient, a dermal absorption rate of 27 μg/cm^2/h has been calculated for human skin in contact with a saturated aqueous solution (3 mg/mL).

Experimental animal data suggest that 1,1,2,2-tetrachloroethane is extensively metabolised by rats and mice following inhalation and oral and intraperitoneal administration. Biotransformation of 1,1,2,2-tetrachloroethane may involve oxidative dechlorination pathways and a reductive pathway – leading to a carbon-centred radical and subsequent lipid peroxidation in the liver. CO_2 – in exhaled air – is the main metabolite while dichloroacetic acid (a peroxisome proliferating agent) is the main urinary metabolite. Rats expired 4 and 14 times more unmetabolised tetrachloroethane following inhalation and oral administration when compared to mice, which might render rats more sensitive to CNS effects.

When 2 male volunteers were exposed to increasing concentrations (for 10 minutes to each concentration), no effects were reported at levels up to 90 mg/m^3 (13 ppm) (at which the odour was detected), while mucosal irritation and dizziness were reported at levels of 1000 mg/m^3 (144 ppm) and higher. The committee did not find human data on skin irritation and sensitisation.

From animal data, the committee concludes that liquid 1,1,2,2-tetrachloroethane is strongly irritating to the skin and mucous membranes. The committee did not find experimental animal sensitisation studies. Following single inhalation exposure, effects were reported only at thousands of mg/m^3. In rats, the 4-hour LC_{50} values were ca. 7000 mg/m^3 (1000 ppm) or higher.

These data do not suggest the need for a short-term exposure limit.

In studies on workers occupationally exposed to 1,1,2,2-tetrachloroethane – published before 1965 – , the liver, the gastrointestinal tract, and the nervous system were the target organs. In one of these studies, viz., a survey on workers of Indian, bangle-facturing, cottage industries, exposure was poorly characterised. Further, there was additional exposure to acetone, which was not given any attention, and dermal contact to both 1,1,2,2-tetrachloroethane and acetone. Due to these kinds of drawbacks in design, the committee is of the opinion that these

studies are inappropriate to assess clear dose-response relationships from which an 8-hour health-based occupational exposure limit might be derived.

Experimental animal studies with repeated exposure by inhalation supported that the CNS and the liver are target organs. However, the studies available suffer from several flaws such as insufficient number of animals, inappropriate exposure regimens, very high exposure concentrations, limited number of end points examined, and/or insufficient reporting. Therefore, the committee considers them not suitable to serve as a basis for the establishment of a health-based recommended exposure limit.

In repeated-dose oral studies, the liver was the most sensitive organ. In gavage studies, repeated doses of 200-300 mg/kg bw induced such severe toxicity in rats that experiments were broken off untimely. Doses of 1,1,2,2-tetrachloroethane of 104 mg/kg bw affected the liver (increased weights accompanied by mild to moderate cytoplasmic vacuolisation).

In 14-week diet studies in rats and mice, the committee could not establish NOAELs, since effects were observed in male rats and female mice at the lowest doses tested in these species and sexes. In male rats given daily doses of 20 mg/kg bw, there was an increase in the incidence of cytoplasmic vacuolisation of hepatocytes of minimal to mild severity in 7/10 animals; in female mice given 80 mg/kg bw, 2/10 animals showed minimal to mild hepatocytic hypertrophy.

In carcinogenicity studies in which examinations were limited to observation of signs of toxicity, body weight recordings, and (histo)pathology (after a 32-week treatment-free period), non-neoplastic findings included signs of toxicity at time-weighted average gavage doses of 62 mg/kg bw/day in male rats and of 43 mg/kg bw/day in female rats (the lowest doses tested) and decreased survival at 284 mg/kg bw/day in mice (NOAEL: 142 mg/kg bw/day). 1,1,2,2-Tetrachloroethane was carcinogenic to male and female B6C3F_1 mice causing statistically significant increases in the incidences of hepatocellular carcinomas following oral (gavage) administration of time-weighted average doses of 142 or 284 mg/kg bw/day, for 78 weeks (sacrifice after 90 weeks), but not in male and female Osborne-Mendel rats at time-weighted average doses of up to 108 mg/kg bw/day. 1,1,2,2-Tetrachloroethane had a weakly tumour-initiating and a more strongly tumour-promoting activity in Osborne-Mendel rats.

From *in vitro* and *in vivo* mutagenicity and genotoxicity data, the committee concludes that 1,1,2,2-tetrachloroethane is not a stochastic genotoxic compound.

1,1,2,2-Tetrachloroethane affected the reproductive tissues of male rats at dietary doses of 40 mg/kg bw/day and higher and of female rats at 170 mg/kg bw and higher, indicating that it might compromise fertility. However, the committee

did not find valid studies addressing the potential effects of 1,1,2,2-tetrachloroethane on fertility or pregnancy outcome.

Based on the data on carcinogenicity and mutagenicity, the committee concludes that 1,1,2,2-tetrachloroethane has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern. Therefore, 1,1,2,2-tetrachloroethane is classified as a suspect (non-genotoxic) carcinogen (comparable with EU category 3(B)).

For the time being, the committee considers 1,1,2,2-tetrachloroethane as a suspect, non-genotoxic carcinogen and, therefore, a threshold approach warranted. Both in human and experimental animal inhalation and oral studies, the liver seems the target organ. The committee considers that the data from inhalation studies are inappropriate and takes oral studies as a starting point for deriving a health-based recommended occupational exposure limit (HBROEL). The committee prefers the 14-week diet studies[45] to the carcinogenicity studies[49], in which no haematology and clinical chemistry analysis were done and (histo)pathology only after a 32-week exposure-free period. In the diet studies, no NO(A)EL could be established and rats were more sensitive than mice. At 20 mg/kg bw/day, the lowest level tested in rats, there was an increase in the incidence of cytoplasmic vacuolisation of hepatocytes of minimal to mild severity (average grade: 1.3 on a scale of 1-4) in 7/10 male rats. At 40 mg/kg bw, the next higher dose, hepatocellular vacuolisation was seen in 9/10 males (severity grade: 2.0) and in 10/10 females (severity grade: 1.7). At doses of 80 mg/kg bw and higher, there were increases in the incidence and severity of hepatocellular hypertrophy and necrosis.

Although hepatocellular vacuolisation by itself is not necessarily an adverse effect, the committee is of the opinion that in this case vacuolisation marks the first step leading to more severe effects such as hypertrophy and necrosis. Therefore, the committee takes the LOAEL of 20 mg/kg bw/day as the starting point for deriving a health-based occupational exposure limit (HBROEL).

7.2 Recommendation of a health-based occupational exposure limit

7.2.1 1,1,1,2-Tetrachloroethane

The committee is of the opinion that the data on the toxicity of 1,1,1,2-tetrachloroethane are insufficient to allow the recommendation of a health-based occupational exposure limit.

However, generally, the toxicological data – among which those from the study by Bucher[43] in which male rats were exposed by gavage to similar levels of 1,1,1,2- and 1,1,2,2-tetrachloroethane for 21 days – suggest that 1,1,1,2-tetrachloroethane is less toxic than the 1,1,2,2 isomer. Therefore, the committee is of the opinion that applying the occupational exposure level of 1,1,2,2-tetrachloroethane for 1,1,1,2-tetrachloroethane is justifiable.

7.2.2 *1,1,2,2-Tetrachloroethane*

The committee takes the LOAEL of 20 mg/kg bw/day from the study by Cha[45] in which rats received 1,1,2,2-tetrachloroethane in their diets, 24 hours/day, 7 days/week, for 14 weeks.

For extrapolation to an HBROEL, the following aspects are taken into account: the absence of a NOAEL, the difference between experimental conditions and the exposure pattern of the worker (i.e., semichronic vs. chronic, but intermittent exposure), and interspecies and intraspecies variation.

The committee notes the mildness of the key effect and the gap between the doses inducing the mild (vacuolisation) and more severe (hypertrophy/necrosis) effects. Further, in the experimental animal study, the animals had continuous access to the test compound without any exposure-free period to recover. On the other hand, workers are exposed 8 hours/day, 5 days/week allowing recovery. Therefore, the committee considers a factor of only 2 justified for the absence of a NOAEL and does not apply a factor for difference in 'exposure duration'. For intraspecies and interspecies variation, the committee takes a total factor of 10.

Taking the LOAEL of 20 mg/kg bw and applying the total extrapolation factor of 20, the committee recommends a health-based occupational exposure limit of 7 mg/m^3 for 1,1,2,2-tetrachloroethane, assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%.

In view of the large gap (a factor of about 180) between the lowest dose (142 mg/kg bw/day) found to increase the number of hepatocellular carcinomas in mice and the recommended exposure limit (7 mg/m^3, equivalent to a daily dose of 1 mg/kg bw,), the committee considers this limit as cancer preventive for workers.

Skin notation

According to the committee, a 'skin notation' is warranted if the amount absorbed by both hands and underarms (total surface area: 2000 cm^2) during a 1-hour contact with the liquid could amount to more than 10% of the amount

absorbed via the lungs following exposure to the occupational exposure limit for eight hours (provided that this limit is based on systemic rather than on local effects).

A quantitative dermal absorption rate of 27 µg/cm^2/h has been calculated for contact with a saturated aqueous solution (3 mg/mL). Using this rate, it can be calculated that an amount of 54 mg (27 µg/cm^2/h x 2000 cm^2 x 1 h) can be taken up by dermal contact, which is far more than 10% of the amount absorbed via the lungs at an 8-hour exposure to the proposed exposure limit (viz., 70 mg).

This absorption rate is based on a skin permeability coefficient, which was calculated using an equation in which this coefficient is described as a function of the molecular weight and the octanol-water partition coefficient (see [61]). EPA states that such figures should only be used at steady state conditions, which usually occur only for prolonged exposure to an infinite dose.

Although the committee feels that there are much uncertainties in using this aforementioned dermal absorption rate for the quantitative comparison underlying the recommendation of a skin notation, such as the relevance of mathematically derived data with respect to the actual dermal exposure of the worker, the data indicate a skin penetrating potential. The committee considers, therefore, a skin notation warranted.

7.3 Groups at extra risk

The committee has not identified groups at extra risk.

7.4 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for 1,1,2,2-tetrachloroethane of 7 mg/m^3 (1 ppm), as an 8-hour time-weighted average concentration, and a skin notation.

For 1,1,1,2-tetrachloroethane, no health-based occupational exposure limit can be recommended.

7.5 Additional consideration

The committee concluded that the toxicological database does not allow the recommendation of a health-based occupational exposure limit for 1,1,1,2-tetrachloroethane (see Section 7.1.1).

However, generally the toxicological data – among which those from the study by Bucher[43] in which male rats were exposed by gavage to similar levels of 1,1,1,2- and 1,1,2,2-tetrachloroethane for 21 days – suggest that 1,1,1,2-tetrachloroethane is less toxic than the 1,1,2,2 isomer. Therefore, the committee is of the opinion that applying an occupational exposure level of 7 mg/m^3 for 1,1,1,2-tetrachloroethane, as an 8-hour time-weighted average, is justifiable.

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden
- R.B. Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- P.J. Boogaard
  toxicologist; SHELL International B.V., The Hague
- P.J. Borm
  toxicologist; Centre of Expertise in Life Sciences, Hogeschool Zuyd, Heerlen
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  professor of risk assessment in occupational epidemiology; IRAS, University of Utrecht, Utrecht
- T.M. Pal
  occupational physician; Dutch Centre for Occupational Diseases, Amsterdam
- I.M. Rietjens
  professor of toxicology; Wageningen University, Wageningen.
- H. Roelfzema, *advisor*
  ministery of Health, Welfare and Sport, The Hague

- T. Smid
  occupational hygienist/epidemiologist; KLM Health Safety & Environment, Schiphol; and, professor of working conditions, Free University, Amsterdam
- G.M.H. Swaen
  epidemiologist; Dow Chemical Company, the Netherlands
- R.A. Woutersen
  toxicologic pathologist; TNO Quality of Life, Zeist
- P. Wulp
  occupational physician; Labour Inspectorate, Groningen
- J.T.J. Stouten, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released 2005 for public review. The following organisations and persons have commented on the draft document:

- R. Zumwalde
  National Institute of Occupational Safety and Health (NIOSH), USA

Annex **D**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at which inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{10}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{10}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit

ppb	parts per billion (v/v)10^{-9}
ppm	parts per million (v/v)10^{-6}
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit
STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice a day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram

FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	Guinea pig maimisation test
GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration
MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	Relative Risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring

BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography
IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	Relative Risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Part II

Arbete och Hälsa: Tetrachloroethane

1996:28

DECOS and NEG Basis for an Occupational Standard

Tetrachloroethane

Marita Luotamo
Vesa Riihimäki

Nordic Council of Ministers

ARBETE OCH HÄLSA VETENSKAPLIG SKRIFTSERIE

ISBN 91-7045-403-5 ISSN 0346-7821

Arbetslivsinstitutet
National Institute for Working Life

ARBETE OCH HÄLSA
Redaktör: Anders Kjellberg
Redaktionskommitté: Anders Colmsjö,
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Arbetslivsinstitutet,
171 84 Solna, Sverige

ISBN 91-7045-403-5
ISSN 0346-7821
Tryckt hos CM Gruppen

Preface

An agreement has been signed by the Dutch Expert Committee for Occupational Standards (DECOS) of the Dutch Health Council and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents which could be used by the national regulatory authorities in both the Netherlands and in the Nordic Countries.

The document on health effects of Tetrachloroethane was written by Dr Marita Luotamo and Dr Vesa Riihimäki from the Finnish Institute of Occupational Health, Helsinki, Finland, and has been reviewed by the DECOS as well as by the NEG.

