Ethanol (ethyl alcohol)

Evaluation of the health effects from occupational exposure

Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *'Ethanol (ethyl alcohol)'*
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Mijnheer de staatssecretaris,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan ethanol ('alcohol'). Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over ethanol is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. De Commissie Beoordeling Carcinogeniteit van Stoffen heeft geadviseerd over de carcinogeniteit.

Twee zaken zijn van belang om hier onder uw aandacht te brengen: de beschikbaarheid van de onderzoeksgegevens en de wijze waarop de risico's zijn berekend.

Ten eerste de onderzoeksgegevens. Een mogelijk gevolg van langdurige blootstelling aan ethanol op de werkplek is kanker. Voor normstelling zijn de meest relevante vormen van kanker borstkanker (bij vrouwen) en darmkanker (bij mannen en vrouwen). Ook bij lage blootstellingen bestaat daarop namelijk een kleine kans. De Commissie Beoordeling Carcinogeniteit van Stoffen heeft vastgesteld dat voor borstkanker een genotoxisch werkingsmechanisme niet is uit te sluiten. Dat wil zeggen dat ethanol (of een metaboliet) directe veranderingen in de DNA-structuur kan veroorzaken. Volgens de huidige wetenschappelijke inzichten is daarbij geen absoluut veilig niveau van blootstelling aan te geven. Daarom berekent de Gezondheidsraad concentratieniveaus in de lucht op de werkplek die horen bij vooraf door de overheid bepaalde kankerrisico's.

De commissie heeft zich bij het berekenen van deze risico's gebaseerd op studies naar de gevolgen van het drinken van alcoholische consumpties. Daarover is namelijk al veel bekend. Deze gegevens zijn ook geschikt om de risico's van het inademen van ethanol op te werkplek te bepalen omdat voor borstkanker een genotoxisch werkingsmechanisme niet is uit te sluiten. In dat geval geldt dat de totale belasting relevant is voor het risico op kanker. Dus ongeacht de blootstellingroute.

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Het tweede punt is hoe die risico's vervolgens zijn berekend. Voor stoffen waarvoor geen veilig niveau van blootstelling aan te geven is, berekent de commissie normaliter de concentratieniveaus die horen bij een kans op 4 extra sterfgevallen per 1000 en 4 per 100 000.

In dit geval heeft de commissie echter rekening gehouden met het feit dat ethanol ook in lage concentraties aanwezig is in het bloed van mensen die nooit alcohol consumeren. Dit wordt waarschijnlijk veroorzaakt door stofwisselingsprocessen in het lichaam. Ook deze endogene ethanolconcentratie in het bloed draagt bij aan het risico op borstkanker, meent de commissie. Zij schat dat van elke 1000 vrouwen die overlijden er mogelijk 4 sterven aan borstkanker die wordt veroorzaakt door een levenslange, van nature voorkomende ethanolbelasting.

Wat betekent dit voor de berekening van het risico dat samenhangt met concentraties op de werkplek? Normaal gesproken wordt met twee risico's gewerkt. Berekenen we het concentratieniveau dat hoort bij een kans op 4 extra gevallen van overlijden door borstkanker per 100 000 sterfgevallen, dan resulteert dat echter in een blootstelling die veel lager is dan de endogene ethanolbelasting. De commissie acht het schatten van dit concentratieniveau op de werkplek daarom niet relevant.

Daarom heeft zij alleen het concentratieniveau berekend dat hoort bij een kans op 4 extra gevallen van overlijden aan borstkanker per 1000 sterfgevallen. Dat risico treedt op bij een blootstelling aan 1300 milligram ethanol per kubieke meter lucht gedurende 40 arbeidsjaren. Het is dus vergelijkbaar met het risico op borstkanker door de ethanol die toch al in het bloed aanwezig is.

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 Milieu.

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
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to:

the State Secretary of Social Affairs and Employment

No. 2006/06OSH, The Hague, July 10, 2006

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

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Contents

12 Ethanol (ethyl alcohol)

Samenvatting

Vraagstelling

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

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan ethanol. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór januari 2006 zijn verschenen.

Fysische en chemische eigenschappen

Ethanol (CAS-nummer 64-17-5) is een heldere kleurloze vloeistof met een karakteristieke geur. De geurdrempel bedraagt ca. 95 mg/m^3. Ethanol mengt goed met water en organische oplosmiddelen. Het heeft een hoge dampspanning.

Ethanol is momenteel een van de meest gebruikte organische chemicaliën die worden toegepast in industriële en consumentenproducten, voornamelijk als intermediair bij de productie van andere chemicaliën (waaronder aceetaldehyde, ethylacrylaat en ethylchloride) en als oplosmiddel. Daarnaast zit ethanol (‘alcohol’) in alcoholische dranken.

Ethanol wordt endogeen in het menselijk lichaam gevormd. De concentratie ethanol in het bloed van mensen bedraagt gemiddeld 0.27 mg/l.

Monitoring

Door de Amerikaanse instanties NIOSH* en OSHA** zijn methoden beschreven voor het bepalen van de concentratie ethanol in lucht. Deze methoden zijn gebaseerd op gaschromatografische analyse (GC-FID***).

Een methode voor de bepaling van ethanol in bloed met behulp van GC-FID is beschreven door de NIOSH. Voor het bepalen van de concentratie ethanol in de uitgeademde lucht zijn diverse aparaten op de markt.

Grenswaarden

De huidige bestuurlijke grenswaarde voor ethanol in de lucht op de werkplek bedraagt in Nederland 1000 mg/m^3. Ook in Zweden geldt dit als grenswaarde, terwijl in Duitsland de zogeheten MAK-waarde**** 960 mg/m^3 bedraagt. De grenswaarden in het Verenigd Koninkrijk en Denemarken en de door de American Conference of Industrial and Governmental Hygienists (ACGIH) vastgestelde Threshold Limit Value (TLV) bedragen 1900 mg/m^3 (1000 ppm).

In Duitsland geldt tevens een STEL-waarde***** van 4800 mg/m^3 (2500 ppm) voor een blootstelling gedurende dertig minuten; met als restrictie dat deze maximaal twee keer per werkdag mag voorkomen. Zweden kent een grenswaarde voor blootstelling gedurende vijftien minuten van 1900 mg/m^3 (1000 ppm).

Duitsland heeft ethanol geclassificeerd als kankerverwekkende stof in categorie 5, wat inhoudt dat de genotoxisch-carcinogene activiteit zo laag is dat bij de MAK-waarde van 960 mg/m^3 geen noemenswaardige bijdrage aan het kankerrisico voor de mens te verwachten is. Voor de reproductietoxische effecten geldt een classificatie in groep 2, wat wil zeggen er dat geen reden tot zorg is bij de MAK-waarde van 960 mg/m^3.

*	National Institute for Occupational Safety and Health
**	Occupational Safety and Health Administration
***	Gas chromatografie (GC) met een vlamionisatie dectector (FID)
****	Maximale Arbeidsplatzkonzentration
*****	Short Term Exposure Limit

Kinetiek

Na inademing wordt ethanol voor ongeveer 60 procent door de longen geabsorbeerd. Blootstelling via de ademhaling aan 1900 mg/m^3 ethanol gedurende vier uur (bij rust), kan in het bloed tot ethanolconcentraties van ongeveer 2 mg/l leiden. Ethanol kan ook via de huid worden opgenomen. Voor het berekenen van de interne dosis na huidblootstelling kan voor de snelheid van opname van ethanol door de huid een waarde van 0,7 mg per cm^2 huid gedurende 1 uur worden gehanteerd; Deze waarde zal in het algemeen de werkelijke opnamesnelheid overschatten.

Ethanol wordt na orale opname efficiënt in het lichaam opgenomen (voor meer dan 90 procent). De ethanolconcentratie in het bloed wordt door verscheidene factoren beïnvloed, waarvan voedselinname en geslacht de belangrijkste zijn. Na het drinken van twee alcoholische consumpties (ongeveer 20 gram ethanol) kan de ethanolconcentratie in het bloed binnen een uur een maximale waarde bereiken van ongeveer 300 mg/l. Daarna neemt de concentratie weer snel af; binnen enkele uren is bij mensen de ethanolconcentratie weer op het niveau zoals dat normaal in het lichaam voorkomt (endogeen niveau).

Inhalatoire blootstelling aan 1900 mg/m^3 (overeenkomend met ongeveer 11 gram ethanol per dag[*]) resulteert dus in een maximale ethanolconcentratie in het bloed die 10-100 keer kleiner is dan de bloedconcentraties na het drinken van één alcoholische consumptie (bevattend 11 gram ethanol). Veel effecten zijn gerelateerd aan de maximale ethanolconcentratie in het bloed; In die gevallen is de commissie van mening dat de gezondheidsrisico's na inhalatoire blootstelling aan ethanol zullen worden overschat als wordt uitgegaan van risico's na consumptie van vergelijkbare hoeveelheden. Voor de genotoxisch carcinogene effecten daarentegen is niet de maximale ethanolconcentratie in het bloed maar de totale interne ethanolbelasting[**] van belang. Zoals hierboven aangegeven geldt hiervoor dat de interne ethanolbelasting na het drinken van een alcoholische consumptie vergelijkbaar is met het inademen van 1900 mg/m^3 ethanol gedurende acht uur.

De lever is bij de mens het belangrijkste orgaan voor de afbraak van ethanol. Dat gebeurt in twee stappen: eerst wordt ethanol omgezet in aceetaldehyde en aansluitend wordt azijnzuur gevormd.

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* Uitgaande van 10 m^3 ingeademde lucht per achturige werkdag en 60 procent opname door de longen.

** Het begrip 'interne ethanolbelasting' kan worden omschreven als het product van de bloed-ethanolconcentratie en de tijd dat die ethanolconcentratie in het lichaam aanwezig is (AUC ofwel Area Under the Curve)).

Effecten

Bij mensen

Wanneer mensen korter dan een uur eenmalig worden blootgesteld aan concentraties ethanol kleiner dan 1900 mg/m^3 (1000 ppm) ondervinden ze geen irritatie in de luchtwegen of andere klachten. Bij blootstelling aan concentraties hoger dan 3000 mg/m^3 worden klachten als hoesten, droge keel en prikkeling van de neus gemeld. Blootstelling aan concentraties van 17 000 mg/m^3 en hoger worden als onverdraaglijk beschreven.

Bij hoge concentraties in de lucht is ethanol zeer irriterend voor de ogen. Herhaalde blootstelling van de huid aan 95 procent ethanol leidt niet tot huidirritatie, maar kan door ontvetting wel een droge huid veroorzaken. Occlusief contact daarentegen kan tot roodheid en verdikking of verharding van de huid leiden. Het kan ook irritatieve contact dermatitis en non-immunologische netelroos veroorzaken.

De meeste gegevens over de gevolgen voor mensen van langdurige blootstelling aan ethanol hebben betrekking op de consumptie van alcoholische dranken. Verscheidene epidemiologische onderzoekers rapporteren dat het verband tussen blootstelling aan ethanol en de algemene gezondheidstoestand van een persoon een U- of J-vorm lijkt te hebben: bij lage consumptieniveaus is er sprake van een gezondheidskundig gunstig effect, zoals een verminderd risico op hart- en vaatziekten, terwijl bij hogere niveaus de gezondheidstoestand achteruit gaat.

De meest kritische effecten na blootstelling aan ethanol betreffen kanker, levercirrose, vermindering van de vruchtbaarheid en afwijkingen bij het nageslacht. Uit epidemiologisch onderzoek blijkt dat bij consumptie van minder dan 10 tot 12 gram per dag waarschijnlijk geen levercirrhose zal optreden. De Commissie Alcohol en zwangerschap van de Gezondheidsraad concludeerde in een begin 2005 uitgebracht advies dat bij inname van deze hoeveelheden ethanol er wel effecten op de vruchtbaarheid en de ontwikkeling van het nageslacht kunnen optreden; Ook stelde deze commissie dat langdurige blootstelling aan 1 tot 10 gram ethanol mogelijk effecten kan veroorzaken op de vruchtbaarheid en op de ontwikkeling van het nageslacht (onder andere een verhoogde incidentie van spontane abortussen, foetale dood, en vroeggeboortes en een verkorte zwangerschap).

Met betrekking tot carcinogeniteit lijken borstkanker en colonkanker het meest relevant: onderzoek naar de gevolgen van langdurige consumptie van

alcoholhoudende dranken laten een verhoging van het voorkomen van deze vormen van kanker zien. Uit een gecombineerde analyse van de gegegevens over borstkanker volgt dat een consumptiepatroon van 10 gram ethanol per dag het risico op borstkanker met 10 procent verhoogd. Een kwantitatieve schatting van het risico op colonkanker door alcoholgebruik is niet beschikbaar.

Bij proefdieren

De laagste dosis waarbij proefdieren stierven na inademing van ethanol bedroeg bij muizen 55 000 mg/m^3 (7-uurs-blootstelling) en 25 000 mg/m^3 bij ratten (22-uurs-blootstelling). In één onderzoek gingen ratten na inhalatie van 385 mg/m^3 gedurende 45 minuten gedragsveranderingen vertonen. Na twee dagen blootstelling aan vergelijkbare concentraties trad het effect niet meer op en was kennelijk een tolerantie ontwikkeld.

De laagst gerapporteerde letale dosis bij blootstelling van de huid van konijnen bedraagt 20 g per kilogram lichaamsgewicht. Bij konijnen veroorzaakt occlusieve blootstelling aan zuivere ethanol milde irritatie van de huid en van de ogen.

Uit het schaarse proefdieronderzoek naar de gevolgen van herhaald inademenvan ethanol volgt dat hoge luchtconcentraties (resulterend in ethanolconcentraties in het bloed van meer dan 1700 mg/l), slechts geringe toxiciteit bewerkstelligen.

Na herhaalde orale blootstelling blijkt ethanol bij proefdieren effecten te veroorzaken in alle organen, het meest in de lever. Na toediening van ethanol via vloeibaar voedsel gedurende een periode van 30 dagen werden verhoogde concentraties van vetzuren en triglyceriden in de lever waargenomen.