V.J. Feron
Chairman
DECOS

P. Lundberg
Chairman
NEG

Contents

Abbreviations

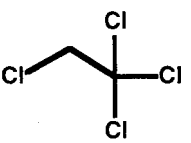
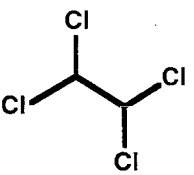
TCA	=	Tetrachloroethane
1,1,1,2-TCE	=	1,1,1,2- Tetrachloroethane
1,1,2,2-TCE	=	1,1,2,2- Tetrachloroethane
P450	=	cytochrome P450
OEL	=	occupational exposure level
MTD	=	maximum tolerated dose
GSH	=	glutathione (reduced)
ESR	=	electron spin resonance
DEN	=	diethylnitrosamine
GGT+	=	γ-glutamyl-transferase positive
ASAT	=	aspartate amino-transferase
ALAT	=	alanine aminotransferase
NOAEL	=	no-observed-effect-level

1. Introduction

Tetrachloroethanes are ethanes in which four hydrogen atoms have been replaced by chlorine. Tetrachloroethane has two isomers: 1,1,1,2- and 1,1,2,2-tetrachloroethane (in this report abbreviated 1,1,1,2-TCE and 1,1,2,2-TCE, respectively). 1,1,1,2-Tetrachloroethane has not been used on an industrial scale, whereas 1,1,2,2-TCE has been manufactured and used extensively. The latter isomer has mainly been used as a solvent for cellulose acetate, fats, waxes, greases, rubber and sulphur and as a dope in the varnish to render aeroplane wing surfaces impervious to moisture and air. It has also been used in electrical equipment and supplies, chemicals and allied products, electric, gas and sanitary services. 1,1,2,2-Tetrachloroethane has been used from the beginning of this century until its high toxicity was detected, and after the 1970s its use has nearly ended.

According to European Parliament and Council Directive (24), 1,1,1,2- and 1,1,2,2-TCE may not be used in concentrations equal to or greater than 0.1% by weight in substances and preparations placed on the market for sale to the general public.

2. Substance identity

	1,1,1,2-TCE	1,1,2,2-TCE	Ref.
CAS number	630-20-6 *	79-34-5 *	
Systematic name	Tetrachlororethane asymmetric tetra	Tetrachlororethane symmetric tetra	
Synonyms		Acetylene tetrachloride [1], 1,1-dichloro-2,2,-dichloroethane, tetrachloroethane, symtetrachloroethane	[1] (81)
Trade name	Not commercially available	Acetosal, Bonoform, Cellon Westron, 1,1,2,2-Czterochlorethan (Polish), NCI-C03554 RCRA Waste Number U209	
Molecular formula	$ClHC\text{-}CHCl_3$	$Cl_2HC\text{-}CHCl_2$	
Structural formula			
Molecular mass	167.84	167.84	

* CAS number 25322-20-7 (tetrachloroethane, isomer not specified)

3. Physical and chemical properties ((86), if not otherwise stated)

	1,1,1,2-TCE	1,1,2,2-TCE	Reference
Melting point	-68.7°C		
Boiling point	130.5°C	146.5°C	
Relative density	.5468		
Vapour pressure	0.66 kPa (20°C) [1]	0.680 kPa (20°C)	[1] (93)
Saturation concentration in air	0.65 % (20°C) (= 45.3 g/m^3)	0.67% (20°C) (= 46.7 g/m^3)	
Solubility in water	No data available	0.3% w/w (20°C)	(9)
Solubility	Soluble in ethanol, diethylether, acetone, benzene, chloroform	Soluble in methanol, ethanol, benzene, diethyl ether, petroleum ether, carbon tetrachloride, chloroform, carbon disulphide, dimethyl-formamide, oils [2]	[2] (111)
Refractive index	no data available	1.4918	(9)
Conversion factors	1 cm^3/m^3 = 6.87 mg/m^3 [4] 1 mg/m^3 = 0.146 cm^3/m^3 (25°C, 101.3 kPa)	1 cm^3/m^3 = 6.87 mg/m^3 [3] 1 mg/m^3 = 0.146 cm^3/m^3 (25°C, 101.3 kPa)	[3] (9) American convention
Conversion factors	1 cm^3/m^3 = 6.96 mg/m^3 1 mg/m^3 = 0.144 cm^3/m^3 (20°C, 101.3 kPa)	1 cm^3/m^3 = 6.96 mg/m^3 1 mg/m^3 = 0.144 cm^3/m^3 (20°C, 101.3 kPa)	European convention (used in this report)

1,1,1,2-Tetrachloroethane is a colourless, non-flammable, heavy liquid. In general 1,1,1,2-TCE is more stable than its symmetrically substituted isomer. Thermal decomposition at 500-600°C yields trichloroethylene and hydrogen chloride (105).

1,1,2,2-Tetrachloroethane is a colourless, non-flammable, heavy liquid with a sweetish odour. 1,1,2,2-Tetrachloroethane is sufficently stable and can be stored without adding stabilizers, if there is no exposure to moisture, air and light.

In this document all the tetrachloroethane concentrations in air are given first as mg/m^3 (= 1,000 x mg/L) and after that the units used in the original article. The conversion factor used in this report is that of the European convention (see above in this Section).

4. Production, occurrence and use

Neither 1,1,1,2-TCE nor 1,1,2,2-TCE are known to occur as natural products.

4.1. 1,1,1,2-Tetrachloroethane

1,1,1,2-Tetrachloroethane was first synthetized by A. Mouneyrat in 1898. It is a common by-product of many industrial chlorination reactions of C_2 hydrocarbons but it is not produced on an industrial scale (105).

4.2. 1,1,2,2-Tetrachloroethane

1,1,2,2-Tetrachloroethane was first synthetized in 1869, and the first industrial scale production process was developed in 1903. 1,1,2,2-Tetrachloroethane became the first chloroethane to be produced in high tonnage before World War I, and was mainly used as a solvent for cellulose acetate, fats, waxes, greases, rubber and sulphur. Due to its relatively low cost, non-flammability and good solvent capacity, tetrachloroethane was widely used in industry for many years (5). Its main use was as an additive in varnish applied to aeroplane wing surfaces to render them impervious to moisture and air (75, 105). The isomer was also used in electronic and pesticide industry and as an organic intermediate in the production of trichloroethylene from acetylene (78). Tetrachloroethane was used by the U.S. Army during World War II to impregnate clothing for protection against mustard gas (76), in fabric cleaning and clothes "spotting", artificial silk industry and in the manufacture of artificial pearls (5). Due to the toxicity of 1,1,2,2-TCE, it has largely been replaced since World War II by less toxic solvents (75): Subsequent to the replacement of trichloroethylene by 1,1,1-trichloroethane and the development of more economical processes for the production of tetrachloroethylene, 1,1,2,2-TCE has become less important for the production of chlorinated solvents (105). Currently the trend has partly been reversed by international agreements prohibiting the use of 1,1,1-trichloroethane for environmental reasons.

In the U.S.A., approximately 222 million kg were produced in 1967, but the production declined to about 17 million kg by 1974 (U.S. International trade Commission, 1977 in (93)). No data of the chemicals use in Europe and Japan are available (44). In Norway, 0.6 tonnes of 1,1,2,2-TCE was produced or imported in 1994 (Petter Kristensen, National Institute of Occupational Health, personal communication). In Denmark, no 1,1,2,2-TCE was produced, imported or used (Adolf Schaich Fries, Institute of Occupational Health, Denmark, personal communication). In Finland, 1,1,2,2-TCE was not produced, imported or used.

1,1,2,2-Tetrachloroethane is currently produced by catalytic addition of chlorine to acetylene. In most processes the substance is a non-isolated intermediate which is immediately thermally cracked for the production of trichloroethylene. However, it may also be isolated as a by-product and used as a feedstock in the production of trichloroethylene, tetrachloroethylene, and 1,2-dichloroethylene. Consequently 1,1,2,2-TCE may appear as a minor impurity in the previous end products. Due to toxicity concerns and new processes for manufacturing chlorinated

ethylenes, the manufacture of 1,1,2,2-TCE as an end product now appears to be very limited (1). It may be used as a solvent, insecticide or fumigant; however, it is not currently registered for use as a pesticide in the USA (1).

5. Occupational exposure

In 1976, NIOSH reviewed the exposure levels of 1,1,2,2-TCE in occupational settings (75). A study from that late 50s and others from the 60s are reported. In a relatively old study from 1957 the levels of tetrachloroethane (TCE) was measured in a penicillin factory in which TCE was used as an extraction liquid (48). The concentrations of TCE ranged from 10-1,700 mg/m^3 (1.5-247 ppm) measured in 170 air samples taken from different points of the process and from within the ventilation systems.

In a study conducted in India, hundreds of workers were exposed to tetrachloroethane in 23 different bangle-manufacturing plants; the measured average 1,1,2,2-TCE concentrations in air varied from 63-680 mg/m^3 (9-98 ppm) (59).

Horiguchi and coworkers reported 1,1,2,2-TCE concentrations from 520-1,570 mg/m^3 (75-224 ppm) in three Japanese artificial pearl producing factories (40). Subsequently two factories ceased using 1,1,2,2-TCE, and the remaining factory succeeded in reducing the level of 1,1,2,2-TCE to around 140 mg/m^3 (20 ppm) (40).

In an Italian study, 75 workers at two plants were exposed to tetrachloroethane during four different process events: i) tetrachloroethane production via chlorination of acetylene; ii) production of tri- and tetrachloroethylene from TCE; iii) storage and loading of TCE; and iv) quality control laboratories of the two plants (32). At the first activity the average minimum concentration was 2.5 mg/m^3 (0.37 ppm), average maximum 9.1 mg/m^3 (1.33 ppm) with a single peak at 22 mg/m^3 (3.2 ppm). During maintenance and unusual circumstances the concentrations ranged from 34-103 mg/m^3 (5-15 ppm) with occasional peaks at 278 mg/m^3 (40 ppm).

6. Sampling and analysis of substance at workplace

Few contemporary reports concerning industrial hygiene measurements of tetrachlororethanes have been found. Obviously there has not been pressing needs to method development in recent times.

NIOSH (75) has presented an analytical method for 1,1,2,2-TCE ("Analytical method for 1,1,2,2-tetrachlororethane", Appedix II). The sample is collected in a charcoal tube (in the range of 0.5-15 ppm), and tetrachlororethane is desorbed, and an aliquot analyzed by a gas chromatograph. The coefficient of variation for the total analytical and sampling method at the air concentration range of 16-70 mg/m^3 (2.3-10 ppm) was 0.057 (75).

Three different passive samplers (Abcor, duPont, 3M) were assessed for the measurement of 1,1,2,2-TCE in air, and the results were compared with infrared measurements. The passive samplers showed an average bias of +2.0%, at the level of 21 mg/m^3 (3 ppm) (25).

Four portable chromatographs using electron capture and photoionization detectors were compared under field conditions for the analysis of 1,1,2,2-TCE. The electron capture detector had a lower limit of detection compared to the photoionization detector (50).

A gas chromatographic-mass spectrometric analysis using methylene chloride extraction, DB-Wax-30N capillary column and temperature programming, detected 1,1,2,2-TCE as a leached rubber stopper component (17).

7. Toxicokinetics

Table 1 shows partition coefficients between important (biological) media for 1,1,1,2-TCE and 1,1,2,2-TCE, measured *in vitro*. Tetrachloroethanes are quite soluble in blood and tissues.

7.1. Uptake

Inhalation uptake in humans has been measured indirectly as exhaled TCE. A volunteer inhaled 2.5 mg of uniformly ^{38}Cl-labelled 1,1,2,2-TCE vapour from a 150 mL bulb, held their breath for 20 seconds, and exhaled through an activated-charcoal trap (two exhalations). Ninety-seven per cent of the inhaled tetrachloroethane was retained; and only 3.3% of the inhaled tetrachloroethane vapour was exhaled during the following hour (67).

Information on dermal absorption in humans has not been located. However, the human skin permeability coefficient for 1,1,2,2-TCE was calculated as 0.009 cm/h (106); the corresponding steady state flux of 1,1,2,2-TCE from a saturated water solution (3 mg/ml) is therefore 27 µg per cm^2 and h. These relatively low values imply that the skin penetration potential of the compound is limited. However, compared to the inhalation uptake at the occupational exposure limit (OEL) of 1 ppm = 7 mg/m^3, extensive skin contact may lead to significant absorption. As an example, if the palms of both hands (about 400 cm^2) are in contact with 1,1,2,2-TCE, skin uptake would amount to approximately 10.8 mg per hour.

7.2 Distribution

When adult C57B1 mice were injected ^{14}C-1,1,2,2-TCE *i.v.* (3 mg/kg; 9 µCi in 20 µL DMSO), highest concentrations of irreversibly bound metabolites were found between 1 and 240 min in the respiratory and gastrointestinal systems (olfactory and tracheobronchial mucosa, mucosa of the oral cavity, tongue, nasopharynx, esophagus and cardiac region of the forestomach), in the liver, in the contents of the gallbladder, as well as in the inner zone of the adrenal cortex and in the interstitium of the testis (23).

Table 1. Partition coefficients for 1,1,1,2-TCE and 1,1,2,2-TCE *in vitro* (at 37°C)

Partition coefficient		1,1,2,2-TCE		
Octanol / water (log_{10}-value)	2.6 1)	2.4	7)	1) ICIS in (45)
				7) (1)
Oil / water		370	3)	3) (84)
Oil / air		6358	4)	7) (31)
Blood / air	41.7 2)	142	5)	2) (31)
		72.6	6)	5) (28)
				6) (67)
Serum / air		78.2	6)	6) (67)
Liver / blood	2.12 2)	1.38	2)	2) (31)
Muscle / blood	0.95 2)	0.71	2)	2) (31)
Fat / blood	51.5 2)	26.5	2)	2) (31)

The oil/blood partition coefficient calculated on the basis of blood/air and oil/air coefficients is 45

7.3. Biotransformation

One dose of ^{14}C-1,1,2,2-tetrachloroethane was injected *i.p.* into female albino mice (dose 0.21-0.32 g/kg) and the elimination of radioactivity was followed for 3 days. Half of the dose (45-61%) was expired as carbon dioxide and 28% (range 23-34%) of the activity was excreted with the urine. Around 16% of the dose retained in the animal and less than 4% was expired unchanged (113). Metabolites identified in the urine collected for 24 h (mean activity %) were dichloroacetic acid (27%), trichloroacetic acid (4%), trichloroethanol (10%), oxalic acid (7%) and glyoxylic acid (0.9%). From these data, the authors proposed the following metabolic scheme for 1,1,2,2-TCE which has been supplemented with some more recent data (36, 78) (Fig. 1). The biotransformation seems to involve a stepwise hydrolytic cleavage of carbon chlorine bonds via dichloroacetic acid (and through subsequent intermediates) to glyoxylic acid (a, b, c, Fig. 1). More recent evidence show that the hydroxylation of 1,1,2,2-TCE yielding 1,1-dichloroacetyl chloride (route k) is the predominant metabolic pathway to dichloroacetic acid (36). Glyoxylic acid is further metabolized (m, n). Some of the tetrachloroethane undergoes (enzymatic and non-enzymatic) dehydrochlorination to trichloroethylene (d), which gives rise to trichloroethanol and trichloroacetic acid (e, f, g), and some is oxidized to tetrachloroethylene (h), which contributes to the formation of trichloroacetic acid and oxalic acid (i, j). There is also evidence for a reductive dechlorination pathway (l) to form a carbon centered radical (78).

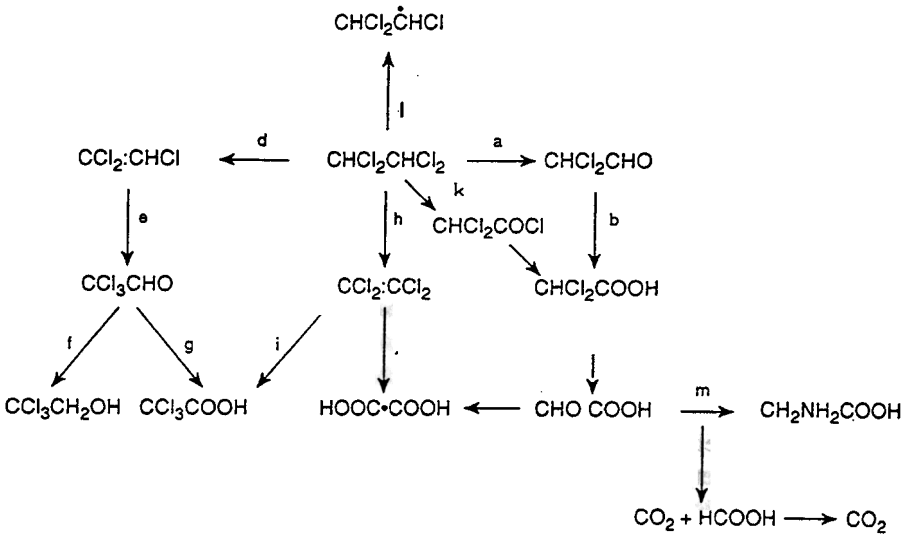


Figure 1. Suggested metabolic pathways of 1,1,2,2-TCE (1)

Wistar rats (about 70 g, 6-8 animals per group) of both sexes were given 1,1,1,2-TCE and 1,1,2,2-TCE (2.78 mmol/kg = 467 mg/kg) *i.p.*, or were exposed to the vapour of the compounds (1,374 mg/m^3 = 200 ppm) for 8 h and urine was collected for 48 h. Total trichlorocompounds, trichloroacetic acid and trichloroethanol were determined colorimetrically by the Fujiwara reaction under conditions described by Tanaka and Ikeda (95). The authors point out, that these estimates using the Fujiwara reaction also include other metabolites than trichloroacetic acid and trichloroethanol. After inhalation exposure, the urinary excretion of total trichlorocompounds in rats was 199 mg/kg bw from 1,1,1,2-TCE and 8.2 mg/kg bw from 1,1,2,2-TCE. 1,1,1,2-Tetrachloroethane gave rise to large amounts of trichloroacetic acid (39.4 mg/kg bw) and trichloroethanol (159.6 mg/kg bw) excretion, whereas 1,1,2,2-TCE yielded less than one-twentieth of these amounts (1.7 and 6.5 mg/kg bw, respectively). After a single *i.p.* injection of 1,1,1,2-TCE, the total 48-h urinary excretion of trichlorocompounds was 114.2 mg/kg bw, trichloroacetic acid excretion was 16.9 mg/kg bw and trichloroethanol excretion was 97.3. mg/kg bw. After an *i.p.* injection of 1,1,2,2-TCE, the amounts of these metabolites were 2.1, 1.3, and 0.8 mg/kg bw, respectively (42).

Male Osborne-Mendel rats and male B6C3F1 mice were given 1,1,1,2-TCE and 1,1,2,2-TCE at two dose levels, the maximum tolerated dose (MTD) and 1/4 MTD orally as unlabelled compounds 5 days per week for 4 weeks, followed by a single

dose of the corresponding radiolabelled compound in corn oil. The doses of 1,1,1,2-TCE were 200 mg/kg and 50 mg/kg to rats and 400 mg/kg and 100 mg/kg to mice. The doses of 1,1,2,2-TCE were 100 mg/kg and 25 mg/kg to rats, and 200 mg/kg and 50 mg/kg to mice (66). A similar proportion of the dose of 1,1,2,2-TCE was exhaled unchanged in experiments with rats (7.03%) and mice (9.69%), while rats exhaled much more 1,1,1,2-TCE unchanged (34.14%) than mice (5.89%), At least three fourths of the dose was found as CO_2, in excreta, or in carcass, and 78.7% and 67.8% of 1,1,2,2-TCE, and 64.8 and 84.3% of 1,1,1,2-TCE in rats and mice, respectively, were considered to be metabolized. Apart from CO_2 trichloroethanol and trichloroacetic acid were major metabolites from both 1,1,1,2- and 1,1,2,2-TCE (66).

Studies with rodents suggest that the main metabolic pathway for 1,1,1,2-TCE gives rise to trichloroethanol and trichloroacetic acid while the corresponding pathway for 1,1,2,2-TCE yields dichloroacetic acid. Although not depicted in Fig. 1, further detailed studies on the metabolism of dichloroacetate and trichloroacetate in mice and rats have demonstrated a potential for the formation of toxic metabolites (acid chloride intermediate from dichloroacetate and dichloroacetyl free radical from trichloroacetate) (54).

Metabolism of 1,1,1,2-TCE involves both oxidative and reductive pathways; both processes are dependent on phenobarbital-inducible forms of cytochrome P450 (abbreviated P450)(10). The former leads to trichloroethanol as the major metabolite (60). Under unaerobic conditions 1,1,1,2-TCE was metabolized extensively in rat liver microsomes to 1,1-dichloroethylene (the main metabolite) and 1,1,2-trichloroethylene. The ratio of the two was about 25:1 (99). Oxygen strongly inhibited the reduction of 1,1,1,2-TCE *in vitro*. 1,1,1,2-TCE was metabolized to 1,1-dichloroethylene (75-220 higher concentrations) and 1,1,2-trichloroethylene also *in vivo,* (99). The reductive pathways involve one- or two-electron reductions. The former leads to a free radical formation, and this reactive product can either interact with membrane components and induce lipid per-oxidation, producing 1,1,2-trichloroethane, or bind covalently with nucleic acids and proteins. The latter reaction, which is the preferred pathway, yields 1,1-di-chloroethylene (10).

Metabolism of 1,1,2,2-TCE *in vitro* (rat liver microsomes) was shown to be P450 dependent (11, 13, 47). In another study, 1,1,2,2-tetrachloroethane was oxidized to dichloroacetic acid by rat liver microsomes and purified P450b (35). 1,1,2,2- Tetrachloroethane was metabolized by rat liver microsomes *in vitro* only through an oxidative route, no reductive metabolism was observed (70, 99, 100, 102). However, an *in vivo* CD1 mouse study with 1,1,2,2-TCE indicated the formation of a carbon centered radical, presumably $CHCl_2\dot{C}HCl$, and subsequent lipid peroxidation in the liver (78), suggesting that in addition to the oxidative biotransformation, some reductive metabolism of 1,1,2,2-TCE occurs. Microsomal and cytosolic GSH-transferases have also been shown to contribute to the metabolism of both 1,1,2,2- and 1,1,1,2-TCE (10, 11).

A reconstituted monooxygenase system from phenobarbital treated rats, which lacked aldehyde dehydrogenase, effectively metabolized 1,1,2,2-TCE to dichloro-

acetic acid (36). This finding suggests that dichloroacetaldehyde is not an obligatory intermediate in dichloroacetic acid formation and gives support to the involvement of an acyl chloride intermediate. A partially purified antibody against the major phenobarbital-induced form of P450 (CYP2B subfamily), which was capable of inhibiting 46% of 7-ethylcoumarin deethylase activity, inhibited almost to the same extent the formation of dichloroacetic acid from 1,1,2,2-TCE in phenobarbital treated intact rat liver microsomes (36).

Metabolic activation of 1,1,2,2-TCE in male BALB/c mouse liver microsomes, and binding to calf thymus DNA, was enhanced by phenobarbital pretreatment and inhibited by addition of SKF 525A or diethylmaleate. The latter suggested that even glutathione conjugation (microsomal and cytosolic GSH-transferases) have an important role in the metabolic activation of 1,1,2,2-TCE in the mouse (11). In contrast, another study showed that addition of glutathione to incubates containing microsomal and cytosolic fractions of the C57B1 mouse olfactory mucosa and liver decreased the binding of 1,1,2,2-TCE to macromolecules, indicating that glutathione-mediated activation does not play a major role in binding (23).

The hepatic rate of 1,1,1,2-TCE metabolism in fed male and female Wistar rats measured *in vitro* ranged from 6.9-8.1 nmol/g liver tissue and minute. The corresponding values for 1,1,2,2-TCE were 13.3-15.7 nmol/g liver tissue and minute (69). These rates of metabolism were increased 2-4 fold by fasting (69), and 4-5 fold by chronic ethanol consumption (85). While these treatments did not increase the total microsomal P450 content, they suggested that ethanol inducible P450 isoform, CYP2E1, is involved in TCE metabolism. However, male Wistar rats given methanol, isopropanol or ethanol as one large dose by gavage 18 h prior to challenge, or the same alcohols in drinking water for 5-6 weeks and then challenged by an *i.p.* injection of 140 or 240 mg/kg 1,1,2,2-TCE, showed no or very small serum ALAT elevations compared to control animals that had not received alcohols (51). In contrast, alcohol pretreatments which are known to induce CYP2E1 mediated metabolism enhanced hepatotoxicity of chloroform and carbon tetrachloride in the same study. Therefore it appears unlikely that CYP2E1 is involved in the metabolic activation of 1,1,2,2-TCE.

Metabolic kinetic constants of 1,1,1,2-TCE and 1,1,2,2-TCE were determined *in vivo* by a gas phase method. Male Fischer 344 rats (200-300 g) were exposed by inhalation to a constant concentration for 6 hours 2,450 mg/m^3 (352 ppm) of 1,1,1,2-TCE and 2,440 mg/m^3 (350 ppm) of 1,1,2,2-TCE and then placed in 2.5-liter exhaled breath chambers with fresh air flow. The chamber effluent was serially analyzed for test chemical. Optimized maximum metabolic rates (V_{max}) were 6.39 mg/kg/h (= 38.7 µmol/h) for 1,1,1,2-TCE and 12.9 mg/kg/h (= 71.5 µmol/h) for 1,1,2,2-TCE (31).

1,1,1,2-Tetrachloroethane was transformed to 1,1-dichloroethylene with an apparent K_m of 19.50 mM, V_{max} of 59.0 nmol/min/mg in the presence of oxygen by microsomes from phenobarbital-treated male Holzman rats (102). In hepatic microsomes from Aroclor 1254 treated rats, the apparent V_{max} for 1,1,2,2-TCE dechlorination was 18.2 nmol/min/mg protein and the Km 0.55 mM (83).

7.4. Elimination

About half of an *i.p.* administration of ^{14}C-1,1,2,2-TCE given to female albino mice was excreted over 3 days, for further details, (see Section 7.3 "Biotransformation") (8, 113).

According to basic toxicokinetic principles the temporal characteristics of distribution of the free compound to tissues are a function of the compound's solubility, tissue volumes and tissue blood flows. These factors determine the attainment of equilibrium, often expressed by tissue time constants: the time for the tissue to reach 63% of the equilibrium concentration (21). Elimination by washout is a reverse phenomenon to distribution. Concerning fatsoluble substances that are rapidly metabolized, mobilization from adipose tissue may be rate-limiting for elimination. A calculation of the tissue time constant for TCE in human fat (based on perfusion rate 0.32 L/min, tissue volume 14.5 L and oil/blood partitioning of 45) would yield a time constant of 34 hours. Thus it may be predicted that the unbound compound would not have a long residence time in the human body.

8. Methods of biological monitoring

Due to its limited industrial exposure, there are no reports concerning biological monitoring methods for the assessment of exposure to TCE. It can be envisaged that TCE could be measured in blood for biological monitoring purposes like other chlorinated aliphatic hydrocarbons. Similarly, analysis of metabolites such as trichloroacetic acid and trichloroethanol could be used for biomonitoring purposes, but the drawback is that they are not specific to TCE.

However, because there are no data on the relationship between TCE concentrations in ambient air and tissues (blood) among occupationally exposed workers, and because the essential toxicokinetic data on metabolism and excretion in humans are lacking, meaningful application of biological monitoring for TCE is not possible.