Proefdieronderzoek naar de carcinogeniteit van ethanol na langdurige inhalatoire blootstelling zijn niet beschikbaar. Omdat de opzet van de proefdieronderzoekn na langdurige orale blootstelling onvoldoende is, kan de commissie hieruit geen conclusies trekken.

Er is geen overtuigend bewijs dat ethanol genotoxisch is, dat wil zeggen schade toebrengt aan het erfelijk materiaal (DNA). Er zijn in enkele genotoxiciteitstesten echter wel positieve resultaten waargenomen. Omdat aceetaldehyde (een metaboliet van ethanol) een genotoxisch carcinogeen is en dit een rol zou kunnen spelen bij het ontstaan van kanker na ethanol blootstelling, adviseert de Commissie Beoordeling carcinogeniteit van stoffen van de Gezondheidsraad ethanol als een genotoxisch carcinogeen te beschouwen.

Inhalatoire blootstelling van ratten aan ethanolconcentraties tot 30 400 mg/m^3, resulterend in bloed-ethanolwaarden van ongeveer 500 mg/l, had geen effect op de mannelijke vruchtbaarheid. Orale inname van 10 g ethanol per kg lichaamsgewicht per dag of meer resulteerde daarentegen bij ratten in een verminderd reproductievermogen, verlaagde testosteronwaarden in het serum, en verminderd gewicht en atrofie van de testikels. Bij mannelijke muizen was na een orale blootstelling van maximaal 0,6 gram ethanol per kg lichaamsgewicht per dag geen verandering in paargedrag en zwangerschapsuitkomst waarneembaar. Uit ander onderzoek volgde dat orale inname van hoge doseringen ethanol (meer dan 2 gram per kg lichaamsgewicht per dag), geen invloed had op het paargedrag van vrouwelijke dieren.

Volgens de Commissie Alcohol en zwangerschap van de Gezondheidsraad resulteert inademing van concentraties ethanol tot 38 000 mg/m^3 gedurende de dracht bij vrouwelijke ratten niet in effecten op het nageslacht. Wel werden er bij deze blootstelling, die leidde tot een alcoholconcentratie in het bloed van ongeveer 2500 mg/l, toxische effecten bij de moederdieren gevonden. Blootstelling van mannelijke ratten aan concentraties tot 30 400 mg/m^3 resulteerde niet in veranderingen in de nakomelingen. Zowel in ratten als in muizen is de ontwikkeling van het nageslacht verstoord na orale toediening van grote hoeveelheden ethanol gedurende de dracht, resulterend in bloed-alcoholconcentraties van ongeveer 2000-6000 mg/l.

Evaluatie en advies

In dit advies beoordeelt de Commissie WGD de gevolgen van beroepsmatige blootsteling aan ethanol. De commissie realiseert zich dat het drinken van alcoholische dranken een grotere bijdrage zou kunnen leveren aan de dagelijkse ethanolblootstelling dan de beroepsmatige blootstelling. Zij houdt hier echter geen rekening mee bij de schatting van het risico van beroepsmatige blootstelling omdat de commissie zich bij het bepalen van het risico baseert op de ook aanwezige niet-drinkers.

Gezondheidskundige advieswaarde (gemiddeld over 15 minuten)

De gegevens over de effecten van inhalatoire blootstelling op mensen zijn schaars. Op grond van hetgeen wel bekend is, meent de commissie dat kortdurende, eenmalige blootstelling aan ethanolconcentraties gelijk aan of kleiner dan 1 900 mg/m^3 (1000 ppm) gedurende 1 uur, waarschijnlijk geen irritatie van de luchtwegen of andere klachten geeft. Bij concentraties groter dan 3000 mg/m^3

worden klachten als hoesten, droge keel en prikkeling van de neus gemeld. De commissie adviseert daarom een advieswaarde van 1900 mg/m^3, gedurende 15 minuten (STEL).

Gezondheidskundige advieswaarde (gemiddeld over 8 uur)

De commissie beschouwt de verhoogde kans op borstkanker na langdurige blootstelling aan ethanol als het kritische effect. Op advies van de Commissie Beoordeling carcinogeniteit van stoffen, concludeert de commissie dat ethanol bewezen kankerverwekkend is voor de mens (overeenkomend met EU-categorie 1) en dat een genotoxisch mechanisme niet kan worden uitgesloten. De Commissie WGD leidt daarom voor ethanol concentratieniveaus (HBC-OCRV*) af in de lucht die samenhangen met een kans op 4 extra sterfgevallen door kanker per 1000 en 4 per 100 000.

Voor het afleiden van de risicogetallen gaat de commissie uit van onderzoek naar borstkanker. De commissie concludeert dat het regelmatig drinken van één alcoholische consumptie (ongeveer10 gram ethanol) per dag, de kans op borstkanker verhoogt met 7 tot 10 procent. Op basis van het hogere getal (overeenkomend met een RR van 1.1) schat de commissie de volgende risicogetallen:

- 4×10^{-5} gedurende 40 jaar beroepsmatige blootstelling aan 13 mg/m^3
- 4×10^{-3} gedurende 40 jaar beroepsmatige blootstelling aan 1300 mg/m^3.

Ethanol komt echter van nature voor in het bloed, ook bij mensen die nooit alcohol consumeren. Dit betekent dat ook deze mensen een interne ethanolbelasting hebben. Voor de door het lichaam zelf aangemaakte ethanol varieert een levenslange belasting van 8 tot 35 (mg/l)×jaar. De bijdrage tot de ethanolbelasting na beroepsmatige blootstelling gedurende 40 jaar aan 13 mg/m^3 bedraagt ongeveer 0,2 (mg/l)×jaar. De commissie is van mening dat deze bijdrage wegvalt binnen de endogene ethanolbelasting en de variatie daarin. Dat betekent dat deze bijdrage het risico op borstkanker niet wezenlijk beïnvloedt. Daarom vindt de commissie het niet zinvol een risicogetal te adviseren, dat hoort bij een kans van 4 extra sterfgevallen door borstkanker per 100 000.

Zoals de commissie reeds hierboven heeft aangegeven is er een kans op 4 extra sterfgevallen als gevolg van borstkanker per 1000 bij 40 jaar beroepsmatige blootstelling aan 1300 mg ethanol per kubieke meter.

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* HBC-OCRV: Health based calculated occupational cancer risk value, ofwel risicogetal.

Vervolgens heeft de commissie beoordeeld of bij een beroepsmatige blootstelling aan 1300 mg/m^3, andere effecten dan kanker kunnen optreden bij werknemers. De Commissie Alcohol en zwangerschap van de Gezondheidsraad concludeerde dat zowel effecten op de ontwikkeling van het nageslacht als effecten op de vruchtbaarheid kunnen worden waargenomen na consumptie van 1 tot 10 gram per dag. De Commissie Alcohol en zwangerschap geeft in haar advies ook aan dat deze effecten niet te relateren zijn aan de eerder genoemde ethanolbelasting maar aan de maximale ethanolconcentraties in het bloed. Omdat de maximale ethanolconcentraties in bloed na het drinken van 10 gram ethanol 10-100 keer hoger ligt dan de bloed ethanol concentratie na inhalatoire blootstelling aan 1300 mg/m^3, is de commissie van mening dat een blootstelling aan 1300 mg/m^3 voldoende bescherming biedt tegen deze effecten. Andere gezondheidseffecten treden op bij hogere ethanol blootstelling.

Huidnotatie

De commissie heeft tevens beoordeeld of voor ethanol een huidnotatie nodig is. Omdat dermale blootstelling een substantiële bijdrage kan leveren aan de interne belasting, stelt de Commissie WGD een huidnotatie voor.

Advies

De Commissie WGD schat dat de ethanolconcentratie in de lucht die samenhangt met een kans op 4 extra sterfgevallen door borstkanker per 1000 sterfgevallen (4×10^{-3}) bij een beroepsmatige blootstelling gedurende 40 jaar, 1300 mg/m^3 bedraagt.

Daarnaast beveelt de commissie een advieswaarde voor een kortdurende blootstelling van 15 minuten van 1900 mg/m^3 (STEL) aan en een huidnotatie.

Executive summary

Scope

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

In the present report the committee discusses the consequences of occupational exposure to ethanol. The committee's conclusions are based on scientific publications prior to January 2006.

Occurrence, physical and chemical properties

Ethanol (CAS registry number 64-17-5) is a clear, colourless liquid with a pleasant characteristic odour. The odour threshold is about 95 mg/m^3. Ethanol is miscible with water and organic solvents. It has a high vapour pressure.

Ethanol is currently one of the largest-volume organic chemicals utilised in industrial and consumer products, primarily as an intermediate in the production of other chemicals (ie acetaldehyde, ethylacrylate and ethylchloride) and as a solvent. In addition, ethanol is is present in alcoholic beverages.

Besides, ethanol is present endogenously in humans, which leads to a blood alcohol concentration of 0.27 ($^{+}$/_ 0.17) mg/l.

Monitoring

Methods for the determination of ethanol in air have been described by NIOSH* and OSHA**, and are based on GC-FID.

A method for analysing ethanol in blood by GC-FID has been described by NIOSH. In addition, ethanol can be determined in human breath samples as well.

Limit values

The current occupational exposure limit in the Netherlands and Sweden is 1000 mg/m^3 (500 ppm), whereas in Germany the limit is 960 mg/m^3 (500 ppm). In the UK, Denmark, and the USA the occupational exposure limit is about 1900 mg/m^3 (1000 ppm).

Short-term exposure limits have been set in Germany at 4800 mg/m^3 (2500 ppm; 30-min value) and Sweden 1900 mg/m^3 (1000 ppm; 15-min value). In Germany, ethanol has been assigned in Category 5 for carcinogenic effects (*i.e.* the genotoxic carcinogenic potential is so low that the MAK*** value (500 ppm) will not represent an unacceptable risk level), in Group C for genotoxic effects (*i.e.* the substance is shown to be genotoxic in studies performed in mammals), and Group 2 for reproduction toxic effects (*i.e.* no need for concern at exposure levels at/lower than the MAK level).****

Kinetics

Inhaled ethanol is absorbed by the lungs for about 60%. Exposure to 1900 mg/m^3 by inhalation for 4 hours, results in blood concentrations of approximately 2 mg ethanol/l (at rest). As a worst case estimate, a penetration rate of 0.7 mg/cm^2/h can be used to calculate the internal dose after dermal exposure.

Orally consumed ethanol is efficiently absorbed (>90%). The blood ethanol concentration is influenced by a number of factors of which food intake and gender are the most important. Consuming two alcoholic beverages (~20 gram etha-

* National Institute for Occupational Safety and Health
** Occupational Safety and Health Administration
*** Gas chromatography with flame ionization detector
**** Maximale Arbeidsplatzkonzentration

nol) results in a maximal blood concentration of approximately 300 mg ethanol/l; the maximal concentration in blood is reached within a hour, but the concentration is decreased rapidly and the blood ethanol concentration has reached endogenous levels after several hours.

Inhalatory exposure to 1900 mg/m^3 ethanol (corresponding to 11 gram ethanol per day[*]) results in a maximal ethanol concentration in blood which is 10 to 100 times lower than the maximal blood ethanol concentration after drinking one alcoholic beverage (approximately 11 gram ethanol). Most effects are related to the maximal ethanolconcentration in blood. In that case DECOS is of the opinion that the health risks after oral exposure to ethanol will overestimate the risk of inhalatory exposure to comparable levels of ethanol. For the genotoxic carcinogenic effects, however, the total internal exposure[**] is the relevant exposure estimate. The total internal exposure (or AUC) after drinking one glass of beer is comparable with the AUC after eight hour exposure to 1900 mg/m^3 ethanol.

The human liver is the main site of ethanol oxidation. Ethanol degradation occurs in two steps, first the formation of acetaldehyde with a subsequent formation of acetic acid.

Ethanol and acetaldehyde are oxidized by a wide range of enzymes and each of these enzymes may occur in different isoenzymes. Overall, ethanol and acetaldehyde are efficiently metabolized in Caucasians. A healthy subject is considered to metabolize between 6 and 9 g ethanol per hour. The significance of the first-pass metabolism is most likely limited.

Effects

Human data

Short-term inhalatory exposure to ethanol for one hour will not cause irritation or other effects below concentrations of 1900 mg/m^3 (1000 ppm). Concentrations higher than 3000 mg/m^3 might result in transient cough, dry throat and tickling of the nose. Levels over 40,000 mg/m^3 (21,000 ppm) are suffocating.

In concentrated form, ethanol is very irritating to the eyes. Non-occlusive, repeated dermal exposure to 95% ethanol does not cause skin irritation, but may

* Assuming that 10 m^3 air is inhaled per 8-hour working day and a lung retention of 60 percent.
** the total internal exposure is the product of the bloodalcoholconcentration (BAC) times the period present in the body, (*i.e.* the Area Under the Curve AUC).

cause dry skin due to defatting. Occlusive contact, in contrast, may induce erythema and induration (thickening/hardening of the skin). It may also induce irritant contact dermatitis and non-immunologic urticaria.

Most human data on the effects of long term exposure to ethanol concern the consumption of alcoholic beverages. Several epidemiological studies reported that the dose-effect curve for ethanol and overall mortality appears to be U- or J-shaped; beneficial effects due to the consumption of low levels of ethanol are observed, like a reduced risk of coronary heart disease.

The most critical non-carcinogenic effects in humans appear to be liver cirrhosis and effects on the development of offspring and fertility. Epidemiological studies suggest that consumption levels below 10-12 grams of ethanol per day, will probably not cause liver cirrhosis. However, the Committee on Alcohol consumption and reproduction concluded that at these consumption levels effects on fertility and development have been reported. Even long term oral exposure to levels of 1-12 gram ethanol per day might result in effects on the development (like increased incidence of spontaneous abortion, foetal death, pre-term delivery and decreased length of gestation) and fertility, according to the Committee on Alcohol consumption and reproduction.