9. Mechanisms of toxicity

In tissue binding studies adult male Wistar rats and male BALB/c mice were injected *i.p.* with ^{14}C-labelled-1,1,2,2-TCE (11) and 1,1,1,2-TCE (10) (127 µCi/kg bw). Some of the animals were daily pretreated with 100 mg/kg bw *i.p.* phenobarbitone injections for 2 days before sacrifice. Fasted animals were killed 22 h later, their organs (liver, kidney, lung, stomach) were removed, pooled and processed in order to obtain DNA, RNA and proteins (10, 11). 1,1,2,2-Tetrachloroethane was bound covalently to DNA and RNA, and to proteins of the microsomal and cytosolic fractions in liver, lung, kidney and stomach. With the exception of the lung, specific activity in the DNA from mouse organs was higher

than that from rat organs. On the contrary, the binding to RNA and proteins was higher in rat organs than in mouse organs. Cytosolic fractions from all assayed organs of the mouse and from rat liver and, to a lesser extent from rat lung, enhanced binding of 1,1,2,2-TCE to macromolecules *in vitro*. The interaction of 1,1,2,2-TCE with synthetic polyribonucleotides was clearly higher in the presence than in the absence of NADPH and GSH. The highest specific activity was observed in poly(G) (11).

After *i.p.* injection of 1,1,1,2-TCE, binding to DNA was highest in mouse lung. With the exception of stomach, DNA and RNA from mouse organs were labelled more than DNA from rat organs. Labelling of proteins and especially RNA from organs of both species was higher than labelling of DNA. Microsomes isolated from the liver of both species were efficient in bioactivating 1,1,1,2-TCE, and gave similar binding values. Binding to DNA was also mediated by microsomes isolated from the mouse lung and to a smaller extent by microsomes isolated from the mouse kidney. Microsomal fractions from lung and kidney of the rat and from stomach of both species were ineffective (10).

When both tetrachloroethanes were given to rats and mice orally (66) (for experimental details, see Section 6.3 of that Ref.), 1,1,2,2-TCE was extensively covalently bound to hepatic proteins both in rats and mice; covalent binding was much lower for 1,1,1,2-TCE. Since both tetrachloroethane isomers have caused hepatic tumours (adenomas and/or carcinomas) in mice, and since especially 1,1,2,2-TCE showed genotoxicity in some short term assays *in vitro*, it is of interest to note the reactivity of the compounds to DNA. The magnitude of binding to the mouse and rat liver DNA (covalent binding index) was higher for 1,1,2,2-TCE than for 1,1,1,2-TCE; the interaction with DNA was found comparable to recognized weak-to-moderate carcinogens (61). Concerning metabolism in the liver, studies suggest that tetrachloroethane isomers may be metabolically activated to acylchlorides or free radicals; toxicity may ensue from protein binding and lipid peroxidation. Metabolism of 1,1,2,2-TCE in the mouse was associated with reduced hepatic levels of a wide variety of microsomal P450 enzymes and haeme, indicating damage to the liver endoplasmic reticulum (78). With electron spin resonance (ESR) spectroscopy spin-trapping *in vivo* techniques the authors found evidence in support of the occurrence of a free radical species, presumably $CHCl_2\dot{C}HCl$ free radical. There was evidence of concomitant peroxidation of polyunsaturated fatty acids based on characteristic conjugated diene signals in lipids extracted from liver microsomes of 1,1,2,2-TCE treated mice (78).

10. Effects in animals and *in vitro* studies

10.1. Irritation and sensitisation

Liquid 1,1,2,2-TCE is a strong irritant of the skin and mucosal membranes (93). No reports were located concerning sensitisation. No data were found in 1,1,1,2-TCE.

10.2. Acute toxicity

Acute toxicity data on 1,1,1,2-TCE and 1,1,2,2-TCE are given in Tables 2 and 3, respectively.

The anaesthetic potency of TCE has been demonstrated in different studies: cats exposed to 5,700 mg/m^3 (830 ppm) developed narcosis in 4 h and deep narcosis in 5 h, while the cats exposed to 57,000 mg/m^3 (8,300 ppm) reached light narcosis in 25 min and deep narcosis in 40 min (56).

In different inhalation exposures to TCE lasting a few hours (55, 77) mice assumed a lateral position at 7,460-10,000 mg/m^3 (1,091-1,455 ppm), lost their reflexes at 9,996-14,990 mg/m^3 (1,450-2,180 ppm) and died at 40,000 mg/m^3 (5,820 ppm).

Table 2. Acute toxicity data for 1,1,1,2-TCE source: RTECS(R) (80) (extracted from RTECs)

Species	Acute toxicity	Route	Dose
rat	LD_{50}	oral	670 mg/kg
rat	LC_{50}	inhalation	14,600 mg/m^3 (2,100 ppm) / 4h
mouse	LD_{50}	oral	1,500 mg/kg
mouse	LD_{50}	intraperitoneal	1,275 mg/kg
rabbit	LC_{50}	inhalation	19,500 mg/m^3 (2,800 ppm) / 4h
rabbit	LD_{50}	skin	20,000 mg/kg

Table 3. Acute toxicity data for 1,1,2,2-TCE source: RTECS(R) (80) (extracted from RTECs)

Species	Acute toxicity	Route	Dose
human	TDLo	oral	30 mg/kg
human	TCLo	inhalation	1,000 mg/m^3 /30 min
rat	LCLo	inhalation	7,000 mg/m^3 (1,000 ppm)/4h
rabbit	LDLo	subcutaneous	500 mg/kg
guinea pig	LDLo	intraperitoneal	500 mg/kg
dog	LDLo	oral	300 mg/kg
dog	LDLo	intravenous	50 mg/kg
cat	LCLo	inhalation	19,000 mg/m^3/45 min
rat	LD_{50}	oral	250 mg/kg
mouse	LC_{50}	inhalation	4,500 mg/m^3/2h
mouse	LD_{50}	intraperitoneal	821 mg/kg
mouse	LD_{50}	subcutaneous	1,108 mg/kg

TDLo/TCLo = lowest published toxic dose/concentration

LDLo/LCLo = lowest published lethal dose/concentration

10.3. Short-term exposure

Most studies available are relatively old (between 1911 and 1962) and are conducted by the inhalation and injection routes. The isomer of TCE was usually not specified, but it is likely that it was 1,1,2,2-TCE. Two relatively recent studies from 1972 conducted with rats administered 1,1,2,2-TCE by the inhalation and oral routes are also available.

Inhalation exposure of cats to TCE, ranging from 4,880 mg/m^3 (710 ppm) to 41,900 mg/m^3 (6,100 ppm), resulted in prostration to deep narcosis dose-dependently (57). In another study, two cats and two rabbits were exposed to 1,1,2,2-TCE at concentrations of 800-1,100 mg/m^3 (116-160 ppm) for 8-9 h/day, 6 days/week, for 4 weeks: all animals showed initial stage of prostration, but no remarkable changes in body weight, behaviour, body temperature or blood studies (56). When mice were exposed to high concentrations of TCE 41,060 mg/m^3 (5,900 ppm) for 3 h daily 3/10 mice died within one week; at 45,900 mg/m^3 (6,600 ppm) for 3 h 4/10 mice died within one week; at 48,700 mg/m^3

(7,000 ppm) for one 2-hour period per week, 5 mice died after first exposure, three more after the third and the remaining mouse died after the fifth exposure (41). Six rats were exposed to 62,600 mg/m^3 (9,000 ppm) of TCE for 2 h/day, 2 days/week, one rat died after the second exposure, two after the fourth, and the remaining three rats died after the 11th exposure. Among these last three rats two had decreased red blood cell counts and haemoglobin levels, but no significant change was found in the white blood cell counts. The mice and rats showed congestion in tissues and fatty degeneration of the liver (41).

Fiessinger and co-workers (26) reported toxic effects of TCE to mice after repeated inhalation exposure (groups of four mice in 17 L chamber with Petri dish containing 10-20 mL of TCE, for 1-1.5 hours, the evaporation did not exceed 1.5 mL for each exposure). Some of the mice were comatose by the end of the exposure period, exhibited convulsive movements and staggering of the limbs. After the eighth exposure or a total of 10 hours, the mice had bristly hair, had lost weight and were anorectic. Between 8th and 28th exposures hepatic lesions developed, the liver became yellowish, and signs of centrilobular degeneration with some fatty infiltration were seen.

Müller (68) reported several studies of the effects of TCE on mice, guinea pigs and one rabbit. Mice were exposed to TCE via inhalation (inhalation chamber TCE concentration was 80,000 mg/m^3 (11,400 ppm), at the beginning of the exposure). After six hours deeply anaesthetized mice were removed to fresh air. After repeating the exposure the next day with the same mice, the animals had convulsions and died within a few hours. At autopsy fatty degeneration of the liver particularly in the peripheral of lobes was shown and also focal fatty degeneration of the renal tubular cells.

Müller (68) injected (*i.v., i.p., s.c.*) TCE into an unspecified number of guinea pigs and mice. The animals died in convulsions shortly after an injection dose of 0.2 mL (no other test doses were reported), and autopsies revealed no morphologic changes. Injecting mixtures of TCE in olive oil, glycerin or paraffin subcutaneously into an unspecified number of guinea pigs produced similar effects: the guinea pigs died within a few hours in convulsions (no morphologic changes were apparent at autopsy). In paraffin the lethal dose of TCE was 0.7 mL. When administered in five injections over 14 days (cumulative dose 0.7 mL, details on the schedule of individual injections were not given), no clinical signs were seen preceding death, other than body weight losses, but autopsies revealed liver and kidney damage (68).

One rabbit was given an *i.v.* dose of 0.2 g TCE and the animal went into immediate narcosis, apparently recovered after about 15 min, but died after 30 hours. The autopsy indicated liver enlargement with pasty, fine yellow fields, and the microscopic examination showed severe coarse- and fine-droplet fatty degeneration of the parenchymal cells, especially in the periphery of the lobes (68).

One adult male monkey (*Macaca cynomolga Linné,* 4.5 kg) was injected subcutaneously with TCE (50% v/v) 5 mL on day 1, 2 mL on day 4, 1 mL on day 19, 2 mL on day 20, 4 mL on day 29. The animal showed periods of

unconsciousness and became comatose and died two days after the last injection (its body weight decreased from 4.5 to 3.3 kg at death). There were no remarkable changes in the total white blood cell count, but the differential count showed lymphopenia and neutrophilocytosis (41).

Two short term rat studies of 1,1,2,2-TCE with more modern designs and objectives were located.

The subacute inhalation toxicity of 1,1,2,2-TCE was investigated with rats as part of a large study involving also the chronic toxicity design and an in-built reproductive toxicity study (87, 33). Groups of 7 male rats (strain not defined) exposed to 13.3 mg/m^3 of 1,1,2,2-TCE for 2, 4 or 8 days (within 10 days), other groups of 7 rats that received 4 g of ethanol by gavage at the end of the first, third and the seventh exposure, and appropriate controls, were studied with haematological and biochemical methods, and histological and histochemical investigations were performed on the liver, kidney, lung, brain (cerebrum and cerebellum), adrenal, testis and the thyroid gland. There were no changes in the body weight and organ weights, and some inconsistent changes were noted in serum proteins over the course of the experiment. The liver and renal lipid concentrations were unchanged. Histopathological examination of the liver showed slight inflammatory changes with periportal round-cell infiltration and even small necrotic foci; there was moderate accumulation of lipid in liver cells throughout the course of the study. The authors report that one rat in the tetrachloroethane *plus* ethanol group at the termination on day 2 and five rats in the TCE group at the termination on day 4 showed testis atrophy. Apparently there was no atrophy among the rats terminated on day 10. Therefore the findings were not consistent for an effect by tetrachloroethane and moreover, it is doubtful if such a histopathological change could have developed within a few days of exposure. There were no remarkable findings in other organs (changes found in the thyroid cannot be evaluated).

In an oral study, 1,1,2,2-TCE was given to groups of 10 male rats (of undefined strain) in doses of 3.2, 8, 20 or 50 mg/kg by gavage for up to 7 days (during up to 10 days) with and without exposure to elevated temperature (35^{o}C), and the liver, kidney, thyroid, adrenal, testis, spleen and trachea/oesophagus were examined with histological, enzyme histochemical and histoautoradiographic techniques (34). In the same paper the authors report on their subchronic toxicity investigations which lasted for 60 or 150 days. However, distinction between results from the short and long term administration is not made. It is stated that the histological changes (in the liver, kidneys, testis and the thyroid gland) were dose dependent, whereas the histochemical and autoradiographic findings were more dependent on the duration of the experiment (for further details, see Section 10.4).

10.4. Long term exposure

Dogs were given 150 times 1 mL doses of tetrachloroethane over a 1-year period (the route of exposure and the specifics of the experiment were not given): early symptoms consisted of gastrointestinal upset, diarrhoea, intestinal haemorrhage,

followed by jaundice and marked ascites with continued administration, the liver was hypertrophic after 1 year and returned to normal size within 3 months after the exposure stopped (2).

Adult male cynomolgus monkey (weigth 7 kg), was exposed to tetrachloroethane during 9 months for a total of 190 exposures (2 h/day, 6 days/week concentrations ranged 13,900-27,800 mg/m^3 (2,000-4,000 ppm) during the first 20 exposures, 6,960-13,900 mg/m^3 (1,000-2,000 ppm) for the next 140 exposures and 20,900-27,800 mg/m^3 (3,000-4,000 ppm) for the rest of the experiment). The monkey developed diarrhoea and anorexia after the 12th exposure, and at and after the 15th exposure it became nearly unconscious 20-60 min after the beginning of each exposure. Red blood cell counts and haemoglobin levels decreased reversibly during the 3-4 months. Histologically no definitive changes in heart, lungs, kidneys, pancreas and testes were seen, but the central zone of liver had marked vacuolization of the cytoplasm (41).

Schmidt et al. (87) conducted a subchronic study with male rats involving inhalation of 13.3 mg/m^3 of 1,1,2,2-TCE for 9 months. After 110 days 7 exposed rats and 7 sham-exposed controls were killed for examinations, and at the end of the inhalation period (265 days) corresponding groups of 7 rats were killed and examined. The remaining animals were allowed to recover until day 325 when again groups of 7 animals were terminated for examinations. The rest of the animals (apparently about 150 rats) were kept until their natural death. Daily exposures lasted 4 h/day but it is not specified how many days per week. Histochemical investigations were performed on the liver but the results are inadequately reported in a companion paper (33). The longevity of the rats was not affected by exposure. The authors report that apart from one special examination, all the slight deviations found occurred only at one point in time (exposed group: somewhat lower body weight at 4 months, increased liver lipids at 7 months). The special examination was a biological test of the ACTH activity of the hypophysis of the study animals (an increase, viz. a decreased concentration of ascorbic acid in the adrenal after injecting a pituitary extract, was found which was greatest at 4 months and lessened towards the end of the experiment). Therefore one has to conclude that the effects by 1,1,2,2-TCE found in the study were very slight. No further conclusions can be drawn because only a very limited number of end points were examined and because only one exposure level was used.

In the previously mentioned oral study with male rats, 1,1,2,2-TCE was given by gavage to groups of 10 rats at dose levels of 8 or 20 mg/kg during 60 days and at 3.2 or 8 mg/kg during 150 days (34). The authors report that the most remarkable findings concerned the liver but some effects were noted also in the kidney, testis and the thyroid. The lowest dose of 3.2 mg/kg was given as a threshold for chronic effects. However, the study is impossible to evaluate because no quantitative data on the dose-effect and dose-response relationships were given.

Another subchronic inhalation study of 1,1,2,2-TCE with female Sprague Dawley rats involved exposure to a single level of 560 ml/m^3 (= ppm), which

would correspond to 3,900 mg/m^3, for 5 or 6 hours per day, 5 days/week, for 15 weeks (103). Routine haematology and the histopathology of the liver, kidneys, lungs, ovaries, uterus and adrenal glands were examined. A slight decrease of the haematocrit level, increased relative liver weight, signs of hepatic hyperplasia (increased number of binucleated cells and transient increase of DNA synthesis), granulation and vacuolization of the liver were found. There were no effects in the kidneys, lungs, reproductive organs and adrenals.

It is also worth noting that the oral (gavage) carcinogenicity studies of 1,1,1,2-TCE (72) with Fischer 344/N rats and B6C3F1 mice, and 1,1,2,2-TCE (71) with Osborne-Mendel rats and B6C3F1 mice (for further details, see Section 10.6) involved the gross and microscopic examination of major tissues and organs: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice) and bile duct, pancreas, oesophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, pancreatic islets, testis, prostate, brain, uterus, mammary gland, and ovary. For rats, the non-neoplastic, inflammatory, degenerative and proliferative lesions that were seen in the 1,1,2,2-TCE dosed and control animals were similar in number and kind to those lesions occurring naturally in aged rats. For mice, a large number in the high dose level group died at weeks 69 and 70; histopathological examination revealed acute toxic tubular nephrosis as the apparent cause of death. In addition, hepatocellular carcinomas were found in most of these mice (71). In the carcinogenicity studies with 1,1,1,2-TCE (72) mice of each sex developed behavioural signs of central nervous system (CNS) toxicity (weakness, inactivity, loss of coordination) from week 51 at the high dose level (500 mg/kg) while among rats, CNS involvement was observed from week 44 at the high dose (250 mg/kg).

10.5. Mutagenicity and genotoxicity

Genotoxicity studies with 1,1,1,2-TCE are given in detail in Table 4, and corresponding studies with 1,1,2,2-TCE are shown in Table 5.

Testing in *Salmonella* with a variety of strains (forward and reverse mutations) have given mostly negative results for 1,1,1,2-TCE. In *in vitro* mammalian test systems the substance gave positive responses for sister chromatid exchange in Chinese hamster ovary cells without metabolic activation (30) and in the mouse lymphoma forward mutation assay with metabolic activation (96, 97). There was no induction of DNA repair in rat hepatocytes (64, 110). Only one *in vivo* study with 1,1,1,2-TCE was located. It concerned mitotic recombination in *Drosophila* and gave a negative result (107).

There is a wealth of mutagenicity and genotoxicity information on 1,1,2,2-TCE from *in vitro* studies, but limited data concerning *in vivo* studies. Most tests with a variety of *Salmonella* tester strains have given negative results (five out of eight studies available), while three have shown some positive findings, one of them with metabolic activation (63). 1,1,2,2-TCE induced prophage lambda in *Escherichia coli* in a test system which contained metabolic activation (but not its absence) (19).

1,1,2,2-Tetrachloroethane caused gene conversions and mitotic recombinations in *Saccharomyces cerevisiae* at high concentrations (7). *In vitro* studies with mammalian cells have shown positive sister chromatid exchange responses in Chinese hamster ovary cells and BALB/c-3T3 cells (30, 12). DNA repair tests with mouse and rat hepatocytes have yielded negative results (64, 110). Both positive and negative results were obtained in cell transformation assays with BALB/c-3T3 cells (64, 12, 13).

Assesment of unscheduled DNA synthesis and S-phase synthesis in mouse primary hepatocytes derived from animals that had been gavaged with one dose of 1,1,2,2-TCE gave negative results (65). Two *in vivo* studies with *Drosophila* detecting either sex-linked lethal mutations (112) or mitotic recombination (107) were negative.

Overall, while the genotoxicity studies of 1,1,2,2-TCE do not give a consistent picture, the substance appears to have some potential for genotoxicity. This may relate to the observed covalent binding of 1,1,2,2-TCE to the DNA in liver and other tissues (10, 11, 23) and consequent DNA damage.

10.6. Carcinogenicity

10.6.1. 1,1,1,2-TCE

Groups of 50 male and 50 female B6C3F1 mice, were administered 0, 250 or 500 mg/kg bw of 1,1,1,2.TCE (>99% pure with traces of chloroethane and ethylene derivatives) in corn oil by gavage on five days a week for 103 weeks (low dose) or 65 weeks (high dose). All high dose animals died or were killed when moribund after 65 weeks. Thirtyeight control males, 34 low dose males, 41 control females and 31 low dose females survived till the end of the study, i.e., 103 weeks. In spite of low survival there was a statistically significant dose-related increase in the incidence of hepatocellular adenomas in males: 6/48, 14/46 and 21/50 in control, low dose and high-dose animals, respectively, as well as in females: 4/49, 8/46 and 24/48. There was also a dose-related increase in the incidence of hepatocellular carcinomas in females: 1/49, 5/46 and 6/48 (45, 72).

Groups of 50 male and 50 female Fischer 344/N rats, were similarly administered 0, 125 or 250 mg/kg/b.w. of 1,1,1,2-TCE (>99% pure with traces of chloroethane and ethylene derivatives) for 103 weeks, and the animals were killed at 104 weeks. 29, 25 and 21 control, low dose and high-dose males, and 29, 27 and 24 females survived until the end of the study. A statistically significant increase in the incidence of fibroadenomas of the mammary gland was observed in low dose females: controls 6/49; low dose 15/49 and high-dose 7/46 (45, 72).

Table 4. Genotoxicity studies with 1,1,1,2-TCE

Species	Strain/cells	Measured endpoint	Test conditions	Results		Reference
				without activation	with activation	
Bacterial systems						
S. typhimurium	TA1535 TA1537 TA98 TA100	Reverse mutations	Dose levels ranging 0.01 - 1 mg/plate			(37)
S. typhimurium	TA98 TA100 TA97 TA104	Reverse mutations	Dose levels ranging 0.01 - 1 mg/plate	+ + + +		(93)
S. typhimurium	TA1535 TA1537 TA98 TA100	Reverse mutations	S-9 mix from Osborne Mendel rats or B6C3F1 mice; closed system			(64)
S. typhimurium	TA100	Reverse mutations	Tedlar® vapourization desiccator			(108)
S. typhimurium	TA97 TA98 TA100 TA102	Reverse mutations	Three methods used for each strain: classical Ames-test, spot test, preincubation			(63)
S. typhimurium	BA13/BAL13	Forward mutations	Dose levels ranging 0.06 - 2979 nmol			(82)
Yeasts						
Saccharomyces cerevisiae	D61.M	Chromosome loss cold-interruption standard incubation	Dose at maximum 0.97 - 1.15 mg/ml 0.77 - 1.34 mg/ml			(109)

Species	Strain/cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Fungi						
Aspergillus nidulans	Diploid P1	Chromosome missegregation	0.0125 - 0.05 % (v/v)	+		(14)
In vitro mammalian systems						
CHO cells		SCE	15.8 - 400 µg/ml (- S-9) 248 - 348 µg/ml (+ S-9)	+		(30)
CHO cells		Chromosome aberrations	455 - 506 µg/ml (-S-9) 348 - 443 µg/ml (+S-9)			(30)
Mouse	Lymphoma cell line L5178Y/TK	Forward mutations	Lowest positive dose 200 µg/ml		+	(96, 97)
Rat hepatocytes	Osborne Mendel	DNA repair				(64)
Rat hepatocytes	Osborne Mendel	DNA repair	9.5 x 10-4 M			(110)
BALB/C-3T3 cells		Cell transformation	Closed system			(64)
In vivo						
Drosophila melanogaster		Mitotic recombination	1,000 or 2,000 ppm feeding	-		(107)

Table 5. Genotoxicity studies with 1,1,2,2-tetrachloroethane

Species	Strain/cells	Measured endpoint	Test conditions	Results		Reference
				without activation	with activation	
Bacterial systems						
S. typhimurium	TA1530 TA1535 TA1538	Reverse mutations	10 µmol/plate			(3)
S. typhimurium	TA1535 TA108 TA1537 TA1538 TA98	Reverse mutations	Up to 4 mg/plate			(57)
S. typhimurium	TA1535 TA1537 TA98 TA100	Reverse mutations	Dose levels ranging 0.01 - 1 mg/plate			(37)
S. typhimurium	TA98 TA100 TA97 TA104	Reverse mutations	Dose levels ranging 0.01 - 1 mg/plate	+ + + +		(93)
S. typhimurium	TA1535 TA1537 TA98 TA100	Reverse mutations	S-9 mix from Osborne Mendel rats or B6C3F1 mice; closed system			(64)
S. typhimurium	TA100	Reverse mutations	Tedlar® vapourization desiccator			(108)

Species	Strain/cells	Measured endpoint	Test conditions	Results		Reference
				without activation		
S. typhimurium	TA97 TA98 TA100 TA102	Reverse mutations	Three methods used for each strain: classical Ames-test (A), spot test (S), preincubation		+(A) +(A) +(S)	(63)
S. typhimurium	BA13/BAL13	Forward mutations	Dose levels ranging 0.06-2,979 nmol			(82)
E.coli	polymerase deficient pol A+/pol A1-	DNA damage				(3)
Escherichia coli		Induction of prophage lambda	7.4 - 473 µM		+	(19)
Yeasts						
Saccharomyces cerevisae	D7	Gene conversion mitotic recombination	3.1, 5.2 or 7.3 mM			(7)
Saccharomyces cerevisae	D7 XV185-14C	Gene conversion reversion				(73)
Fungi						
Aspergillus nidulans	diploid P1	Chromosome missegregation	0.01 - 0.04 % (v/v)			(14)
In vitro mammalian systems						
CHO cells		SCE	16.8 - 168 µg/ml (-S-9) 451 - 558 µg/ml (+S-9)			(30)

Table 5. Cont.

Species	Strain/cells	Measured endpoint	Test conditions	Results		Reference
				without activation	with activation	
CHO cells		Chromosome aberrations	455 - 506 µg/ml (-S-9) 348 - 443 µg/ml (+S-9)	-	-	(30)
BALB/c-3T3 cells		SCE	1,000 µg/ml (-S-9) 500 µg/ml (+S-9)	+	+	(12)
Mouse hepatocytes	B6C3F1	DNA repair				(64)
Rat hepatocytes	Osborne Mendel	DNA repair				(64)
Rat hepatocytes	Osborne Mendel	DNA repair	9.5 - 10-5 M			(110)
BALB/c-3T3 cells		Cell transformation	Closed system			(64)
BALB/c-3T3 cells		Cell transformation	125 ,000 µg/ml	+	+	(13)
BALB/c-3T3 cells		Cell transformation	31 - 500 µg/ml with or without promoting treatment with TPA			(12)
<u>In vivo - in vitro</u>						
Mouse primary hepatocytes	B6C3F1	UDS	50 - 1,000 mg/kg by gavage; hepatocyte isolation 2 or 12 h later			(65)
Mouse primary hepatocytes	B6C3F1	S-phase synthesis	200 - 700 mg/kg by gavage; hepatocyte isolation 24 or 48 h later	- (male mouse) equivocal (female mouse)		(65)
<u>In vivo</u>						
Drosophila melanogaster		Sex-linked recessive lethal mutations	800 ppm by injection 1,500 ppm feeding			(112)
Drosophila melanogaster		Mitotic recombination	500 or 1,000 ppm feeding	-		(107)

10.6.2. 1,1,2,2-TCE

Groups of 50 male and 50 female B6C3F1 mice were administered technical-grade 1,1,2,2-TCE in corn oil by gavage on 5 days per week. Initially, high-dose animals received 200 mg/kg bw/day, and low dose animals received 100 mg/kg bw/day; after 18 weeks the doses were increased to 300 and 150 mg/kg bw/day. Test animals were maintained at these levels for 3 weeks, followed by 5 weeks at 400 and 200 mg/kg bw/day and 52 weeks at 300 and 150 mg/kg bw/day (total treatment time 78 weeks). The measured, time-weighted average doses were 142 (low dose) and 284 (high dose) mg/kg bw/day. Animals were killed and necropsied 12 weeks after the last dose. Groups of 20 male and 20 female mice were given corn oil for 78 weeks and killed after 91 weeks; another control group of 20 male and 20 female mice were fed the standard diet for 90 weeks. By 90 weeks, only 1 male that received the high dose was still alive, whereas 34% of females lived to that time. In males, hepatocellular carcinomas occurred in 2/19 untreated controls, in 1/18 vehicle-treated controls, in 13/50 low dose animals and in 44/49 high-dose animals; in females, the respective incidences were 0/19, 0/20, 30/48 and 43/47, respectively (44, 71).