With respect to carcinogenicity the most relevant types of cancers appear to be breast and colorectal cancer. All the available data concern the association of the consumption of alcoholic beverages and these cancer types. Pooled studies or meta-analyses can be used to estimate the cancer risk in a quantitative way. Adequate studies are only available for breast cancer, resulting in a RR of 1.1 per each 10 grams of ethanol per day consumed. Such studies are not (yet) available for colorectal cancer.

Animal studies

The lowest lethal dose by inhalation is 55000 mg/m^3 in mice (7 hrs exposure) and 25000 mg/m^3 in rats (22 hrs). In one study, behavioural depression occurred in rats inhaling 385 mg/m^3 for 45 minutes.

The lowest reported lethal dermal dose for rabbits is 20 g/kg body weight. In rabbits acute occluded exposure to 95% ethanol caused mild irritation. 96% ethanol is mildly irritating to the eyes of the rabbit.

Available animal studies with repeated inhalatory exposure were only limited in design. From the available data it may be concluded that at high concentrations

(*i.e.* resulting in blood alcohol concentrations > 1700 mg/l), only slight toxicity was observed.

After repeated oral administration to animals, ethanol appears to affect all organs with the liver as main target organ. Increased hepatic concentrations of fatty acid and triglycerids were observed after a 30-day administration of a liquid diet containing ethanol.

No long-term inhalation carcinogenicity studies in animals have been found. Because in the long-term oral exposure studies in rats and mice the MTD was not reached, these studies are of limited value to evaluate the carcinogenic potential of ethanol.

There is no convincing evidence that ethanol is genotoxic. In a limited number of *in vitro* as well as *in vivo* genotoxicity tests, however, ethanol gave positive results. Because a role for one of the major metabolites of ethanol, *i.e.* acetaldehyde (a known genotoxic carcinogen) can not be excluded, the committee on the Evaluation of the carcinogenicity of chemical substances of the Health Council concluded that ethanol should be considered a genotoxic carcinogen.

According to the Committee on Alcohol consumption and reproduction of the Health Council, exposure of rats by inhalation to concentrations upto 30,400 mg/m^3 ethanol, resulting in blood alcohol levels of about 500 mg/l, did not cause changes in male fertility. Oral intake of ethanol (ca. 10 g ethanol/kg bw/day or higher), in contrast, resulted in decreased reproductive performance, decreased serum testosterone levels, decreased testicular weight and testicular atrophy in rats. An oral study in male mice at levels up to 0.6 g ethanol/kg bw/day) did not influence mating behaviour and pregnancy success. Other studies into the fertility effects of ethanol have shown that oral intake of high ethanol doses (> 2 g/kg bw/day) before and during pregnancy had no influence on mating behaviour of females.

In addition, the Committee on Alcohol consumption and reproduction concluded that exposure of female rats by inhalation during pregnancy to concentrations upto 38,000 mg/m^3 ethanol, resulting in blood alcohol levels up to about 2500 mg/l, did not cause developmental toxicity although maternal toxicity was observed. Exposure of male rats to concentrations up to 30,400 mg/m^3 did not show changes in paternal offspring. Teratogenic effects, however, were observed in rats and mice following oral intake of large amounts of ethanol during pregnancy, resulting in blood alcohol levels of about 2000-6000 mg/l. No teratogenic effects were observed in mice after oral intake of ethanol resulting in blood alcohol levels of about 200 mg/l.

Health based occupational exposure limit

In this report, DECOS evaluates the effects of occupational exposure to ethanol. Although the committee acknowledges the fact that drinking alcoholic beverages might be a more important source of ethanol exposure, this exposure is not taken into consideration for the assessment of the effects after occupational exposure.

Recommendation of an HBROEL, 15-min TWA (STEL)

Although the human data are limited, the committee is of the opinion that inhalatory exposure to 1900 mg/m^3 ethanol for one hour will probably not cause local or systemic effects in man. Exposure to higher concentrations will result transient cough, dry throat and tickling in the nose. In conclusion, DECOS is of the opinion that exposure to 1900 mg/m^3 for 15 minutes will be low enough to protect workers for effects after short term exposure. DECOS therefore recommends a short term exposure limit of 1900 mg/m^3.

Recommendation of an HBC-OCRV

The committee considers the development of breast cancer after exposure to ethanol as the critical effect. Based on the advice of the Committee Evaluation of Carcinogenic substances that ethanol is known to be carcinogenic to humans (comparable to EU category 1) and that a genotoxic mechanism cannot be excluded, DECOS calculates occupational cancer risk values for ethanol.

From the available meta-analysis and pooled studies, the committee concluded that drinking of one glass of alcoholic beverage (~10 gram ethanol) per day would increase the risk for breast cancer with 7-10%. From a RR of 1.1, DECOS calculates the following HBR-OCRVs:

- 4×10^{-5} for 40 years of occupational exposure to 13 mg/m^3
- 4×10^{-3} for 40 years of occupational exposure to 1300 mg/m^3.

However, ethanol is present in the human body of non-drinkers as well, which results in a total internal ethanol dose for lifetime (80 years) of 21.6 (±13.6) (mg/l)×year. On the other hand, occupational ethanol exposure to 13 mg/m^3 (corresponding to a extra cancer risk of 4×10^{-5}) gives an internal dose of approximately 0.2 (mg/l)×year. DECOS is of the opinion that an internal dose of 0.2 (mg/l)×year as a result of occupational exposure to 13 mg/m^3 is negligible as compared to the internal dose due to the endogenous ethanol concentration in

blood ((22 mg/l)×year). Consequently, the committee considers the relevance of the calculation of an HBC-OCRV corresponding to a risk of 4×10^{-5} doubtful.

Therefore, DECOS estimates the HBC-OCRV of 1300 mg/m^3 corresponding to an additional breast cancer risk of 4×10^{-3}.

Subsequently, the committee evaluated whether this HBC-OCRV of 1300 mg/m^3 is low enough to protect workers against other toxic effects. According to the Committee on Alcohol consumption and reproduction, first signs of developmental toxicity and effects on fertility manifest after drinking one alcoholic consumption per day or less (<10 gram ethanol per day). However, for these effects DECOS is of the opinion that the blood alcohol concentration (BAC) is the relevant exposure parameter. Considering the fact that the maximal alcohol concentration in blood after one (oral) drink is approximately 10-100 times higher than the ethanol concentration in blood after inhalatory exposure to1300 mg/m^3, the committee is of the opinion that a HBC-OCRV of 1300 mg/m^3 is low enough to protect against these effects. Other toxic effect manifest after exposure to higher exposure levels.

Skinnotation

At request of the minister of Social Affairs and Employment, the committee judged wheteher for ethanol is skin notation is needed. As dermal exposure can substantially contribute to the body burden of ethanol, DECOS recommends a skin notation.

Health based calculated occupational cancer risk value

DECOS calculates an HBC-OCRV of 1300 mg/m^3, resulting in a breast cancer risk of 4 additional death cases per 1000 ($4*10^{-3}$) deaths for 40 years.

In addition, DECOS recommends a short term exposure limit (STEL) of 1900 mg/m^3 twa 15 minutes and a skin notation.

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). This evaluation should, if possible, lead to a health-based recommended exposure limit for the concentration of the substance in the air. Such an exposure limit cannot be derived if sufficient data are not available, or if the toxic action cannot be evaluated using a threshold model. In the latter case, an exposure-response relationship is recommended for use in regulatory standard setting.

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

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of ethanol. The members of DECOS are listed in Annex B. The draft document has been prepared by PMJ Bos, JHE Arts, RA Bausch-Goldbohm, S Dekkers, HFJ Hendriks, K Nordheim and C de Heer of the Toxicology Division of TNO Nutrition and Food Research, Zeist, the Netherlands.

With respect to the genotoxic properties of ethanol, DECOS has consulted the Committee on the Evaluation of the carcinogenicity of chemical substances of the Health Council of the Netherlands. The committee's advice is included in Annex D.

In 2005, the DECOS released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The DECOS has taken these comments into account in deciding on the final version of the report.

1.3 Data

Since the amount of data on ethanol is enormous, the committee has chosen a specific strategy for gathering the necessary data. Several evaluations were used as starting point for the literature search [1-4,5]. These evaluations were used as basis. If considered relevant the original publications were studied. The literature search was limited to reviews published in recent years, *i.e.* covering 1997-2001, in addition to the literature covered by the regarding evaluations. Key words were: ethanol or 64-17-5 and toxic* or adverse or kinet* or metabol* or expos* or development* or reproduct* or embryo* or feto* or foeto*. For the evaluation of the effects of ethanol on reproduction, DECOS followed the evaluation of the Committee on Alcohol Consumption and Reproduction of the Health Council of the Netherlands, published in 2004[6].

For the preparation of this document, literature has been retrieved from online databases such as Medline, Toxline and Chemical Abstracts-plus (last update online search was performed on 21 May 2001). For specific aspects, more recent literature was consulted afterwards.

The search resulted in 1043 hits. The abstracts were screened and a selection of approximately 120 reviews was chosen for further evaluation. Original publications, referred to in these reviews, were studied when considered relevant.

In addition, HSDB, RTECS, Merck, EINECS (on CD-ROM) and IPCS (online) were also consulted (last update: 21 May 2001).

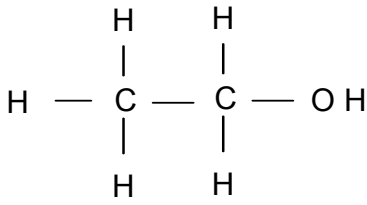
In 2004, an additional search was performed in Pubmed using the keywords: inhalat* and ethanol; breast cancer and ethanol. References published between 2004 and 2006 were no reason for the committee to adjust her recommendation.

Chapter 2

Identity, properties and monitoring

2.1 Identity

2.1.1 *Structure*



2.1.2 *Chemical names and synonyms/registry numbers*[*]

Name	:	ethanol
Synonyms	:	ethyl alcohol; absolute alcohol; anhydrous alcohol; dehydrated alcohol; ethyl hydrate; ethyl hydroxide
Molecular formula	:	C_2H_6O
Structure formula	:	CH_3CH_2OH
CAS registry number	:	64-17-5
EINECS number	:	200-578-6
EC number	:	603-002-00-5
RTECS number	:	KQ6300000

* Data from [7]

2.2 Physical and chemical properties*

Molecular weight	:	46.07
Melting point (100 kPa)	:	-114.1°C (–117°C[11])
Boiling point	:	78.2°C (79°C[11,12])
Relative density at 20°C with saturated vapour/air mix (air = 1)	:	1.04
Density (den^{20}_4)	:	0.7893 g/l
Solubility (relative scale, 5 = miscible, 20°C)	:	water 5; ethanol 5; ether 5; acetone 5
Log $P_{octanol/water}$	:	-0.3
Relative vapour density (air = 1)	:	1.6
Relative density (water = 1)	:	0.8
Vapour pressure at 25°C	:	7.9 kPa (5.8 kPa at 20°C[11])
Flash point, closed cup	:	12°C (13°C[9,11]; 14°C[10])
Odour threshold	:	95 mg/m^3 (~50 ppm[13])
Odour	:	pleasant, characteristic
Appearance at 20°C	:	clear, colourless liquid
Maximum vapour concentration in % at 25°C	:	6.58
Explosive limits/flammability by volume in air	:	lower, 3.3%; upper, 19%
Autoignition temperature	:	363°C (422.78[8]; 793.0[10])
Conversion factors	:	1 ppm = 1.9 mg/m^3 (in air) 1 mg/m^3 = 0.53 ppm (in air) 1 mg/l (blood) = 0.022 mM (in blood) 1 mM (in blood) = 46 mg/L (blood)

2.3 EU Classification and labelling**

2.3.1 Classification:

Highly flammable [F]

2.3.2 Labelling:

Highly flammable [R 11]
(Keep out of the reach of children) [(S 2)]
Keep container tightly closed [S 7]
Keep away from sources of ignition - No smoking [S 16]

* Data from [7-12]
** Data from [14]

2.4 Validated analytical methods

2.4.1 *Environmental monitoring*

NIOSH method 1400 is suitable for measuring the concentration of ethanol in air[15]. A known volume of air is drawn through coconut shell charcoal to trap the organic vapours present. The analyte is desorbed with carbon disulphide containing 1 percent 2-butanol, and quantified. The sample is then separated and analysed by a gas chromatograph with a flame ionisation detector (GC-FID). The recommended sample volume is 0.1 to 1 litre at a flow rate of 0.05 l/min, with a working range of 16-1000 ppm (30-1900 mg/m^3) for a 1-litre sample. Accuracy is ±14%.

OSHA method no.100 is recommended for measuring the ethanol concentration in air[16]. Samples are collected by drawing air through two 8-mm o.d. Anasorb 747 sampling tubes connected in series. The front tube contains 400 mg of adsorbent, and the back tube 200 mg. The samples are desorbed with a 60/40 *N,N*-dimethyl-formamide/carbon disulfide solution and analysed by a gas chromatograph using a flame ionisation detector (GC-FID). The recommended air volume and sampling rate is 12 litres at 0.05 l/min. With a reliable quantitation limit of 0.68 ppm (1.29 mg/m^3), the target concentration is 1000 ppm (1900 mg/m^3). This method was developed to improve a similar OSHA method (based on NIOSH 1400 described above), as this method appeared to be prone to sample migration and has a low sampling capacity.

2.4.2 *Biological monitoring*

NIOSH method 8002[15] may be used to measure the concentration of ethanol in blood by gas chromatography with flame ionisation detection (GC-FID). A sample of venous blood is drawn into a 5 ml heparin-coated vacuum tube after 2 hrs of exposure. The detection range is 0.01-0.6 mg/ml with a recovery of 0.98 at 0.05 mg/ml blood.

Alcohol can be determined in breath samples of humans as well (in mg per liter breath) by a variety of methods. The most commonly used alcohol breath analyzers quantify the ethanol concentration by measuring the absorbtion of infrared radiation[17]. Since other infrared absorbing components (eg acetone in diabetics) may be present in breath, it is essential to measure at numerous wavelengths. Differentiation between two infrared-absorbing components and quanti-

tation of one of them requires at least measurement at two wavelengths (usually 3.4 and 9.5 μm) plus, usually, a third isosbestic point in the spectrum.