Groups of 50 male and 50 female Osborne-Mendel rats were administered technical-grade 1,1,2,2-TCE in corn oil by gavage on 5 days per week. High-dose animals received 100 mg/kg bw/day; in males, this was increased after 14 weeks to 130 mg/kg bw/day for 18 weeks followed by 9 cycles of 4 weeks at this dose and 1 week treatment-free (total 78 weeks); in females, the dose was reduced after 25 weeks to 80 mg/kg bw/day for 7 weeks followed by the cyclic treatment as above at this dose level for 45 weeks. Low dose males received 50 mg/kg bw/day for 14 weeks and 65 mg/kg bw/day for 64 weeks; females received 50 mg/kg bw/day for 25 weeks and 40 mg/kg bw/day for 53 weeks. All groups were maintained for a further 32 weeks on a standard diet without treatment. Time-weighted average doses were 62 mg/kg bw/day and 108 mg/kg bw/day in males and 43 and 76 mg/kg bw/day in females. Groups of 20 animals of each sex were administered corn oil alone; further groups of 20 males and 20 females served as untreated controls. Weight gain was consistently lower in high-dose groups than in low dose and control groups. Fifty per cent of the high-dose males, 40% of the high-dose females, 50% of the low dose males and 58% of the low dose females lived more than 105 weeks. The incidences of tumours in treated and control rats were not significantly different for any tumour type; however, 2 of 49 males treated with the high-dose developed hepatocellular carcinomas and an additional rat had a neoplastic nodule of the liver, compared with 0/20 vehicle controls (44, 71).

In the initiation protocol of an initiation-promotion study, 10 male Osborne-Mendel rats per group were subjected to partial hepatectomy, and were dosed with either 1,1,1,2-TCE (1.2 mmol/kg bw in corn oil) or 1,1,2,2-TCE (0.59 mmol/kg bw in corn oil) 24 h later followed by six days 0.05% phenobarbital in the ground chow diet for seven weeks. In the treatment with promotion protocol, 24 h after 2/3 partial hepatectomies the rats were administered diethylnitrosamine (DEN)

i.p., and five days later the rats received 1,1,1,2- or 1,1,2,2-TCE in corn oil. When administered in the promotion protocol after initiation with DEN, 1,1,2,2-TCE induced significant increases in γ-glutamyl-transferase positive (GGT+) foci above control levels. 1,1,2,2-TCE also induced significant increases in GGT+ foci when administered in the promotion protocol without DEN initiation (92, 64). Thus the study suggested that 1,1,2,2-TCE may be a complete carcinogen with a weak initiating activity and a stronger promoting activity.

Pulmonary adenoma response of a sensitive strain (A/St) of male mice was investigated in a 24 week study in which 80, 200 or 400 mg/kg 1,1,2,2-TCE was injected *i.p.* 3 times a week into groups of 20 animals for a total of 24 injections (98). The highest dose produced an elevated lung tumour response which was close to statistical significance; however, only 5 rats survived to the end of study in the high dose group.

In summary, both tetrachloroethane isomers caused hepatocellular adenomas and/or carcinomas in both sexes of the mouse. 1,1,2,2-TCE also acted as a weak initiator and a strong promotor when initiated with diethylnitrosamine in an initiation/promotion bioassay with rats. The mechanism of tetrachloroethane induced liver carcinogenesis is unclear. It is noteworthy that tetrachloroethane metabolites trichloroethylene, tetrachloroethylene, dichloroacetic acid and trichloroacetic acid have been shown to be carcinogenic in rodents (for review, see IARC, (43)) and more specifically, that the two chlorinated acids have induced hepatic tumours in B6C3F1 mice (6). While some of the proposed mechanisms of tumorigenesis such as peroxisome proliferation may be of lesser relevance to humans, other mechanisms that lead to DNA and protein binding, lipid peroxidation and hepatocellular damage mediated by free radicals and acyl chlorides may also be operative.

10.7 Reproductive and developmental toxicity

Reproductive toxicity has been reviewed by IARC (44, 45) and European Union (94).

No reproductive disturbances were observed in rats exposed orally or by inhalation to 1,1,1,2-TCE, although neonates born to exposed females died within two days of birth (104). (The IARC working group noted that the report did not specify whether or not the newborn animals had themselves been exposed to 1,1,1,2-TCE, and control animals were not described (45).)

Treatment of AB-Jena and DBA mice with daily intraperiteonal injections of 300-400 mg/kg bw/day tetrachloroethane in olive oil during organogenesis gave some indication of an embryotoxic effect (at most doubling of postimplantation loss) in AB-Jena mice but not in DBA mice (88).

When male rats (strain not defined), exposed to 14 mg/m^3 (2 ppm) of tetrachloroethane for 9 months, were mated with untreated females, there were no effects in the reproductive outcome (no change in litter size, average foetal weight, male-to female sex ratio, growth rate, or neonatal mortality) (87).

10.8 Other studies

Male Wistar rats (6 rats at each concentration and 20 rats as controls) were exposed to 1,1,2,2-TCE at 68.7, 687, 6,870 mg/m^3 (10, 100 and 1,000 ppm) by inhalation for 6 hours. At 24 hours after the single inhalation exposures at 68.7 and 687 mg/m^3 (10 and 100 ppm), the average serum aspartate aminotransferase (ASAT = SGOT) values were 144 and 206 units, respectively, while the control rats showed an average value of 110 units. The corresponding average serum alanine aminotransferase (ALAT = SPGT) values were 51 and 53 units, respectively, for the exposed groups and 41 units for the control group. Four of the six rats exposed at 6,870 mg/m^3 (1,000 ppm) for 6 hours died within 24 hours after the start of the exposure. Histologic examinations performed at necropsy after 24 and 120 hours of recovery in the 70 , 690 and 6,900 mg/m^3 (10, 100 and 1,000 ppm) groups showed no definite changes in the liver, heart, kidney, spleen, brain or bone marrow (18).

Groups of 10 male Wistar rats were exposed to 1,1,2,2-TCE at 410-4,200 mg/m^3 (60-600 ppm) for 4 h in an exposure chamber. The threshold concentrations for effects on the liver (alterations in several liver enzymes) were between 400-700 mg/m^3. 1,1,2,2-Tetrachloroethane also transiently increased the ascorbic acid content of the liver (89).

Female Cb mice were exposed to 1,1,2,2-TCE at 4,180 mg/m^3 (600 ppm) for 3 hours in a constant flow exposure chamber. Groups of 6 mice were killed at 0, 4 and 8 hours after termination of exposure; eight female mice were used as controls. Total liver lipids increased to 115, 155 and 216% at 0, 4 and 8 hours, respectively; the triglyceride content increased to 163, 288 and 518%, respectively, while the hepatic ATP content decreased to 75, 59 and 46% of the control values, respectively (101).

Paolini and coworkers (78) studied the biochemical hepatotoxicity of 1,1,2,2-TCE *in vivo* in mice. Groups of 6 male and female Swiss albino mice (CDI strain) were treated *i.p.* with a single 1,1,2,2-TCE dose of 300 or 600 mg/kg bw (corresponding to 20% or 40% of the LD_{50} = 1,476 mg/kg). No significant alteration of microsomal protein content was observed. Hepatic microsomal P450 concentration and activities of several monooxygenases, NADPH-cytochrome *c*-reductase, epoxide hydrolase, UDP-glucuronosyl transferase and glutathione S-transferase were decreased. Microsomal haeme was decreased, accompanied by a decrease in δ-aminolaevulinic acid synthetase and a significant increase in the hepatic heme oxygenase (78).

Swiss albino mice (CD1 strain) were given a single *i.p.* injection of 1,1,1,2-TCE at two dose levels (35 or 70% of the LD50: 376.6 mg/kg bw or 753.2 mg/kg bw). After 24 h, P450 levels were significantly ($p<0.01$) decreased at both doses. Ethoxyresorufin deethylase and pentoxyresorufin O-dealkylase activities were also decreased (4).

1,1,2,2-Tetrachloroethane was applied to the clipped back skin of guinea pigs (1 mL of pure solvent in a glass chamber with 3.1 cm^2 area). Biopsies were taken at different times of exposure for histopathological studies. A moderate karyopyk-

nosis was found at 16 hours and pseudoeosinophilic cellular infiltration occurred at the same point in time but only in the deep and middle parts of dermis (53).

With a view to studying potential for causing immunological glomerulopathy, Brown Norway and Wistar rats were exposed to 516 ppm of 1,1,2,2-TCE, 5 h/day, 5 days/week for 13 weeks (16). Paradoxically, the exposed rats had consistently lower proteinuria than control rats. No histological lesions were found in the kidneys with light microscopy and immunofluorescence. Electron microscopy showed slight deposits within the glomerular basal membrane of the exposed rats.

Groups of 4-5 rabbits were exposed to 2, 10 or 100 mg/m^3 of 1,1,2,2-TCE, 3 h/day, 6 days/week for 8 months and then immunized with typhoid; 50 unexposed rabbits served as controls. In the middle and high dose groups the summary titre of typhoid antibodies was decreased compared to controls. There was also a concomitant increase in the electrophoretic mobility of antibodies toward β- and α-globulin fractions and a decrease in the level of "normal" haemolysis to the Forsman's antigen of sheep erythrocytes (91).

In a study of acute neurochemical effects of 28 different substances, 5 14-week old male Sprague-Dawley rats were given 50 mg/kg 1,1,2,2-TCE in one dose by gavage and two hours later various neurotransmitters and their metabolites were determined in the midbrain, hypothalamus and medulla (49). While there was no change in the acetylcholine concentration of the hippocampus, some monoamine neurotransmitters or their metabolites increased in the midbrain, hypothalamus and medulla. The authors could not find any consistent compound structure-related effects among the study results.

1,1,2,2- Tetrachloroethane induced release of alanine aminotransferase from a rat hepatocyte suspension incubated for 30 to 180 minutes at concentrations of 7.5 and 10 mM (15).

1,1,2,2- Tetrachloroethane was a potent inhibitor of acetylcholinesterase activity in the human blood erythrocyte membrane *in vitro*. The enzyme inhibition was concentration dependent (52).

11. Observations in man

11.1. Acute effects by contact and systemic distribution

In the context of accidental inhalation or ingestion by humans pronounced to extremely severe toxic alterations of the liver and toxic fatty degeneration of the renal tubules were observed (40, 89, 93).

There are also reports concerning non-occupational poisonings due to ingested tetrachloroethane with suicidal intent in some cases (22, 29, 39, 58, 62). The signs and symptoms included early loss of consciousness, progressive CNS depression and death within 9 h.

Eight adult Africans (two females and six males) were mistakenly given 3 ml of tetrachloroethane orally (about 60-70 mg/kg) for eradication of hookworms (90).

Within two hours the patients lost consciousness and three became comatose with absent reflexes and enlarged, fixed pupils. The pulse was barely perceptible and respirations were shallow and rapid. After about half an hour the patients regained consciousness but were slightly confused and complained of slight headache. The recovery was uneventful.

Two male volunteers were exposed to 1,1,2,2-TCE at concentrations ranging from 20 to 2,300 mg/m^3 (2.9-335 ppm), during periods up to 30 min. During 10 min periods of exposure at 20, 30, 90 mg/m^3 (2.9, 4.4 and 13 ppm) the men did not complain of any effects. The odor was detected at 90 mg/m^3 (13 ppm). At 1,000 mg/m^3 (144 ppm) the subjects experienced dizziness after 10 min, mucosal irritation at 12 min and fatigue after 20 min. At 1,800 (262 ppm) a 10 min exposure resulted in dizziness and mucosal irritation of the mouth, eyes, and nose. The highest concentration produced a strong odor which was no more discernible after 3 min. At this concentration 1,1,2,2-TCE produced dizziness in 3 minutes and mucosal irritation in 10 minutes (57).

11.2. Effects of repeated exposure

Already at the beginning of World War I, the adverse health effects of tetrachloroethane (1,1,2,2-TCE) were known due to numerous poisonings of workers in the aircraft industries of Germany, France, England and Holland. In England alone there were 70 reported cases with 12 deaths (5). The deaths were attributed to severe toxic hepatitis with jaundice but there was no development of acute yellow atrophy of the liver. Among German aircraft factory workers 12 out of 15 workers who regularly used varnishes containing 30-50% of 1,1,2,2-TCE became ill. The patients were classified into two groups according to the symptoms: one group showed mainly gastrointestinal disturbances, jaundice and enlarged livers, the second group had neurologic disturbances (tremors, impaired hearing, paresthesias in the extremities, reduced patellar reflexes, headache, anorexia and nausea) (38). In some case reports changes in the blood picture: occurrence of a large number of mononuclear cells, a slight increase of the total white cell count and progressive anaemia were observed (5).

In a penicillin plant where 1,1,2,2-TCE concentrations ranging from 10 to 1,700 mg/m^3 (1.5 to 247 ppm) were measured in different processes, about one half of the workers exhibited adverse symptoms, particularly digestive organ complaints (loss of appetite, bad taste in the mouth, epigastric pain, sensations of pressure in the liver area), headaches, general debility, lack of stamina, loss of body weight, and occasionally painful prurigo. Among the symptomatic workers many had enlarged liver and abnormal liver function tests. All these symptoms were reduced with improvements in the working conditions which lowered the tetrachloroethane concentrations (48). Most workers became symptom-free after improvements in the working conditions which lowered the maximum 1,1,2,2-TCE levels down to 36 ppm (48).

Lobo-Mendonca (59) has reported on a thorough survey of the use of 1,1,2,2-TCE as a solvent for cellulose acetate in the manufacture of bangles in Bombay,

India. The survey involved 23 factories (so-called cottage industries), and 380 workers, representing 80% of the population employed, were examined. The raw material for bangles was cellulose acetate safety film. To dissolve the material a mixture of acetone and 1,1,2,2-TCE in equal proportions was used and during the monsoon some diacetone alcohol was added to prevent haziness. It was estimated that about 900 kg (2,000 pounds) of tetrachloroethane was used every month. The process involved both inhalation exposure and direct dermal contact with liquid 1,1,2,2-TCE. About one half of the workforce was in direct contact with the substance. Air samples were collected in different locations from the breathing zone and analyzed by titration against 0.01N silver nitrate for chloride. Tetrachloroethane concentrations ranged in 14 measurements from 9 to 98 ppm (63-680 mg/m^3).

Among workers with the most severly exposing jobs nervous symptoms and signs were the most frequent: headache and vertigo were complained by more than a third of the workforce and roughly every other person exhibited a fine tremor in the fingers (59). Almost a quarter of these workers also complained of loss of appetite and many had felt nausea, abdominal pain, and had vomited; however liver enlargement was not found. The author reported that the symptom complex of tetrachloroethane poisoning appeared after about 3 months of exposure, and a more consistent series of symptoms was evident after 6 months. There seemed to be a dose response for hand tremor: at 9-17 ppm of 1,1,2,2-TCE (factory G) 14% of workers handling the substance showed tremors, at 40-74 ppm (factory A) the corresponding frequency was 33%, at 50-61 ppm (factory T) 41%, and at 65-98 ppm (factory L) 50%. The author pointed out that the survey was unable to reveal the full impact of exposure on chronic illness and debility because of high labour turnover.

In Italy, 75 persons were exposed to tetrachloroethane in tri- and tetrachloroethylene production, and in laboratory work at two plants. Tetrachloroethane in air varied from the low mean of 2.6 mg/m^3 (0.37 ppm) to the high mean of 9.3 mg/m^3 (1.33 ppm) with a single maximum of 278 mg/m^3 (40 ppm). Clinical examinations of the workers indicated that the pulse rate, circulatory response to postural changes, and the ECG's were not significantly different from "normal" (32).

In a retrospective cohort study, 1,099 white men who had been exposed to 1,1,2,2-TCE while using machinery that impregnated clothing (plus some exposure to dry cleaning solvents) in seven companies, and 1,319 men who served in the same companies but were not involved in the impregnation process, were investigated for mortality experience from 1946 through 1976 (76). There was an additional comparison cohort of 3,166 white men who had been engaged in water solvent processes for cleaning in other companies at the same time period. Expected numbers of deaths were calculated on the basis of the US mortality statistics. There was no increased mortality in any of the cohorts in all diseases, all malignancies, cardiovascular disease, or cirrhosis of the liver. The TCE cohort exhibited the lowest standardized mortality ratio for cirrhosis of the liver (0.48) which may be attributable to exclusion of moderate to heavy drinkers because of

liver toxicity hazard during exposure. The overall cancer mortality in the TCE exposed cohort was somewhat (26%), but not significantly, higher than in the cohort not involved in the impregnation process, and some cancer types occurred more frequently (not significantly different) in the TCE cohort: cancers of the genital organs 3 cases versus 1.65 expected, leukemia and aleukemia 4 cases versus 1.81 expected. The workers had been exposed to TCE for limited periods of time ranging from five weeks to one year, with an average of about five months.

12. Dose-effect and dose-response relationships

There are limited and uncertain data concerning dose-effect and dose-response relationships for tetrachloroethanes (1,1,2,2-TCE) in humans, other than acute effects. Acute human effects by 1,1,2,2-TCE are shown in Table 6. The following evaluation is therefore essentially based on animal data.

12.1 Short term exposure

Studies concerning short term effects of 1,1,2,2-TCE in animals are compiled in Table 7.

Table 6. Acute effects of 1,1,2,2-TCE in humans

	Dose or exposure conc. and duration	Effects	Reference
Ingestion	Unknown	Coma, no corneal reflex, death after 17 h, lung and liver congestion	(39)
Ingestion	Unknown	Unconsciousness, cyanosis, death 12 h later	(22)
Ingestion	Unknown	Coma shortly after ingestion, death after 9 h	(58)
Ingestion	3 ml	Unconsciousness, coma in 2 h after half an hour consciousness was regained, followed by uneventful recovery	(90)
Inhalation	2,330 mg/m^3 (335 ppm) 3-10 min	Dizziness, mucosal irritation	(57)
	1,000 mg/m^3 (144 ppm) 10-20 min	Dizziness, fatigue, mucosal irritation	
	20-90 mg/m^3 (2.9-13 ppm) 30 min	No effect	

Table 7. Short term effects of 1,1,2,2-TCE in animals via inhalation

Species	N	Exposure conc. and duration or dose	Effects	Ref.
Mouse		11,400 ppm for 6 h, exposure repeated next day	Mice: after 6 h, deep anaesthesia, convulsions, death within a few hours on the second day, fatty degeneration of the liver, focal fatty degeneration of the renal tubular cells	(68)
Mouse	10	5,900 ppm, 3 h	3/10 mice died	(41)
	10	6,600 ppm, 3 h	4/10 mice died	
	10	7,000 ppm, 2 h period per week	5/10 died after the first exposure and the last animal died after the 11th exposure	
Rat	6	9,000 ppm, 2 h/day, 2 days/week	One died after first and one after the second exposure 3/6 survived up until the 11th exposure 2/3 surviving rats showed decreased red blood cell counts and haemoglobin levels	
Mouse	4	Exposure in a closed vessel for 1-1.5 h; theoretical max concentration about 7,000 ppm	Coma, convulsive movements and staggering of the limbs; after 8 exposures, bristly hair, weight loss, anorexia; between the 8th and 28th exposures hepatic lesions	(26)
Cat	2	710 ppm	Prostration	(57)
	2	6,100 ppm time not given	Deep narcosis	
Cat	7	830 ppm	Light narcosis in 4 h deep narcosis in 5h	(56)
		8,300 ppm	Light narcosis in 25 min deep narcosis in 40 min	
Cat	2	116-160 ppm	Prostration	(56)
Rabbit	2	8-9 h/day, 6 days/week for 4 weeks		
Rat, male		13.3 mg/m^3 (1.9 ppm) for 2,4 or 8 days within 10 days	No effects on body weights, and organ (liver, kidney, lung, brain, adrenal, testis, thyroid) weights; liver and renal lipid concentrations unchanged, histopathology: slight periportal round-cell infiltration & small necrotic foci, moderate accumulation of lipid in the liver	(87, 33)

Table 8. Long-term studies with 1,1,1,2-TCE in animals

Route of exposure	Species	N	Exposure conc. and duration, or dose	Effects	Ref.
Gavage	Mouse B6C3F1	50 male 50 female	0 and 250 mg/kg bw for 103 weeks 500 mg/kg bw for 65 weeks	CNS toxicity at week 51 of each sex (500 mg/kg), all died or were killed moribund after 65 weeks (high dose) hepatocellular adenomas in males 6/48, 14/46 and 21/50 ($p<0.001$) in females 4/49, 8/46 and 24/48 ($p<0.001$) dose related increase of hepatocellular carcinomas: 1/49, 5/49 and 6/48 in treated females ($p<0.05$)	(72). in (45)
Gavage	Rat Fischer 344	50 male 50 female	0, 125 or 250 mg/kg bw, five days a week for 103 weeks, termination 104th week	CNS involvement from week 44 fibroadenomas of the mammary gland: 6/49, control females 15/49, low dose females 7/46, high-dose females	(72), in (45)

12.2 Long-term exposure

Long-term studies with 1,1,1,2-TCE in animals are compiled in Table 8, and the corresponding studies of 1,1,2,2-TCE are compiled in Table 9.

Table 9. Long-term studies with 1,1,2,2-TCE in animals

Route of exposure	Species	N	Exposure conc. and duration	Effects	Ref.
Inhalation	Monkey cynomolgus	1	1,000-4,000 ppm 190 exposures over 9 months (2 h/day, 6 days/week)	Diarrhoea, anorexia reduction of red blood cells and hemoglobin, vacuolation in liver cells	(41)
Inhalation	Rat Sprague-Dawley	165 rats divided in three groups	560 mL/m^3, 5-6 h/day, 5 days/week, for 15 weeks	Slight decrease of haemato-crit, increased relative liver weight, signs of hepatic hyperplasia, foci of granulation & vacuolization in liver	(103)
Inhalation	Rat male	group size 7 rats	13.3 mg/m^3 (1.9 ppm) for 9 months	Lower body weight at 4 months, increased liver lipids at 7 months, consistently decreased ACTH activity of the pituitary, longevity of rats not affected	(87)

Table 9. Cont.

exposure	Species	N	Exposure conc. and duration	Effects	Ref.
Gavage	Mouse B6C3F1	50 male 50 female Sham treated controls: 20 male 20 female Untreated 20 males 20 females	Low dose: 100-200 mg/kg bw/day (mean 142 mg/kg bw/day) for 78 weeks high-dose 200-400 mg/kg bw/day (mean 284 mg/kg bw/day) for 78 weeks	Large number of high-dose mice died at weeks 69 and 70 of acute toxic tubular nephrosis hepatocellular carcinomas in males: 2/19 untreated controls 1/18 sham-treated controls 13/50 low dose 44/49 high-dose in females: 0/19 sham-treated controls 0/20 untreated controls 30/48 low dose 43/47 high-dose	(71)
Gavage	Rat Osborne Mendel	50 male 50 female sham-treated controls: 20 male 20 female untreated controls: 20 male 20 female	Low dose: males: 50-65 mg/kg bw/day for 64 weeks (mean dose: 62 mg/kg bw/day) females: 40-50 mg/kg bw/day (mean dose: 43 mg/kg bw/day) high-dose males: 100-130 mg/kg bw/day (mean dose: 108 mg/kg bw/day) females: 80-130 mg/kg bw/day (mean dose: 76 mg/kg bw/day) for 78 weeks	Occurrence of tumours in treated and control rats was not significantly different for any tumour type, however, 2/49 males at the high-dose developed hepatocellular carcinomas and an additional rat had a neoplastic nodule compared to 0/20 in sham-treated controls	(71)
Gavage	Rat male	10 rats per group	8 or 20 mg/kg during 60 days; 3.2 or 8 mg/kg during 150 days	Most effects in the liver, some effects in kidney, testis and thyroid; 3.3 mg/kg given as threshold for chronic effects (dose-effect or dose-response data were not provided)	(34)

13. Previous evaluations by (inter)national bodies

International Agency for Research on Cancer concluded that there is inadequate evidence in humans of the carcinogenicity of 1,1,2,2-TCE. No epidemiological data on cancer in humans were available for 1,1,1,2-TCE. There is *limited evidence* in experimental animals for the carcinogenicity of 1,1,2,2-and 1,1,1,2-TCE. Thus, 1,1,2,2- or 1,1,1,2-TCE are not classifible as to their carcinogenicity to humans (Group 3) (46).

In the German MAK-value list 1,1,2,2-TCE is included in group IIC (substances shown to be hazardous during pregnancy without further categorization). Additionally, 1,1,2,2-TCE is classified into group IIIB (justifiably suspected of having carcinogenic potential) (20).

In the late 1970s, the National Institute for Occupational Health (NIOSH) of the United States recommeded that it would be prudent to handle 1,1,2,2-TCE in the workplace as if it were a human carcinogen and that the exposure be minimized (75, 79).

14. Evaluation of human health risks

14.1. Groups at extra risk

There is no firm evidence, mechanistic or otherwise, to indicate particular factors of individual susceptibility to tetrachloroethane toxicity.

14.2. Assessment of health risks

Tetrachloroethanes are recognized hepatotoxins and liver carcinogens in mice (44, 45, 71, 72, 78). In the liver, tetrachloroethanes are metabolically activated to acyl chlorides (1,1,2,2-TCE to dichloroacetyl chloride) or free radicals which may bind to proteins or initiate lipid peroxidation causing toxicity (78). Both compounds, 1,1,2,2-TCE more extensively than 1,1,1,2-TCE, were shown to bind covalently to rat liver DNA (11, 12). Although genotoxicity studies on tetrachloroethanes do not provide a consistent picture of effects, the compounds appear to have some potential for genotoxicity (30, 7, 11, 63, 96). The mechanism of tetrachloroethane induced liver carcinogenesis is unclear. Several metabolites are carcinogenic to rodents and produce in biotransformation reactive intermediates mentioned above. Although some of the proposed mechanisms for tumorigenesis such as peroxisome proliferation may have lesser relevance for humans, other mechanisms that lead to DNA and protein binding and consequent damage, lipid peroxidation and hepatocellular damage mediated by free radicals and acyl chlorides may be operative.

The concentration of 4,900-5,600 mg/m^3 (700-800 ppm) of 1,1,2,2-TCE caused prostration and light narcosis in cats, and some prostration was found in cats and rabbits at 840-1,100 mg/m^3 (120-160 ppm) (56, 57). At high dose levels severe CNS depression, unconsciousness, convulsions and coma, and/or hepatic lesions were noted in mice, rats, guinea pigs and rabbits. Regarding acute effects in humans, two male volunteers exposed to 1,1,2,2-TCE at concentrations ranging from 20 to 90 mg/m^3 (2.9-13 ppm) up to 30 minutes, did not complain of any effects (57). The subjects experienced dizziness after 10 minutes, mucosal irritation at 12 minutes and fatigue after 20 minutes at 1,000 mg/m^3 (146 ppm). A 30 minute exposure at 1,800 mg/m^3 (262 ppm) resulted in dizziness and mucosal irritation of the mouth, eyes and nose. Exposure to 2,330 mg/m^3 (335 ppm) caused dizziness in 3 minutes and mucosal irritation in 10 minutes. Oral ingestion of 3 ml (about 60-70 mg/kg) of 1,1,2,2-TCE by hookworm bearing patients caused lowered consciousness and among some subjects deep coma; the recovery was reported to be uneventful without specific therapy (90).

Long-term animal studies on 1,1,2,2-TCE have yielded ostensibly conflicting results, but inadequate study design and reporting, or the different endpoints used, may explain the discrepancies. No appropriate long-term studies on tetrachloroethanes by inhalation were located. A subchronic inhalation study with 1,1,2,2-TCE with female Sprague-Dawley rats involved exposure to a single level of 560 mL/m^3 (ppm), for 5 or 6 hours per day, 5 days/week, for 15 weeks (103). Routine haematology and the histopathology of the liver, kidneys, lungs, ovaries, uterus and adrenal glands were examined. A slight decrease of the haematocrit level, increased relative liver weight, signs of hepatic hyperplasia (increased number of binucleated cells and transient increase of DNA synthesis), granulation and vacuolization of the liver were found. There were no effects in the kidneys, lungs, reproductive organs and adrenals. Another subchronic inhalation toxicity study with male rats involving inhalation exposure to 13.3 mg/m^3 (1.9 ppm) of 1,1,2,2-TCE for 9 months revealed some slight (mainly biochemical) changes at one time point (out of three) during the course of the experiment: somewhat lower body weight at 4 months and increased liver lipids at 7 months, whilst there was a consistent increase of the ACTH activity of the adenohypophysis (87).