Chapter 3

Sources

3.1 Natural occurrence

Ethanol occurs naturally as a volatile plant isolate e.g. in beans, and is produced by natural fermentation of carbohydrates. Ethanol is also found as a microbial activity product of animal wastes[18]. Some reports also suggest a natural endogenous production of ethanol in humans, possibly by microbes in the gut[1].

Sprung *et al.* measured the blood alcohol concentration (BAC) in man due to endogenous ethanol of 0.27 (+/- 0.17 mg/l)[19].

3.2 Man-made sources

3.2.1 Production

The main industrial production methods of ethanol are (1) synthetic from ethylene, (2) as a by-product of certain industrial operations, or (3) by the fermentation of carbohydrates (sugar, cellulose or starch)[12]. On an industrial scale, the synthetic production of ethanol far exceeds its production by fermentation[12].

The main process of synthesising ethanol from ethylene in the period 1930-ca 1970, was by indirect hydration (sulphuric acid process). It was almost phased out in the early 1970s by the direct hydration process, developed to eliminate the use of sulphuric acid. There are two main categories for direct hydration of ethyl-

ene to ethanol, of which the first is most frequently employed:(1) vapour-phase processes contact a solid or liquid catalyst with gaseous reactants, while (2) mixed-phase processes contact a solid or liquid catalyst with liquid and gaseous reactants[10]. The bulk of ethylene produced in the USA is used to synthesise ethanol. Anhydrous ethanol is manufactured industrially by azeotropic distillation[12].

Alternative processes for synthesising ethanol exist, but are not utilised on a commercial scale[10]. These include hydration of ethylene in the presence of dilute acids (weak sulphuric acid process); hydration of ethyl ether; conversion of acetylene to acetaldehyde, followed by hydrogenation of the aldehyde to ethyl alcohol; and the Fischer-Tropsch hydrocarbon synthesis[10].

Ethanol can be produced by fermentation of any material containing carbohydrates (sugar), however the yield from the three main raw materials, sugars, starches and cellulose, varies. Sugars from e.g. sugar cane, sugar beets, molasses and fruits can be converted to ethanol directly, with a high yield. Starches (from grains, potatoes and root crops) must be hydrolysed to fermentable sugars by enzymes from malt or mould. The fermentation of cellulose (from e.g. wood agricultural residues, waste sulphite liquor from pulp) is possible by pre-treatment with mineral acids, which converts the cellulose to fermentable sugars. However, the final yield will be <35%.

The ethanol is purified after production to remove unwanted impurities, mainly by distillation, fractionation or hydrogenation to convert aldehyde impurities to alcohols. Dependent on the intended use, the resulting product will vary in proof (ie. the percentage of ethanol in an alcohol/water mixture.)[10]

In Europe, the total production of ethanol is 1.2 million tonnes[20]. The non-beverage part of the production is currently (2001) 650,000 tons/year, with 400,000 tons used as a solvent and 250,000 tons for further processing[20]. The yearly capacity in the USA is close to 7 million tons, of which 6 million tons account for beverage and fuel use. Approximately 850,000 tons are used in the industrial setting[20].

3.2.2 *Use*

Historically, ethanol has been used extensively as antifreeze in the US, but is now largely replaced by ethylene glycol. Ethanol has already for a long time been important in the production of acetaldehyde, ethyl acrylate, ethyl chloride, n-butanol and butadiene. Intermittently, high fuel prices and low grain profits have stimulated research into the use of ethanol as a fuel, and a blend of 10% ethanol and 90% petroleum based unleaded fuel (“gasohol”) or neat ethanol has on

several occasions alleviated fuel shortages. In the United States, there is significant use of ethanol as a automotive fuel component[20,21].

Industrial ethanol is currently one of the largest-volume organic chemicals utilised in industrial and consumer products, primarily as an intermediate in the production of other chemicals and as a solvent. The syntheses of ethylene, glycol ethers, vinegar, ethlyamines, ethyl ether and ethyl vinyl ether are all performed with ethanol as a reactant[10]. Ethanol is an essential raw material in the manufacture of drugs, plastics, lacquers, plasticizers, polishes, perfumes and cosmetics[12]. As opposed to the decreasing use as an intermediate in chemical synthesis, its use as a solvent has recently increased substantially. Products include soap and cleaning preparations, solvents, antiseptic agent, vinegar, cosmetics, ink and coating formulations, and pharmaceuticals.

Finally, ethanol is produced for the alcoholic beverages industry[12].

Chapter 4

Exposure

4.1 General population

The main source of alcohol exposure for the general population is by drinking alcoholic beverages. In the Netherlands, about 85-90% of the general population (16-65 years) is drinking alcoholic beverages now and then (CBS-Statline). In addition, man can be exposed orally to ethanol via its natural occurrence in foods like beans and cheese, and via consumption of foods, flavourings, pharmaceuticals[18], although regulation of its use minimises the possibility of oral ingestion[12]. Concentrations in the lima bean, common bean, mung bean and soy bean varied from 2.9-15 mg/kg[18]. The concentration in alcoholic beverages is measured in percent, e.g. wine will contain ~12% ethanol. Dermal exposure occurs via ethanol-containing cosmetics, perfumes and drugs used topically[12]. The possibility for exposure by inhalation of vapours is considerable, due to the diverse applications of ethanol, and its volatile properties[12]. Concentrations in simple hydrocarbon exhaust (benzene, isooctane) were reported to be less than 1.14 mg/m^3. The ambient air concentration varies according to the proximity of the sources. While rural areas had an average of 0.76-1.46 μg/m^3, concentrations in urban areas were 0.95-<190 μg/m^3 (all measured in the USA)[18].

4.2 Working population

Occupational exposure will mainly occur by inhalation, and secondarily via dermal absorption[12]. The exposure happens in settings associated with its manufacture[18], by use in synthesis, use as a solvent, and when released as a product of fermentation, decomposition or combustion[18]. An overview of data available on ethanol exposure in various work-related areas is presented in Table 4.1[4].

Table 4.1 Summary of data available on ethanol external exposure in various work-related areas (adapted from Bessems *et al.*[4]).

Industry	N (measurements)	Range (mg/m^3)	Reference
Explosives[e]	?	230-671	22
Beverage production		0-95[a]	Data provided by industry
Ethanol production		0-570[a]	Data provided by industry
Ethanol distribution		9.5-95[a]	Data provided by industry
Graphics/printing industry[e]	2	4.8-27	23
Graphics/printing industry[e]	7	0.4-4	24
Graphics/printing industry[e]	7	114-593	BAUA database
Graphics/printing industry	10	<LD->361[b]	25
Graphics/printing industry	3	34-97	Data provided by industry
Graphics/printing industry	12	36-333	Data provided by industry
Graphics/printing industry	18	1.9-148[c]	Data provided by industry
Lacquer and/or paint[e]	11	1.9-209	26
Lacquer and/or paint	1	1.9	26
Lacquer and/or paint	9	1.9-253	26
Lacquer and/or paint	6	1.9-9.5	26
Lacquer and/or paint	28	1.9-253	26
Lacquer and/or paint[e]	21	19-175	27
Lacquer and/or paint[e]	1	48.5	28
Lacquer and/or paint	30	?	29
Lacquer and/or paint	4	21.7-214	30
Wood coating	38	?	31
Parquet treatment[e]	26	4.9-181	32
Parquet treatment	13	122-547	32
Parquet treatment	7	638-2823	32
Parquet treatment	27	207-1721	32
Parquet treatment	17	285-1915	32
Parquet treatment	1	5.7	33
Electrotechnic work[e]	4	3.6-5.5	34
Hospital[e]	7	17-179	35
Hospital[e]	?	2.5-14[c]	36
Hospital	?	0.6-69	37
Hospital	?	2.9-88.5	37
Laboratory[e]	?	<0.02-247	38

Laboratory[e]	5	5.9-8[d]	39
Laboratory[e]	?	3.8-25.5[d]	40
Laboratory[e]	?	<190[d]	41
Hairdresser	10	3.8-35.7[c]	42
Hairdresser	195	0.19-55.9	43
Total	2,601	?	44

a Based on 1 minute stationary measurements
b Breakthrough occurred in all (8) of the short-term samples and all (10) of the longer period samples taken in a liquid inks department. Breakthrough leads to an underestimate in the results.
c Stationary measurements
d Stationary and individual measurements
e Data from BAUA database. Built by BAUA (Germany), with reports from German research institutes and international publications on chemical substances.

Table 4.2 is a representation of studies performed on workers occupationally exposed to ethanol. The exposure during an 8-hr shift was measured with stationary and/or personal equipment. In several settings[31,37] the employees were exposed to a number of solvents, and it proved very difficult to allocate complaints to a specific substance. E.g., workers exposed to formaldehyde, ethanol and other solvents via spray painting wood with acid-hardening lacquers complained about effects in the upper mucous membranes, primarily the nose, eyes and throat. These effects were ascribed to formaldehyde, as this was the only solvent where the mean exposure level (0.48 mg/m^3) was higher than that reported to cause irritation (0.1-0.3 mg/m^3)[31].

Table 4.2 Mean (and range) ethanol concentrations during an 8 hr shift measured with stationary or personal equipment.

Working environment	N (number)	Stationary		Personal		Reference
		Mean (AM) mg/m^3	Range mg/m^3	Mean (AM) mg/m^3	Range mg/m^3	
Hairdresser salons	4 (ventilation) 6 (no ventilation)	9.5 / 33[a]	4-36	-	-	42
Hairdresser salons	90[b] and 195[c]	7.9[d]	0.1-43	10.8	0.1-56.6	43
Car spray painting	70[e]	-	-	88.3	22-217	30
Car spray painting	28 (exposed) 18 (nonexposed)	0.6	-	1.7	-	29
Operating room, anaesthetic nurses	148 (air samples) 162 (personal samples)	23.9	-	23.8	-	37
Woodworking factory	38	-	-	17	-	31

[a] Ventilated/Unventilated hairdresser salons
[b] Number of ambient air concentrations measurements
[c] Number of personal concentration measurements
[d] ventilation rates were measured but not related to concentration measurements
[e] 70 personal samples were measured. Only in 4 samples ethanol was detected with a mean of 88.3 mg/m^3

Dermal exposure predominantly occurs by using ethanol as an antiseptic agent. In hospitals, dermal exposure is probably the most important route of occupational exposure.

Chapter 5

Kinetics

5.1 Absorption

Ethanol can be absorbed easily across the surface of the gastrointestinal tract, the lungs and the skin. The passage of ethanol across biological membranes occurs through a process of passive diffusion along concentration gradients. Because of the low molecular weight and high water and lipid solubility, ethanol moves through the same transmembrane channels that allow the passage of water and lipid membranes.

5.1.1 Inhalation

There is a considerable gap in knowledge regarding the respiratory intake of ethanol. The kinetics of inhaled ethanol pose unique situations that differ from the ingestion of ethanol. In humans, for example, approximately 60%[45] or (30-76%)[46] of inhaled ethanol is absorbed. However, the relatively low retention following inhalation appears not to be due to metabolism[47]. Instead, the loss is a result of the wash-in-wash-out effect observed with water-soluble chemicals[48]. Briefly, as a water-soluble gas is inhaled, it dissolves in the mucous lining of the upper respiratory tract. It then diffuses from a region of higher concentration (lumen) to a region of lower concentration (the epithelial cells and blood capillaries). During exhalation, the opposite occurs: the gas diffuses from the epithe-

lial cells and blood capillaries back into the lumen. Consequently, the net result is that some of the gas desorbs and is exhaled as is observed with other water-soluble chemicals such as methanol[48-50].

Campbell *et al.*[51] studied the effect of ethanol exposure on the Blood Alcohol Concentration (BAC) and exposed one male volunteer for 3 hours to 1900 mg ethanol/m^3. Blood samples were taken at 0, 35, 60, 120 and 180 minutes after exposure. Exposure to 1900 mg/m^3 (at rest, ventilation rate was 6 l/min) did not result in detectable amounts of ethanol in venous blood (detection limit was 2 mg/l). The main factors which will affect the respiratory uptake of ethanol are: the concentration of ethanol in the inspired air, the rate of ventilation, and the percentage of lung clearance. Using a kinetic model, the authors calculated that when a human volunteer, engaged in *very heavy* physical activity (ventilation rate of 50 l/min), was exposed to 1900 mg/m^3, a maximal blood alcohol concentration (BAC_{max}) of 20 mg/l (see figure 5.1) would be expected.

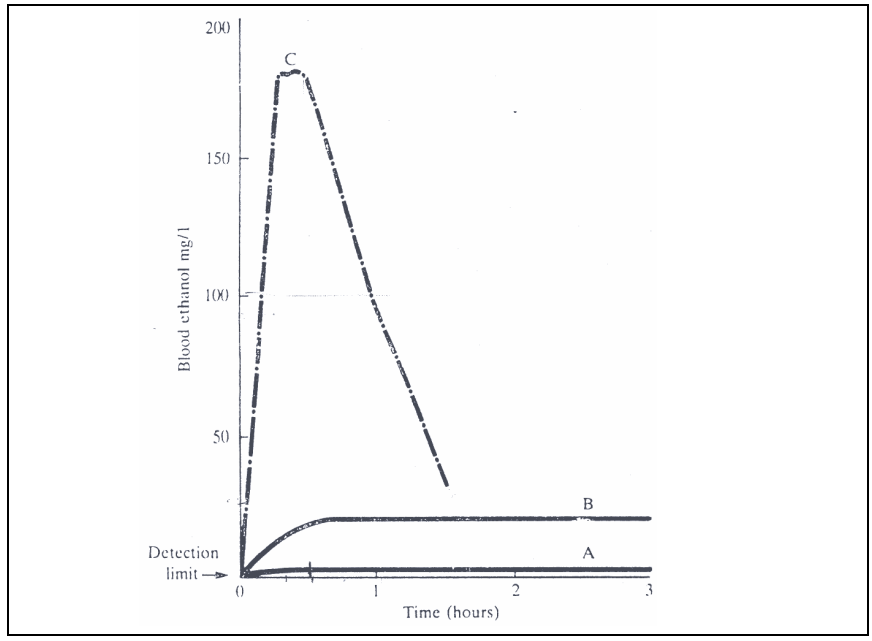


Figure 5.1 Comparison of blood ethanol concentrations after ingestion and inhalation. A = predicted ethanol concentration following inhalation of vapour (1900 mg/m^3) at rest (6 L/min) for 3 hours; B = predicted ethanol concentration following inhalation of vapour (1900 mg/m^3) during heavy work (50 L/min) for 3 hours; C = ingested ethanol (11 g) taken at time 0. 'Detection limits' refer to the detection limit of the analytical method used in this study (2 mg/L) (adapted from Campbell *et al.*[51]).