By the oral route of administration, carcinogenicity studies with 1,1,1,2-TCE (72) and 1,1,2,2-TCE (71) by gavage with rats and mice examined a number of non-neoplastic endpoints, including histopathology of all major organs and tissues. 1,1,1,2-TCE was found to cause at the highest dose level (500 mg/kg for mice and 250 mg/kg for rats) behavioural signs of CNS toxicity towards the end the study. Mice that received the high dose of 1,1,2,2-TCE (282 mg/kg) developed acute toxic tubular nephrosis of the kidneys. In another gavage study, where 1,1,2,2-TCE was administered to rats at dose levels of 8 or 20 mg/kg during 60 days or at 3.2 or 8 mg/kg during 150 days the authors report to have found clear effects in the liver and some effects also in the kidney, testis and thyroid; the lowest dose was given as a threshold for chronic toxicity. However, it was not

possible to evaluate this study because quantitative data on the dose-effect or dose-response were not reported.

It would appear that the NOAELs for non-neoplastic effect endpoints in mice and rats can best be derived from the carcinogenicity bioassays (71, 72) and would correspond to the lower dose level used: i.e. regarding 1,1,1,2-TCE, 250 mg/kg bw/day for mice and 125 mg/kg bw/day for rats, regarding 1,1,2,2-TCE, 142 mg/kg bw/day for mice and 62 mg/kg bw/day for male rats and 43 mg/kg bw/day for female rats. Both substances however caused a clear hepatocarcinogenic response in mice even at the lower dose level.

There are two useful descriptive epidemiological surveys of workers exposed for some time to 1,1,2,2-TCE, one from Hungary (48) and the other from India (59). The air levels of 1,1,2,2-TCE have been measured in both studies with an outdated method but which may however show correctly the magnitude of the substance concentration. The observations reported in these two studies are by and large in agreement, and the more extensive survey in India (59) can be used to summarise the affects of 1,1,2,2-TCE exposure. In the most severely exposed jobs which involved both inhalation of the vapours and direct hand contact with liquid 1,1,2,2-TCE (50% in acetone), more than a third of the workforce complained of headache and vertigo, and roughly every other person exhibited a fine tremor of the fingers. Almost a quarter of these workers also complained of loss of appetite and many had experienced nausea, abdominal pain, and had vomited; however, liver enlargement was not found. The author reported that the symptom complex of tetrachloroethane poisoning appeared after about 3 months of exposure, and a more consistent series of symptoms was evident after 6 months. There seemed to be a dose-response for hand tremor with 1,1,2,2-TCE: at 9-17 ppm (in factory G) 14% of workers handling the substance showed tremors, at 40-74 ppm (in factory A) the corresponding frequency was 33%, at 50-61 ppm (in factory T) 41%, and at 65-98 ppm (in factory L) 50%. The observations of the survey were limited to recording symptomatology and some signs as the workers were reluctant to submit blood and urine for examinations. Moreover, the author pointed out that the study could not disclose the full picture of long-term effects by 1,1,2,2-TCE, because the high labour turnover made the employment periods relatively short, and because the study did not include the workers who had become ill and left the employment.

Human experience would therefore suggest that repeated exposures to 1,1,2,2-TCE may be accompanied by adverse symptoms from the CNS and the gastrointestinal tract at air levels in excess of 10-30 ppm (70-210 mg/m^3); however, skin exposure has also been involved. Acute symptoms of CNS depression and mucosal irritation were observed within 10-20 minutes at 1,000 mg/m^3 (146 ppm) in a volunteer study.

The previous human data concern studies that date back several decades. Currently, the manufacture and use of end products containing 1,1,2,2-TCE (the isomer that has commercial value) is very limited in the US and Europe. However, the substance occurs as a non-isolated intermediate in the closed production

process of trichloroethylene in some chemical industries. Thus, exposure to the tetrachloroethanes in the developed countries is presumably infrequent and low.

Although the database is far from complete, the assessment of health risks caused by tetrachloroethanes can be based on the previous human evidence involving 1,1,2,2-TCE exposure regarding non-genotoxic and non-neoplastic effects. However, the capacity of the compounds to cause liver carcinogenesis in the mouse, to act (1,1,2,2-TCE) as a weak initiator and a strong promoter in an initiation/promotion bioassay with rats, to cause genotoxicity and cell transformation in some *in vitro* assays, and the ability of the compounds to chemically interact with DNA, warrant certain concerns of possible carcinogenic effect in humans. Moreover, although tetrachloroethane-induced cancer was clearly demonstrated only with mice, there is not enough mechanistic data available to decide to what extent the effect is species specific. Human evidence is not helpful: there is one retrospective epidemiological study of cancer mortality in a cohort of workers exposed during World War II to 1,1,2,2-TCE. The study did not show an increased risk, but the study was limited and not sufficient to draw any certain conclusions.

14.3. Recommended basis for an occupational exposure limit

Tetrachloroethane has caused toxic effects in occupationally exposed workers at concentrations varying from 70 to 700 mg/m^3 (10-100 ppm). The target organs are the liver and the gastrointestinal tract, and the nervous system. Similar target organs have been identified in animal studies. Moreover, tetrachloroethane isomers have the capacity to bind to DNA, cause genotoxicity in certain *in vitro* assays, and they possess some carcinogenic activity. It is therefore prudent to consider potential carcinogenicity as the critical effect for tetrachloroethanes.

Several countries have attached the skin notation (indicating skin absorption hazard) to their respective OELs for 1,1,2,2-TCE. In a recent analysis of the industrial hygiene aspects of dermal absorption for 132 chemicals based on physical properties, 1,1,2,2-TCE was included in the list of substances with dermal toxicity potential (27).

15. Research Needs

If tetrachloroethane should still be used in industrial processes causing occupational exposure, it would be important to control the situation carefully with hygienic monitoring and hopefully even with biological monitoring of individual workers. To the latter end, valid methods should be devised. Moreover, it would be highly recommended to institute pertinent health surveillance programmes that may elucidate any adverse effects, including genotoxicity, among the workforce by long term low-level exposures. Further information of the carcinogenic mechanisms of tetrachloroethane is needed for a more complete risk assessment.

16. Summary

Luotamo M and Riihimäki V. Tetrachloroethane. DECOS and NEG Basis for an Occupational Standard. *Arbete och Hälsa* 1996:28.

Tetrachloroethane (TCE) has two isomers: 1,1,1,2- and 1,1,2,2-TCE. The latter has had most use in industry. 1,1,2,2-Tetrachloroethane has caused toxic effects in exposed workers. The target organs are the liver, gastrointestinal tract, and nervous system. In animals tetrachloroethanes: i) are carcinogenic in mouse liver; ii) are genotoxic and can cause cell transformation *in vitro* (1,1,2,2-TCE); iii) acts as a weak initiator and strong promoter in rats (1,1,2,2-TCE); and, iv) interacts with DNA. There is insufficient data on the carcinogenic effects in humans. The critical effect is, therefore, considered to be carcinogenicity for workers exposed to TCE.

Keywords: Tetrachloroethane, occupational exposure, metabolism, hepatotoxicity, genotoxicity, carcinogenicity, human toxicity, risk evaluation, occupational exposure limit

17. Summary in Swedish

Luotamo M and Riihimäki V. Tetrachloroethane. DECOS and NEG Basis for an Occupational Standard. *Arbete och Hälsa* 1996:28.

Tetrakloretan (TKE) består av två isomerer; 1,1,1,2- och 1,1,2,2-TKE. 1,1,2,2-Tetrakloroetan har i huvudsak använts i industrin. 1,1,2,2-Tetrakloroetan har framkallat toxiska effekter hos exponerade arbetare. Målorgan är lever, mag-tarmkanal och nervsystem. Djurförsök visar att tetrakloretaner: i) är cancer-framkallande i lever hos möss; ii) är genotoxisk samt orsakar cell-transformation i flera in vitro tester (1,1,2,2-TKE); iii) är svag initiator och starkt tumörpromotiv hos råttor (1,1,2,2-TKE); och iv) ämnet reagerar med DNA. Det finns endast begränsat med data om cancerframkallande effekter hos människa. Den kritiska effekten bedöms vara cancer hos arbetare exponerade för TKE.

Nyckelord: Tetrakloretan, yrkeshygienisk exponering, metabolism, hepato-toxicitet, genotoxicitet, carcinogenicitet, human toxicitet, riskvärdering, hygieniska gränsvärden

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Submitted for publication 16th December, 1996.

Appendix 1

Permitted or recommended maximum levels of 1,1,2,2-TCE in air. For 1,1,1,2-TCE there is no OEL in the countries listed below.

	ppm	mg/m3	Comments	Year	Ref.
Denmark	1		Skin	1994	
Finland	1	7	Skin	1996	
	3	21	15 min short term		
Germany		7	Skin, IIIB	1996	
Iceland					
Netherlands		7	Skin	1995	
Norway		7	Skin	1995	6
Sweden				1996	7
USA (ACGIH)1		6.9	Skin, A4	1996	8
(NIOSH)	1	7	Skin, C	1994	9
(OSHA)	5	35	Skin	1994	9

C = Potential carcinogen

A4 = Not classifiable as a human carcinogen. (Identified by other sources as a suspected or confirmed human carcinogen)

IIIB = Suspected carcinogen

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Summaries in Swedish and English are placed after the text, preceding the reference list. A summary should be no more than 100 words long. It should begin with complete reference information (see below for format). The texts should be followed by no more than 10 key words, in both Swedish and English.

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2. Borg G. Psychophysical scaling with applications in physical work and the perception of exertion. *Scand J Work Environ Health* 1990;16, Suppl. 1: 55-58.

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b. Chapter in book

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c. Book

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d. Report

1. Landström U, Törnros J, Nilsson L, Morén B, Söderberg L. *Samband mellan vakenhetsmått och prestationsmått erhållna vid körsimulatorstudie avseende effekter av buller och temperatur.* Arbetsmiljöinstitutet, 1988 (Undersökningsrappport 1988:27).

e. Articles written in langueges other than English, French, German or one of the Nordic languages

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f. Article in conference proceedings

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Figures and tables

Figures are placed in the text and numbered in order of appearance. The figure text is below the figure. The tables are placed in the text and numbered in order of appearance. The table text is placed above the table. Tables are normally placed at the top or bottom of a page, or immediately above a subhead.

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1 **H Westberg-Wohlgemuth.** Kvinnor och män märks. Könsmärkning av arbete – en dold lärandeprocess.

2 **T Lindh, S Törnqvist och L-I Andersson.** Exponering för elektriska och magnetiska fält hos anställda inom kraftindustrin.

3 **E Åhsberg och F Gamberale.** Upplevd trötthet efter fysiskt arbete. En experimentell utvärdring av ett trötthetsinstrument.

4 **H Johansson, P Sjölander and R Lorentzon (Eds).** Summaries of lectures during the postgraduate course Neuro-Muscular Systems and Muscle Pain. September 29 – December 12, 1995.

5 **I Holmér, B W Johansson, S Gyllerup och H Lundgren (red).** Kyla på gott och ont.

6 **M Hagberg, L Ekenvall, I-L Engkvist, K Kjellberg, E Menckel, G Persson, E Wigaeus Hjelm, PROSA-gruppen.** Ryggolycksfall i sjukvård. Program mot ryggolycksfall i sjukvårdens arbetsmiljö för sjuksköterskor, undersköterskor och sjukvårdsbiträden i Stockholms län (PROSA).

7 **T Backström.** Accident risk and safety protection in automated production.

8 **L Karlqvist, M Hagberg, G-Å Hansson och K Selin.** Arbetsbord för bildskärmsarbete med datormus – utveckling och utvärdering.

9 **G Björing och G M Hägg.** Undersökning av belastningsergonomiska förhållanden vid manuellt sprutlackeringsarbete inom träindustri.

10 **M Dahlqvist, L Palmberg, B Bergström, U Ekholm, K Eriksson, B Figler, B-M Larsson, P Malmberg, C Müller-Suur, S Siljerud, B-M Sundblad, U Ulfvarson och W Zhiping.** Akut exponering av luftföroreningar i sågverk hos friska försökspersoner.

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12 **S E Mathiassen, J Winkel, P Liukkonen, S Bao och G Björing.** Belastningsergonomi och rationalisering – en fallstudie.

13 **T Nilsson.** White finger symptoms and nerve conduction in relation to work involving exposure to hand-held vibrating tools.

14 **P Westerholm (red).** Psykisk arbetsskada – skadlig inverkan – samband med arbete. Ett vetenskapligt underlag för försäkringsmedicinska bedömningar.

15 **K Wahlstedt, C H Nygård, K Kemmlert, M Torgén och M Gerner Björkstén.** Effekten av en organisationsförändring på brevbärares arbetsmiljö och hälsa.

16 **C-H Nygård and Å Kilbom (Eds.)** Age and learning in working life.

17 **A Toomingas.** Methods for the evaluation of work-related musculoskeletal neck and upper-extremity disorders.

18 **L Olander.** Luftföroreningar i fordon. Halter och åtgärder.

19 **F Gamberale, C Sconfienza och T Hagström.** Värderingar och förhållningssätt till arbete bland ungdomar i Sverige. En kartläggning av ett representativt urval.

20 **D Gavhed.** Thermal responses in man to combinations of work protocols and clothing in cold air.

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27 **U Tikkainen, K Louhelainen and H Nordman.** The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 120. Flour Dust.

28 **M Luotamo and V Riihimäki.** DECOS and NEG Basis for an Occupational Standard. Tetrachloroethane.