In the first of two series of experiments of Seeber *et al.*, volunteers (12 male and 12 female) were exposed to 150, 750 and 1,500 mg/m^3 (80, 400 and 800 ppm) for 4 hours. In the second experiment, volunteers (8 male and 8 female) were exposed for 4 hours to (1) 1,900 mg/m^3 (1000 ppm) constantly or to (2a) with an hourly change between 190 and 3,610 mg/m^3 (100 and 1900 ppm, respectively) or (2b) 3610 mg/m^3 and 190 mg/m^3 (1900 and 100 ppm). The ventilation rate of the volunteers was approximately 6 l/min. The maximal measured blood alcohol concentrations (BAC_{max}) in the first experiment increased from 0.23, 0.85 to 2.1 mg/l (at 150, 750 and 1500 mg/m^3 respectively) and in the second experiment to 0.66-5.6 mg/l. There was a linear correlation ($r_{xy} = 0.49$, $p < 0.001$) between the ethanol exposure in the air and the blood ethanol concentrations[52].

A PBPK model for inhaled ethanol was developed by Lester and Greenberg in both mice and rats and both models were compared. Subsequently, these models have been applied to the human situation mainly in relation to inhaled ethanol vapors during refuelling. The model was very accurate to predict rat, mice and human (male) blood ethanol concentrations[53]. Most modellers compare their modelling data with this study of Lester and Greenberg. Simulated blood ethanol concentrations in human males following exposures to 95 and 1,140 mg/m^3 (50 and 600 ppm) ranged from 7 to 23 μM (320-1100 mg/l) and from 86 to 293 μM (4000-13000 mg/l), respectively[54]. The model was criticized for the incorrect breathing rates used for subjects[55] (Table 5.1). Conolly *et al.* adjusted the PBPK model, and calculated that at an exposure level of up to 9,500 mg/m^3 (5,000 ppm) for 8 hours whilst "sitting awake" (ventilation rate 9 l/min) ethanol metabolism was not saturated and the liver was able to metabolize the ethanol at the rate at which it entered the body.

Table 5.1 Ethanol blood levels at end of simulated exposures using PBPK model of Conolly *et al.*, 1999.

Case	Exposure		Exposure duration	Worker at rest[a]		Light exercise[b]	
				CVM[c]	AUC[d]	CVM[c]	AUC[d]
	(mg/m^3)	(ppm)	(hr)	mM (mg/l)	(mM-hr)	(mM) (mg/l)	(mM-hr)
1	950	500	8	0.045 (2)	0.342	0.060 (3)	0.470
2	1,900	1,000	8	0.091 (4)	0.688	0.126 (6)	0.985
3	9,500	5,000	8	0.496 (20)	3.72	4.82 (20)	24.1
4	38,000	20,000	8	18.7 (830)	79.8	97.9 (4600)	410
5	64,000	33,800[e]	8	52.4 (2400)	218	185 (8500)	778
6	20,000	10,614	4	1.54 (70)	4.81	20.7 (950)	43.4

[a] ICRP "sitting awake"' breathing rate for males 0.54 m^3/hr = 540 l/hr = 9 l/min
[b] ICRP "light exercise", breathing rate for males 1.5 m^3/hr = 1,500 l/hr = 25 l/min
[c] Maximal mixed venous concentration (mM)
[d] Area under the curve for the mixed venous concentration (mM-hr)
[e] Lower flammability limit (3.38% by volume - CRC Handbook)

Based on these results, it may be calculated that if a working man uses 10 m^3 of breath per working day at 1900 mg/m^3 (1000 ppm), pulmonary intake (at an absorption efficiency of 60% (see above)) may increase up to 11.4 g of ethanol. This corresponds to drinking a little over 1 standard glass of an alcoholic beverage.

5.1.2 Oral

Ethanol ingestion is rarely instantaneous. Even in research settings, ingestion may be spread out over some time. Therefore, the input model may be better described by a constant rate input process (zero-order) than an instantaneous input. Absorption across the intestinal wall is most likely diffusion-limited and a first order model (concentration dependent) would provide a simple description. A combination of a "constant rate input" model for input and a "diffusion-limited first order" model for diffusion will provide the simplest description of the absorption phase. The model may be further refined by accounting for transfer from the stomach to the small intestine where the bulk of ethanol absorption takes place, by introducing a lag time in the zero-order input model or by introducing a first order model for gastric emptying[56].

Ethanol is absorbed over almost the entire length of the digestive tract. More than 90% of all ethanol consumed is absorbed. Absorption from the mouth and oesophagus is minimal. Ethanol absorption from the stomach may vary between 10 and 30%[57]. The remainder of the ethanol is absorbed from the duodenum and small intestine. Some absorption may also occur in the large intestine.

Saliva and digestive juices may dilute ethanol consumed. Israel *et al.*[58] suggested that dilution is substantial, because he observed that 10%-20% (v/v) ethanol solutions resulted in a maximum ethanol concentration of about 3% (v/v) in the jejunum.

Gastrointestinal absorption is determined by many different factors including ethanol dose and concentration. Also, an efficient blood circulation will maintain the concentration gradient throughout the intestinal mucosa and maintain a high rate of absorption by rapidly removing the ethanol absorbed. The presence or absence of food and its composition may affect ethanol absorption and its subsequent metabolism[59].

Blood alcohol concentration (BAC) and Area Under the Curve (AUC) after oral exposure

Blood alcohol profiles were determined in human volunteers (n = 8) after drinking one or two beers. The BAC_{max} reached after consuming 6 gram ethanol (one bottle of light beer) was 80 mg/l (ranging from 20-110 mg/l). Drinking 20 g ethanol (two bottles 'normal' beer) resulted in a maximum BAC of 320 mg/l (empty stomach) or 240 mg/l (after a meal)[60].

From these BACs the AUCs (Area under the curve, ie BAC * time) were determined in human volunteers (n = 8) after two beers. The AUC after drinking 20 g of ethanol was approximately 150 mg/ml x hour (empty stomach) or 130 mg/ml x hour (after a meal).

Influence of ethanol dose and concentration on blood ethanol concentration

Ethanol is absorbed at a high rate. The quantity of ethanol absorbed per unit of time across the gastric or intestinal mucosa is directly proportional to the concentration gradient between gastric or intestinal lumen, epithelial cells, capillaries and the portal vein. Several studies have shown that increasing doses of ethanol when provided in the same concentration, result in a proportionately higher maximal blood ethanol concentration (or blood alcohol concentration; BAC_{max}), whereas the rate of absorption (represented by the slope of the ascending part of the BAC curve) is not affected[60,61].

Varying the concentration of ethanol consumed appears to have an effect on the slope of the ascending part of the BAC curve. This was shown in experiments varying the concentration of ethanol without changing the dose[62,63] and by experiments lowering the concentration by adsorbing ethanol using active charcoal[64]. This relation is, however, not linear for all concentrations applied in healthy human volunteers. Pikaar *et al.*[61] also showed that relatively high concentrations of ethanol (29,000 mg/l) did not increase the BAC further, probably because these concentrations delay gastric emptying.

Gastric emptying and intestinal absorption

Gastric emptying is an important factor in determining the availability of ethanol to the duodenum and jejunum. Since the absorption is much higher from the

intestines than from the stomach, gastric emptying is relevant to ethanol absorption.

Every physiological or pharmacological factor affecting gastric emptying may modify the absorption rate of ethanol. The presence of food in the stomach is the most important factor in delaying gastric emptying, which prolongs the absorption of lower dosages of ethanol. Delaying ethanol absorption in the intestine, results not only in a lower BAC_{max}, but also in a smaller area under the blood ethanol curve (area-under-the-curve, AUC) compared with drinking on an empty stomach[65]. The amount of ethanol absorbed may be lower, because a larger part of the ethanol may be metabolised during the presence in the stomach and the intestine. Alternatively, the ethanol is absorbed into the blood over a longer period of time, which means that the elimination of ethanol has already set in. Also, the AUC may not reflect the bioavailability of ethanol under these conditions. AUC only accurately reflects bioavailability if entry into the body and exit from it are first-order processes[66]. This however, may not be the case in ethanol metabolism; ethanol elimination proceeds over a wide concentration range as a pseudo zero-order process[56].

Type of food

Foods enriched with proteins have been suggested to be more successful in reducing the maximum BAC as compared to foods enriched with carbohydrate or fat[61]. Fats have often been considered to exert a stronger effect than carbohydrates because of the stronger inhibitory effect of fat on gastric emptying. In a recent experiment, however, fats and carbohydrates did not differ in their effect on BAC_{max}[65]. McFarlane *et al.*[67] show that the delay in ethanol absorption caused by dietary fats exactly paralleled the delay in gastric emptying, there was no direct interaction between the food and the ethanol.

Clarke *et al.*[68] and others studied the effects of various carbohydrates, like fructose, sorbitol and glucose. Carbohydrates lower the BAC, possibly by retention in the intestinal lumen of water and consequently of ethanol. These effects, however, are only observed using extremely high quantities of carbohydrates and may therefore be considered to be physiologically irrelevant.

Some milk and dairy products are considered to be particularly effective in lowering the BAC[69], however others did not confirm this. Presumably, milk taken on an empty stomach has some effect by delaying gastric motility and diluting the ethanol.

In experiments with rats, caffein proves to have a delaying effect on gastric emptying owing to relaxation of the gastric musculature resulting in a lower BAC, particularly when caffeine is administered a quarter of an hour before ethanol was ingested. Overall, the type of food appears to be of little importance, rather the caloric content of the meal may be more important than the type of food from which the calories are derived[70].

Type of alcoholic beverages

The rate of absorption of the three main types of alcoholic beverages (beverages containing ethanol) has been investigated both in a fasted state as well as after eating a meal. Alcoholic beverages consumed on an empty stomach within a short period of time show that the maximum BAC was greatest after spirits and least after beer with the time to reach the peak being shortest with spirits and longest with beer[63]. These results may be explained by the differences in ethanol concentration as well as in differences in volume leading to differences in gastric emptying time.

Similar experiments have been performed drinking alcoholic beverages in combination with a meal. Roine *et al.*[63] described, surprisingly that beer yields a higher BAC_{max} than whiskey. This result is not confirmed by others in a dietary controlled experiment in 11 middle-aged men consuming 40 g of ethanol daily either as beer, wine or spirits with their evening meal using a cross-over design[71]. Dinner consumed with beer or wine yields a lower BAC_{max} (on average 420 mg/l and 440 mg/l, respectively) as compared to dinner with spirits (510 mg/l) after one hour. After three hours spirit consumption still results in a higher BAC_{max} (290 mg/l) as compared to wine consumption (260 mg/l), which is higher as compared to beer consumption (200 mg/l). Similar tendencies are obtained in a short-term dietary controlled experiment[72]. These differences however are small. The amount drunk as well as the presence or absence of food in the stomach is the major determinant of the blood ethanol concentration.

Drugs

Drugs may also alter ethanol absorption, apparently as a result of their effects on gastric motility. These drugs include aspirin, aminopyrine and anticholinergic drugs slowing down absorption whereas cholinergic drugs increase the absorption rate slightly[73].

5.1.3 Dermal

Sixteen healthy volunteers (8 males and 8 females) sprayed test material (aerosol cans containing 44% ethanol next to 55% of hydrocarbon propellant gas) over their entire body for approximately 10 seconds and waited 15 minutes before they dressed. Blood samples were then drawn at 0, 5, 10, 30 and 60 min and analysed together with a blood sample taken before the experiment. The samples were analysed on two different GC columns. In combination these columns could identify ethanol uniquely. With a (high) detection limit of 9 mg/l no ethanol was positively detected in any blood sample[74].

Ethanol penetration has been studied *in vitro* in full thickness skin samples from mice, rats, rabbits and humans. Human samples have been obtained from female breast and male and female abdomen. Dose volumes of 250 µl [^{14}C] ethanol in a 25% (v/v) aqueous solution have been applied in specific chambers on 1.77 cm^2 skin (approximately 28 mg ethanol/cm^2). Sealing the chambers with a glass stopper prevented evaporative losses. Exposure lasted for 6 hours during which effluent samples were collected at various intervals. The fraction of ^{14}C that penetrated the skin was determined from the sum of effluent radioactivity divided by the total amount of radioactivity initially applied. In total 175 experiments were performed over 6 years. Statistically comparisons of permeability constants (k_p) reveals that the order of skin permeability is rabbit>mouse>rathuman. In humans, the permeability constant amounts to $3.4*10^{-3}$ cm/h. No differences in k_p were observed between the different sites of human skin and between males and females. The penetration rate at steady state for human skin was calculated to be 0.67 mg/cm^2/h. For human skin a total mean of 9.9% of the applied dose was recovered from the effluent[75]. However, it is noted that the amount of ethanol remaining in the skin was not determined. The absorption percentage may therefore be underestimated, although Pendlington *et al.* reports relatively low amounts of ethanol in the skin after 24 hours of exposure[74].

Scott *et al.*[76] reported 10 times lower k_p values ($3.17*10^{-4}$ cm/h) for human abdominal skin than Beskitt and Sun[75]. Skin samples were mounted in glass diffusion chambers that consisted of an open-top donor chamber and receptor chamber (volume ~ 5 ml). Regular samples of fluid in the receptor chamber were collected during a 6-hour exposure period. However, from their report the applied dose is not clear, but may possibly be >100 µl/cm^2 of a 1 mg/ml aqueous solution of [^{14}C] ethanol. In that case, it might be questioned whether the dose

was high enough for the assessment the k_p. Hence the validity of the reported k_p is doubted.

Dermal absorption has also been studied *in vitro* with full thickness pig skin with and without occlusion (using parafilm). A dose of 13 µl/cm^2 (10 mg/cm^2) of [^{14}C] ethanol has been applied to the skin for 24 hours. The initial flux rate under occlusion was over ten times greater than the flux rate for non-occluded skin. The maximum flux for the non-occluded skin was reached by the first hour, whereas the flux rate (maximal 0.25 mg/cm^2/h) for the occluded skin peaked at two hours. The recovery from the receptor fluid was 21.17% and 0.97% for occluded and non-occluded skin, respectively. Analyses of upper skin washes, skin digests and cell washes revealed only small amounts of radio-activity. The total recovery was 40% for occluded skin (of which 13.4% was found in the occlusion material) and 2.2% for non-occluded skin. The relatively low recovery was considered to be due to evaporation of ethanol. Evaporation tests revealed half-lives of evaporation ranging from 11.7 seconds for whole pig skin to 24.8 seconds for glass[74].

The penetration of ethanol through full thickness guinea-pig skin has been studied without and with occlusion (parafilm, gel bound or a sealed chamber with a minimal air space) for an exposure period of 19 hours. Volumes of 25 to 500 µl of [^{14}C] ethanol were applied to 5 cm^2 non-occluded skin. Under occlusive conditions 100 µl (approximately 16 mg/cm^2) was applied. Skin penetration was determined by analyses of ^{14}C in the saline receptor fluid. The percentages recovered in the receptor fluid were 0.94, 0.38 and 0.29% for an initial applied dose (non-occlusion) of 50, 100 and 200 µl, respectively, leading to approximately comparable absolute amounts recovered. The results for the dose of 500 µl were unreliable. Under occlusive conditions the percentages recovered in the receptor fluid were 8.1, 23.4 and 27.1% for occlusion with parafilm, gel bound and sealed chamber, respectively. Peak penetrations were observed at approximately 3 hours for occlusion under parafilm, 14 hours for occlusion with gel bound and 18 hours for the sealed chamber. A significant loss of 24% was found when ethanol in saline was pumped through the experimental apparatus with the exception of the diffusion cells, indicating that the amount of ethanol in the effluent is an underestimation[77]. Further, it is noted that the amount of ethanol present in the skin after 19 hours was not determined. This may have led to a further underestimation of the absorbed dose, although Pendlington *et al.* reported limited amounts of ethanol remaining in the skin after 24 hours of exposure[74].

From the above-mentioned studies it is difficult to derive a reliable estimate for dermal absorption of ethanol. The studies differ in concentrations and dose per cm^2 applied as well as exposure duration. Further, often only the amount of ethanol in the receptor effluent was determined. Due to the high evaporation rate of ethanol, a significant amount of the absorbed ethanol may be evaporated before analyses. An absorption percentage cannot be used for the evaluation of the applicability of a skin notation. The absorption percentage is dependent on the specific conditions of exposure and not applicable to other exposure conditions[78]. Beskitt and Sun[75] estimated that their exposure was under "infinite conditions" whereas Pendlington *et al.*[74] observed a decrease in penetration rate after 2 hours. Further, the type of occlusion may also influence the absorption[77] which may explain the higher absorption rate found by Beskitt and Sun[75].

As a worst case estimate (because the experiment was performed under occlusion) the penetration rate of 0.67 $mg/cm^2/h$ calculated by Beskitt and Sun[75] can be used to calculate the internal dose after dermal exposure; a 25% aqueous solution of ethanol was used.

5.1.4 Concluding remarks

Inhalatory absorption of ethanol is relatively low, *i.e.* about 60%. It may be calculated that if a working man uses 10 m^3 of breath per working day at 1900 mg/m^3 (1,000 ppm), pulmonary intake (at an absorption efficiency of 60% will approximately be 11.4 g of ethanol. This corresponds to drinking a little over 1 standard glass of an alcoholic beverage. These intakes correspond well to the low blood ethanol concentrations observed in the few studies performed.

Most (more than 90%) of the orally ingested ethanol is absorbed at a high rate. Ethanol dose and concentration have a direct and proportional effect on the blood alcohol concentration. The presence of food in the stomach, rather than the type of food or the type of alcoholic beverage, is the most important factor decreasing blood alcohol concentrations, because the presence of food significantly decreases gastric emptying.

From *in vitro* studies, the dermal absorption rate is assumed to be 0.67 $mg/cm^2/hr$ (worst case estimate).

5.2 Distribution

5.2.1 Equilibration between blood and tissues

Once ethanol has been absorbed into the bloodstream it is rapidly transported throughout the body and distributed over the total body water. During the initial absorption phase the arterial BAC is higher than the venous BAC, peaking at a higher level and requiring less time to reach the peak. After an arterial-venous equilibrium is reached, the venous BAC remains above the arterial BAC[79]. Equilibrium is reached about 1-1.5 hr after drinking. This will occur sooner in organs highly perfused with blood (brain, lungs, kidneys, liver) than in other organs. It will take a relatively long time for this equilibrium to be reached in inactive skeletal muscular tissue because of the poor perfusion of this tissue. Body fat and skeletal mass absorb hardly any ethanol. Once the equilibrium has been reached the amount of ethanol in the organs will be proportional to the organs' water content. Several studies in humans have confirmed this with great precision[73]. Plasma and serum, which have virtually the same water content, had identical concentrations of ethanol. The plasma to whole blood ratio of ethanol concentration is about 1.12. This could be predicted by the water contents of plasma (about 92%) and whole blood (about 80%).

5.2.2 Volume of distribution: effects of gender and age

It has been clearly demonstrated that the same dose of ethanol per unit of body weight produces widely differing blood ethanol concentrations in different individuals. The reason for such variability originates amongst others from the large intra-individual variations in proportions of body water and body fat. Because of the very low lipid to water partition coefficient of ethanol, the volume of distribution of ethanol in the body is equivalent to the total body water.

On average, standard women have less body water per kilogram of body weight (500 ml/kg) as compared to standard men (600 ml/kg). Consequently, the same quantity of ethanol per kilogram body weight results in a higher BAC in a standard woman (body weight of 60 kg) than in standard men (body weight of 70 kg). In addition, the differences in BAC between men and women are even larger given a fixed dose of ethanol, because women have on average lower body weights than men do. The combination of the higher body weight with a higher proportion of body water in man results in an appreciably larger amount of body water over which the ethanol can be distributed in men.

Goist and Sutker[80] showed that women had higher maximal blood ethanol concentrations than men when they received the same dose of ethanol in grammes per kilogram body weight, but maximal BAC did not differ between women and men when they received the same dose per litre of body water. Watson *et al.*[81] also calculated that the difference between mean observed BAC and mean predicted BAC on the basis of the dose per kilogram body weight in both men and women disappeared when the dosage was corrected for total body water. It is unlikely that gender differences in ethanol toxicokinetics or ethanol-induced performance impairment may be caused by the menstrual cycle and variations in female sex hormones[82,83].

Ageing also plays an important role in determining the BAC by a given dose of ethanol. Jones and Neri[84] found a substantial change of body water from 61% of body weight in healthy men aged 20-29 years to 54% in those aged 50-59 years. This decrease accounted for a major part of the age-related difference in maximal BAC after the same oral dose of ethanol in grams per kilogram.

5.3 Biotransformation

Ethanol is metabolized in several steps (Figure 5.2): it is oxidized first to acetaldehyde. Acetaldehyde is a toxic substance held to be partly responsible for the harmful effects of excessive ethanol intake. Acetaldehyde is subsequently converted into acetate. Most of the acetate is oxidized completely to CO_2 and H_2O.

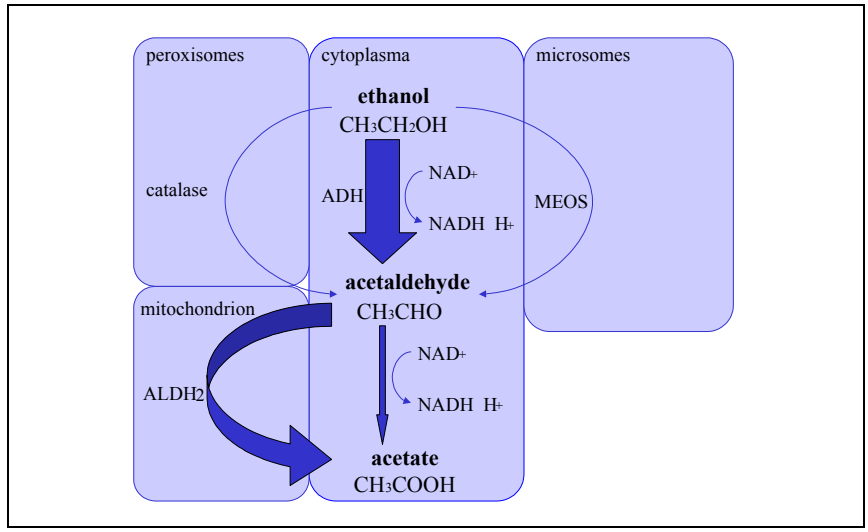


Figure 5.2 Oxidation of ethanol in the hepatocyte.

A small proportion of acetate is incorporated in body tissues as carbohydrates, proteins or lipids. These reactions take place primarily in the liver[85,86].

Although these fundamental pathways for ethanol metabolism are common to all human beings, the enzymes involved can take different forms – so-called isozymes – which result from the substitution of one or more amino acids in the polypeptide chain. The isozymes have different catalytic characteristics which accounts for individual variation in rate of ethanol metabolism.

The maximal amount of ethanol that can be transformed by the human body per hour is estimated in the range of 100-300 mg per kg per hour. This is usually translated into 6-9 g of ethanol per hour for a healthy subject. Considerable inter-individual variations in ethanol metabolism rate have been reported[87]. Both environmental and genetic factors influence the rate of ethanol degradation, like gender and race (Table 5.2). Twin studies indicate that interindividual variability in the rate of ethanol metabolism is under genetic control, a striking similarity in ethanol metabolic rate was observed in identical twins with much greater variability between fraternal twins[88,89].

Table 5.2 Comparative rate of ethanol metabolism in different ethnic and racial groups[90].

Ethnic group	Number of subjects	Metabolic rate ($mg.kg^{-1}.h^{-1}$)
Caucasians		
Europeans	19	86
Europeans	16	108
Europeans	23	108
Europeans	6	103
North Americans	30	93
North Americans	37	104
Canadians	68	108
North Americans	17	112
North Americans	17	145
Hindu Reddis	35	123
Mongoloids		
Chinese	15	137
Chinese	39	127
Mongoloids (mixed)	24	146
Japanese	47	134
Japanese	68	119-138
Native Americans		
Canadian Indians	26	101
North American Indians	30	92
Ojibwa Indians	12	183
North American Indians	17	123
Canadian Eskimos	21	110

5.3.1 *Conversion of ethanol into acetaldehyde*

Three enzyme systems are known to catalyse the conversion of ethanol into acetaldehyde, which differ in intracellular localization:

1 alcohol dehydrogenase (ADH), localised in the cytosol
2 cytochrome P450 oxidizing system (or microsomal ethanol-oxiding system (MEOS)), localised in the endoplasmatic reticulum
3 catalase, localised in the peroxisomes.

The main reaction is the one through ADH. The other two enzyme systems do not seem to play an important part quantitatively, although it has not been established to what extent they exactly contribute to the process (Figure 5.1)[85]. However, these routes may contribute to the toxic effects of ethanol.

ADH-dependent reaction

Human alcohol dehydrogenase (ADH, alcohol:NAD oxidoreductase, EC 1.1.1.1) is a zinc-containing enzyme located almost exclusively in the cytosol of cells. The highest ADH concentrations (approximately 80-90% of the total ADH activity in human tissue) have been found in the liver. ADH activity has also been detected in other tissues, such as the gut, kidneys and lungs.

For the oxidation of ethanol to acetaldehyde catalyzed by ADH, oxidized nicotinamide adenine dinucleotide (NAD+) is needed and NADH is produced in the cytosol. This results in an increased NADH/NAD ratio in the cytosol, with a marked shift in the redox potential[91].

NADH can be reoxidized to NAD+ in the cytosol by reducing other intermediates in metabolism. However, at high ethanol concentration this route can be saturated. In particular pyruvate is reduced to lactate and oxaloacetate is reduced to malate. In addition, NADH and the reduced compounds accumulate in the cytoplasm, inhibiting gluconeogenesis and depressing the activity of the citric acid cycle (Krebs cycle) in the liver. The accumulation of lactate results in hyperlactacidemia, which can cause hyperuricaemia because lactate and urate share the same mechanism for renal tubular excretion[92].

Excess NADH may promote fatty acid synthesis. The increased NADH/NAD ratio raises the concentration of glycerophosphate which favors hepatic triglyceride accumulation by trapping fatty acids. Fatty acids of different sources can accumulate in the liver because of different metabolic disturbances and because of decreased fatty acid oxidation[85].

ADH has a low substrate specificity: it converts not only ethanol, but also other alcohols. ADH is not induced by chronic ethanol consumption.

Human ADH exhibits different molecular forms that have been grouped in classes (for reviews see:[86,93]). Human liver ADH exists in multiple molecular forms which arise from the association of different types of subunits each of molecular weight of 40000 Da into active dimeric molecules. A genetic model accounts for this multiplicity as products of seven loci (ADH1-ADH7) have been described[94]. Polymorphisms occur at 2 loci, ADH2 and ADH3, which encode the β- and γ-subunit. These classes differ from each other in more than 30% of their amino acid sequence and exhibit distinct kinetic properties and specific tissue distribution.

Class I ADH is the classical liver enzyme. Class I is composed of isozymes consisting of α, β, and γ subunits; encoded by the gene loci ADH1, ADH2, ADH3, respectively. These α, β, and γ subunits can associate with one another to form both homodimers and heterodimers.

The ADH2 locus shows three alleles in the human population that encode for β1, β2 and β3 subunits. In the Caucasian population the β1 subunit is predominant. Individuals with β2 subunits represent less than 20% of Caucasians but more than 80% of Japanese and Chinese. Isozymes with β3 subunits are found in 25% of African-Americans but were not detected in other populations. Genetic polymorphism also occurs in the ADH3 locus. The γ1 and γ2 subunits appear with about equal frequency in Caucasians, American and Asian Indians. The γ1 subunit predominates to about 90% over the γ2 subunit in the Asian and African-American population[95].

Class I isozymes are mostly localized in the liver, but several isozymes are also present in the gastrointestinal tract (stomach, small and large intestine) and the kidney. Isozymes with ß subunits are detected in low amount in the digestive tract muscle layers but not in the mucosa. The gastrointestinal mucosa characteristically contains subunits encoded by ADH3. Most of the class I isozymes, with the exception of β3 homodimers, show a low Km for ethanol (0.05 to 5 mM at pH 7).

Class II consists of only one form with π subunits, encoded by the ADH4 gene. It has been described in the liver but not in the gastrointestinal tract and exhibits a high Km for ethanol (Km=34 mM)[96].

Class III is formed by PP subunits encoded by ADH5. It is present in the liver and in the entire gastrointestinal tract. It exhibits a very low activity with ethanol because it cannot be saturated by this substrate. It only contributes to ethanol metabolism at rather high ethanol concentrations. It shows the best kinetic constants as a glutathione dependent formaldehyde dehydrogenase, suggesting that the elimination of formaldehyde is the main physiological function of class III ADH[97].

Class IV ADH was initially described in the stomach mucosa[98], consisting of a homodimer with σ subunits encoded by ADH7 (or ADH6 according to Jörnvall and Höög[93]). It has not been detected in the liver. The Km for ethanol of σσ-ADH is high (37 mM at pH 7.5). It is especially specific for medium and long-chain alcohols. It is predominantly located in the upper gastrointestinal tract, mouth, oesophagus and stomach and represents a metabolic barrier to external alcohol and aldehydes. Individuals exhibiting σ-ADH in the stomach have a higher total ADH activity than those who do not. Ethnic differences in the expression of class IV ADH have been described. A high percentage of Asians lack class IV ADH in the stomach.

A functional new ADH gene, designated ADH6, has been characterized[99]. Both liver and stomach from a Japanese adult female contained the ADH6 mRNA. *In vitro* translation produced a protein with kinetic properties similar to those of human class IV ADH. However, the amino sequence identity is only 60% and therefore ADH6 represents a new class, designated class V ADH. As no class V ADH protein has been detected in either liver or stomach the metabolic significance is not known.

Recently a new gene (ADH8), representing class VI ADH, has been characterized in deer-mouse and rat only. Little is known about the further distribution, but from class separation known to be likely in human, too[100].

Cytochrome P450 oxidizing system (Microsomal ethanol-oxidizing system)

Ethanol may also be oxidized by a cytochrome P450 dependent enzyme system (CYP2E1) in the microsomes of the liver[85], the so called microsomal ethanol-oxidizing system (MEOS). This conversion of ethanol to acetaldehyde needs NADPH and O_2 (Figure 5.1). CYP2E1 has a much higher Km value for ethanol (8-10 mM) as ADH, which means that CYP2E1 only converts ethanol at rela-

tively high blood ethanol concentrations. In this process, the superoxide anion can be formed.

Chronic ethanol consumption results in induction of the human cytochrome P450 2E1 isozyme in MEOS and thereby increasing the contribution of this enzyme to ethanol metabolism. This induction may be responsible for the metabolic tolerance to ethanol in alcoholics. In addition, alcoholics tend to display tolerance to various other drugs. Other microsomal drug-metabolizing enzymes in the liver share many properties with MEOS, including utilization of cytochrome P450, NADPH and O_2. The induction of cytochrome P450 by ethanol consumption may increase the activity of these drug-metabolizing activities in the liver. This could, at least in part, explain the enhanced rate of drug clearance *in vivo* after chronic ethanol consumption[85]. The ethanol dose appears to be important, because induction may occur via two steps: a post-translational mechanism at low ethanol concentrations and an additional transcriptional one at high ethanol levels[101,102].

P450 2E1 has a high capacity of metabolizing ethanol, but also other aliphatic alcohols as well as a number of hepatotoxic agents. It can be induced by various compounds such as acetone, pyrazole and benzene[103]. Respiratory nasal epithelium of humans contained, relative to liver, a low amount of cytochrome P450 and associated biotransformation activities, and a low level of other components of the MEOS[104].

Catalase dependent reactions

The third pathway to convert ethanol into acetaldehyde is by means of catalase enzymes present in the peroxisomes of the liver (Figure 5.1). As early as 1936, Keilin and coworkers (see[105]) pointed out that under certain conditions peroxidation of ethanol might take place. The peroxide needed is generated by NADPH or hypoxanthine oxidation under the influence of oxidases[106] or by β-oxidation of fatty acids such as octanoate, palmitate and oleate in peroxisomes[107]. The formation of H_2O_2 is considered to be the rate-limiting factor.

Some authors mention values of up to 25% for the extent to which catalase accounts for ethanol conversion[107]. However, under physiological conditions, catalase appears to play no major role[85].

The contribution of acetaldehyde to the pathological consequences of chronic ethanol intake is well established, for instance for different forms of cancer in the digestive tract and the upper airways[108]. Acetaldehyde may play a role in other pathological consequences of ethanol abuse including alcoholic liver disease. Evidence suggests an important role of acetaldehyde during acute intoxication causing ”alcohol sensitivity”, which is vasodilatation associated with increased skin temperature, subjective feelings of hotness and facial flushing, increased heart and respiration rate, lowered blood pressure, sensation of dry mouth or throat associated with bronchoconstriction and allergy reactions, nausea and headache, and euphoria. The individual variability in these actions depends on the genetic polymorphisms for the metabolic enzymes involved[108].

However, it is difficult to detect acetaldehyde in human blood samples even during intoxication[109,110]. Nevertheless, a number of reports on human blood acetaldehyde concentrations have been published, but these levels may be explained as artefacts, reconfirming the findings that acetaldehyde concentrations are below detection, *i.e.* <0.5 μM.

Acetaldehyde’s toxicity is, in part, due to its capacity to form protein adducts, resulting in enzyme inactivation, antibody production and decreased DNA repair. Metabolism of acetaldehyde via xanthine oxidase or aldehyde oxidase may generate free radicals, but the concentration of acetaldehyde required is much too high for this mechanism to be of significance *in vivo*. However, acetaldehyde may promote lipid-peroxidation and free-radical mediated toxicity via GSH depletion[85].

5.3.2 Conversion of acetaldehyde into acetate

During the second step in the oxidation process the acetaldehyde generated is converted into acetate by the NAD-dependent enzyme acetaldehyde dehydrogenase (ALDH). Almost all other tissues also contain ALDH; but extrahepatic activity is low.

ALDHs are involved in the detoxification of ethanol-derived acetaldehyde. They could play a role in the conversion of aldehyde intermediates formed during the metabolism of corticosteroids, amino acids, biogenic amines, retinoids, and products of lipid peroxidation[86].

Mammalian ALDHs have been grouped in different classes, based on their structural properties, subcellular localisation, and tissue distribution. Human class 1

and class 2 ALDHs show 68% sequence identity, human class 3 ALDH shares only 35% sequence identity with class 1 or class 2 ALDH. In the human liver at least four different isozymes are described which differ in structural and functional properties. ALDH2 is located in the mitochondria, the other ALDH isozymes in the cytosol[86].

Class 1 ALDH (ALDH1) is represented by the tetrameric enzymes located in the cytosol and is found mainly in the liver but also in both the mucosa and muscular layer of most gastrointestinal tissues. It has a low Km for acetaldehyde, *i.e.* 50μM with a range of range 22 – 483 (amongst others Rashkovetsky *et al.*[111]) and theoretically could contribute to acetaldehyde elimination when class 2 ALDH is not present, although less efficiently than the class 2 enzyme.

Class 2 ALDH (ALDH2) includes the tetrameric forms located in the mitochondria. They are most abundant in the liver but expressed in many other organs. They are present in the stomach and the intestine but barely detectable in the oesophagus. Class 2 ALDH has a very low Km for acetaldehyde (Km 1 μM) with a range of <0.1 to 9 μM, which allows for efficient removal of this component during ethanol consumption.

Class 3 ALDH refers to the dimeric, cytosolic forms, constitutively expressed in stomach, esophagus, saliva, gingiva, lung, cornea and to a much lesser extent, in liver. It is absent or expressed at very low levels in duodenum, jejunum, ileum and colon. Both stomach and cornea enzymes appear to be identical. Class 3 ALDH has been estimated to account for more than 80% of the ALDH activity, measured with a high concentration of aldehyde, in human gastric mucosa. Because of its high Km value for acetaldehyde (Km 88 mM) however, class 3 ALDH contributes little to the elimination of acetaldehyde.

There appear to be interindividual differences in subcellular distribution and amounts of high- and low-Km forms of ALDH in the liver[95]. Caucasians have two active ALDH isozymes: ALDH1 and ALDH2. Many Asians (Mongoloids), about 30-50%, do have an atypical inactive form of ALDH2. The inactive ALDH2 form results in higher acetaldehyde levels in the blood upon ethanol consumption. These high acetaldehyde concentrations are considered as the main cause of the flushing syndrome, manifesting itself in facial flushing, cardiac arrhythmia, headache and vomiting[112]. This reactions are comparable with the reactions caused by disulfiram, an inhibitor of ALDH (Antabuse-reaction). Conceivably, impaired acetaldehyde metabolism could exacerbate the toxic effects of locally generated acetaldehyde.

Several investigations have demonstrated that the ALDH2*2 allele substantially increases the risk for cancers almost throughout the digestive tract as well as cancers in the upper airway tracts[108].

5.3.3 Conversion of acetate

Normally acetate is oxidized in the citric acid cycle, after activation to acetyl-coenzyme A, which is a metabolite central to the whole metabolism. However, the acetate formed in the liver after oxidation of ethanol cannot be oxidized in the citric acid cycle in the liver mitochondria, because of the prevailing high ratio of NADH/NAD+ (see before). Consequently, most of the acetate is released into the circulation and oxidized extrahepatically to CO_2 and H_2O. A small portion of the acetate generated from ethanol is incorporated in tissue components by anabolic reactions.

Normally the endogenous acetate levels are rather low (0.1-0.3 mM). A moderate dose of ethanol can temporarily increase the circulation concentrations of acetate to 0.4 to 0.6 mM[113].

There is a high correlation between the rate of ethanol elimination and the blood acetate concentration. In alcoholics with an elevated rate of ethanol elimination, the acetate concentration in both the hepatic vein and peripheral veins is higher than in controls.

The higher blood acetate levels in alcoholics may have consequences for the heart and the liver, because acetate interferes with lipid metabolism and is the preferred substrate over glucose and lipids in the myocardium[114].

5.3.4 Alternative pathways for ethanol metabolism

When the oxidizing abilities of ADH and ALDH isoenzymes are saturated, the normal metabolic pathways of ethanol may be diverted to a variety of other pathways. The relative importance of the alternate metabolic processes is probably determined by the dose and the duration of ethanol consumption. Alternative pathways for ethanol metabolism contribute to the increased xenobiotic toxicity and carcinogenicity in alcoholics.

A non-oxidative pathway of ethanol metabolism to form fatty acid ethyl esters has been proposed[115]. Acutely intoxicated subjects, had concentrations of fatty acid ethyl esters that were significantly higher than in controls. The reaction is catalyzed by fatty acid ester synthase, which is found primarily in brain, pancreas and heart. Fatty acid ethyl esters may have a role in the production of etha-

nol-induced injury in these organs. The capacity of ethanol to form ethyl esters *in vivo* has been shown[116-118].

As already described, chronic ethanol ingestion may increase the amount of human cytochrome P450 IIE isoenzyme and thereby increase the contribution of this enzyme to ethanol oxidation[85]. The oxidizing system of cytochrome P450 has the potential to generate molecules known as free radicals, which cause tissue injury by inactivation of enzymes and peroxidation of lipids. In addition ethanol inhibits the synthesis of reduced glutathione (GSH), a scavenger of toxic free radicals.

5.3.5 *Elimination by first-pass metabolism*

Studies in human subjects and rats have shown that under certain conditions intravenous administration of a low dose of ethanol results in appreciably higher blood ethanol concentrations than the oral intake of the same amount of ethanol does. This may mean that part of the ingested ethanol has been metabolized before reaching the peripheral blood, because absorption of ethanol from the gastrointestinal is virtually complete. This part of the metabolism is called first pass metabolism and can theoretically occur in liver, stomach or intestines.

Because of the high amount of ethanol metabolising enzymes in the liver, the liver will be the main organ for ethanol metabolism.

There are several lines of evidence that the stomach contributes in first pass metabolism of ethanol as well. Firstly, in gastrectomised patients, blood ethanol concentrations were approximately the same after oral intake and after intravenous infusion of ethanol. In healthy men, intraduodenal infusion of ethanol resulted in significant higher blood ethanol concentrations than normal oral intake of ethanol, which also suggests that bypassing the stomach diminishes first pass metabolism[119].

Secondly, as described before, several isozymes of alcohol dehydrogenase are present in the stomach, with e.g. Japanese exhibiting lower σ-ADH activity than Caucasians, with no difference in the other gastric enzymes. In keeping with this, first pass metabolism was strikingly lower after the intake of a 5% ethanol solution in Japanese than in Caucasians[120]. Frezza *et al.*[121], reported that the activity of stomach ADH was lower in women as compared to men. Other studies, however, showed that gastric ADH activity was not significantly different between men and women[98] or differed only significantly below the age of 50 years[122,123]. So, the relation between gastric ADH activity and gender differences in first pass metabolism is not very consistent.

Although there is some evidence for first pass metabolism to occur in the mucosa of the stomach, the contribution of first pass metabolism to the metabolism of ethanol is still a matter of dispute.

Suggestions for the contribution of first pass metabolism to the total ethanol metabolism vary from 1 to 20%, depending on conditions used in the studies[124].

A randomized cross-over study using concomitant administration of unlabeled and deuterium-labeled ethanol through two different routes (intravenous/oral and intravenous/introduodenal) showed that drinking a moderate dose of ethanol (0.3 g/kg) after a light lunch total first pass metabolism was about 9% and gastric first pass metabolism about 6% without significant differences in men and women[125].

When first pass metabolism is extrapolated from *in vitro* data, its contribution to overall ethanol metabolism is rather small, 1% or less[98]. Parés and Farés[86] calculated that a human stomach of 150 g, assumed to contain 50 g mucosa, will metabolize about 0.9 mg ethanol/minute. This corresponds to approximately 1% of the hepatic activity. Extrapolation of *in vitro* data to the *in vivo* situation, however, is difficult.

Faster or slower gastric emptying can increase or decrease the area under the curve (AUC)[70]. Differences in AUCs measured may have been influenced by differences between the input rate of ethanol in the portal vein and the systemic circulation[126,127,125].

In a later paper, a two-compartment model was applied that accounts for the fall in ethanol concentration that may occur as blood traverses the liver. This two-compartment model predicts that near-complete saturation will occur more abruptly and at a lower blood ethanol concentration (approx. 3 mM) than is the case with the one compartment model. The two compartment model predicts a near constant ethanol elimination rate for blood ethanol concentrations above 3 mM, whereas the one compartment model predicts an increasing elimination rate over a large range of concentrations. The two-compartment model also predicts that first-pass metabolism is extremely sensitive to the rate of ethanol absorption. When absorption was slowed by food ingestion, first pass metabolism accounts for about 50% and 10% of ethanol dosages of 0.15 g/kg and 0.3 g/kg, respectively. When ingested without food, there is negligible first-pass metabolism of even very small dosages (0.15 g/kg). The authors conclude that first-pass metabolism is an unimportant determinant of the blood ethanol response to ingestion of potentially inebriating doses of ethanol[128]. This means that results based on comparisons of AUCs after different routes of administration should be interpreted with care.

The role of first pass metabolism after inhalatory exposure is, however, less relevant.

5.4 Elimination (excretion)

Only a small proportion of ethanol is excreted with urine, breath, breast milk, sweat and tears. Practically all ethanol is eliminated from the body by oxidation via various enzyme systems to CO_2 and H_2O. Generally speaking: 90-98% is oxidized, 1-5% is excreted via the lungs by expiration, and 1-3% is excreted via the other routes, like urine (0.5- 2.0%) and sweat (up to 0.5%)[73].

5.4.1 Pulmonary excretion

The total amount of ethanol lost from the body via breath is small, usually less than 5%. Ethanol diffuses readily from the pulmonary arterial blood into the alveolar air. In principle, ethanol vapor in the breath is in equilibrium with ethanol dissolved in the water of the blood. This equilibrium is expressed as a partition coefficient with a commonly cited average value of 2100 to 1 for blood-to-pure alveolar air. This coefficient, *i.e.* 2100 ml of breath contains as much ethanol as 1 ml of blood in equilibrium, may be used to estimate BAC from the breath measurement. The process is the basis of the well-known breath analyzer tests for estimating BAC.

Gullberg[129] analysed series of simultaneous blood and breath measurements in the literature and observed a skewed distribution with a modal value of about 2200 to 1 and a geometrical mean of about 2300 to 1. The range of distribution observed was wide, however, it ranged from 1700 to 3500. Among other reasons this is caused by the phase in the true BAC curve at which the breath measurements are made. In healthy human subjects, the apparent coefficient was 2130 $\pm$ 150 during the rising phase, 2260 $\pm$ 120 at the peak and 2340 $\pm$ 190 in the descending phase of the curve[130]. Large variation may result from hyperventilation or hypoventilation resulting in disequilibria between alveolar air and blood. Nevertheless, breath measurements give a good approximation of the true BAC, even in routine police use[131].

5.4.2 Urinary excretion

Theoretically, ethanol will appear in the glomerular filtrate at the same concentration as in the water of the blood at the time that the filtrate is being formed in the kidney. In experiments in humans, when capillary blood samples were taken

at the same time as hourly pooled bladder urine, the urine-to-blood ethanol ratio varied with the phase of the BAC curve. The ethanol concentration in blood was higher than in urine during the ascending phase of the BAC curve, whereas the ethanol concentration in blood was lower than in urine during the descending phase of the BAC curve[132].

5.4.3 Elimination by other body fluids and secretions

Ethanol appears in the tears in a concentration proportional to the water content of the lachrymal fluid[133]. When the fluid on the conjunctival surface is exposed to air, ethanol partitions between the fluid and the air, just as it does in the alveoli of the lung. Sweat also contains ethanol in proportion to its water content. The saliva ethanol level is shown to be closely correlated with those in blood and breath[134]. Breast milk of nursing mothers contains ethanol at levels comparable to the corresponding blood samples after ethanol ingestion[135].

5.5 Summary and evaluation

Only limited data are available concerning the kinetics of inhalatory intake of ethanol. Inhalatory absorption of ethanol by the lungs is relatively low, *i.e.* about 60%. Physical activity will increase inhalatory absorption. It is expected that inhaled ethanol will be metabolized by the liver mainly.

There are no data available with a direct comparison of the blood ethanol concentrations after oral or inhalatory ethanol exposure. However, high concentration of ethanol vapours are needed to reach detectable blood ethanol concentrations in humans; for example, inhalatory exposure to 1900 mg/m^3 for 4 hours results in a maximal blood ethanol concentration of approximately 2 mg/l. In comparison, (oral) ethanol consumption (~20 gram) results in BAC_{max} of approximately 300 mg/l in social drinking. In addition, Sprung *et al.* measured a blood alcohol concentration (BAC) in man due to endogenously present ethanol of 0.27 mg/l[19]. The blood alcohol concentration is a highly dynamic parameter, e.g. under social drinking conditions (10-30 g of ethanol per day), blood alcohol concentration will peak during the first hour and after several hours no ethanol will be detectable. This is in contrast with the blood alcohol concentration after inhalatory exposure, which is constant during the exposure.

Data on acetaldehyde pharmacokinetics after inhalatory and oral exposure are scarce, because the analysis of this metabolite is difficult and the acetaldehyde concentration in human blood is low.

Most data concerning the ethanol kinetics are based on oral intake of alcoholic beverages. The presence of food in the stomach, rather than the type of food or the type of alcoholic beverage, is the most important factor for the bioavailability of ethanol, because the presence of food significantly decreases gastric emptying. Most (more than 90%) of the orally ingested ethanol is absorbed at a high rate. After absorption, ethanol will pass the liver first before it will reach the general circulation. The significance of first pass metabolism in the liver, stomach and intestines is debated and considered of minor relevance.

Dose and concentrations of ethanol in air or drinks do have a direct and proportional effect on the blood ethanol concentration. The (maximal) blood ethanol concentration will depend on several factors, like gender, age and genetic predisposition. Genetic predisposition plays a role because many isoenzymes and polymorphisms exist in ethanol metabolising enzymes, which result in different metabolic rates between racial and ethnic groups on the one hand and individuals on the other. Overall, many different ethanol metabolizing enzyme systems exist which result in a large ethanol elimination capacity. A healthy subject is considered to eliminate between 6 and 9 g of pure ethanol per hour.

Dermal absorption is difficult to estimate. As a worst case (experiments were performed under occlusion) estimate the penetration rate of 0.7 $mg/cm^2/h$ can be used to calculate the internal dose after dermal exposure.

Chapter 6

Effects in man

6.1 Irritation and sensitisation

Inhalation studies in humans indicate that ethanol causes concentration-related irritation of the mucous membranes (Table 6.1). The odour threshold is ~95 mg/m^3 (50 ppm; from graph) for a 1-3 seconds exposure to ethanol, while after this short exposure period eye irritation was reported by subjects at approximately 95,000 mg/m^3 (50,000 ppm; read from graph). In this study, both eye irritation and odour thresholds were measured in a two-alternative, forced-choice procedure in which subjects had to choose the stronger of two stimuli, one being blank, until the participant got five correct choices of the same concentration step in a row. The latter concentration was taken as the threshold[13].

No adverse effects on well being (rated on a 7 points scale comprising tension, tiredness, complaints, and annoyance) were (self)reported by 16 subjects, experimentally exposed for 4 hours to constant ethanol air concentrations of 150 mg/m^3 (80 ppm), 750 mg/m^3 (400 ppm) or 1,500 mg/m^3 (800 ppm) in an inhalation chamber[52,136]. A 4-hour exposure to 1,900 mg/m^3 (1,000 ppm) had no adverse effect either. In contrast, an average concentration of 1,900 mg/m^3 (1,000 ppm) for 4 hour, resulting from hourly changing levels of either 190/ 3,610/190/3,610 mg/m^3 or 3,610/190/3,610/190 mg/m^3 was characterised as ‘unpleasant’ by the volunteers. Increasing the concentration directly from 0 or 190 to 3,610 mg/m^3 caused temporary irritation. The female subjects gave a

stronger response than the males[52,136]. Symptoms included tickling in the throat; irritation of the eyes, nose, throat, skin; blurred vision; unpleasant taste and discomfort[52].

Thirty minutes of exposure to ethanol in a concentration of 3,420-3,800 mg/m^3 (1,800 to 2,000 ppm) causes coughing, dry throat and temporary bronchial

Table 6.1 Acute effects of ethanol inhalation exposure in humans.

Concentration (mg/m^3)	n	Time	BAC (mg/l)	Effect	Reference
95	10 (5/sex)	1-3 sec	NR	Odour threshold	13
150	NR[a]	NR	-	Odour threshold	52,136
150, 750, 1500	24 (12/sex)	4 h	0.2-2.1	No effects	52,136
1,900	1	3 h	<2	No effects	51
1,900	24 (12/sex)	4 h	5.6±1.5	No effects	52,136
1,900 (avg) (alternating conc of 190/3610 mg/m^3)	16 (8/sex)	1 h/conc; total 4 h	NR	Tickling in the throat; irritation of the eyes, nose, throat, skin; blurred vision; unpleasant taste and discomfort. Effects were noted after increasing the conc. from 0 or 190 to 3610 mg/m^3	52,136
~2,600	NR	39 min	NR	Strong alcohol smell headache (after 33 min)	138
3420-3800	6 (4 females/ 2 males)	30 min	NR	Transient cough, dry throat	137
855-3800	1 male	15+5+19 min in a period of 2 h	1.3	Effects not reported. Study aimed at determination of BAC after spraying of lacquer under 'relatively arduous conditions'.	140
4,750-6,346	NR	50 min	NR	Strong alcohol odour, tickling in nose, hot and cold flashes, pressure in forehead	138
14,000	1 male	1 h	NR	Feeling of warmth in upper respiratory tract and occasional moderate feeling of difficulty in 'carrying the inspiration through'	45
10,000-20,000	3	3-6 h	20-460 (3 h); 80-480 (6 h)	Transient (5-10 min) coughing and smarting of the eyes and nose	53
16,245-17,366	NR	64 min	NR	Initially almost unendurable, strong alcohol odour, breathing difficulty, burning eyes, hot flashes, pressure in forehead, fatigue	138
17,000	4	165 min	<50	No subjective symptoms of intoxication; intolerable to anyone entering the room unacclimatized.	139
18,000-19,000	1 male	1 h		Marked feeling of warmth in upper respiratory tract and an urge to cough	45
30,000	NR	NR	NR	Continuous lachrymation and marked coughing; tolerable with discomfort	53
40,000	NR	NR	NR	Almost unendurable	53
95,000	10 (5/sex)	1-3 sec	NR	Eye irritation	13

[a] NR: not reported

constriction[137]. In an old study by Loewy and Von der Heide[138], it was described that the irritation increases with the concentration, causing coughing, stinging in the nose and eyes, burning eyes, breathing difficulties, pressure in the forehead and fatigue starting from 4,750-6,346 mg/m^3 (2,500-3,340 ppm). In contrast, Mason and coworkers did not report subjective symptoms of intoxication after exposure to 17,000 mg/m^3 ethanol for almost 3 hours[139]. Also, Kruhøffer[45] reported only a feeling of warmth in the upper throat while inhaling 14 g/m^3 (7,368 ppm) for 1 hr. Since the Loewy and Von der Heide study was published back in 1918, and several more recent studies report no effects in this range, this study will not be taken into consideration.

The ACGIH concluded that no local irritation will occur below 5,000 mg/m^3 (2,650 ppm) air concentration[8]. Levels over 21,280 ppm (40,432 mg/m^3) are suffocating and unendurable[53]. If the subjects are exercising, adverse effects are observed at lower levels than at rest, due to the increased ventilation rate[141].

In concentrated form (not further specified), ethanol is very irritating to the eyes and causes temporary damage to the conjunctiva and cornea[141]. A 40-50% aqueous solution applied to the eye induces reversible lesions and hyperaemia[142].

Human subjects who had 95% ethanol applied for 24 h under an occlusive patch to the forearm in a modified Draize test did not experience any form of irritation after 24 and 72 hrs. This was also true for a 21-day open skin exposure test, where 0.02 ml 95% ethanol was applied to the lower back every 24 hrs. Exposure under identical conditions with occlusion caused erythema and induration (thickening/hardening of the skin) in the two test subjects towards the end of the test period[143]. Ethanol (50% aqueous solution) was shown to be a weak sensitiser in a 24-hr occluded skin patch test, causing allergic contact dermatitis in rechallenges of sensitive individuals[144]. 93 subjects were exposed three times a week for three weeks, resulting in mild to severe skin irritations. During a rechallenge 17 days later, irritation was evident in 15 cases. Additional challenges provoked allergic response in 6 subjects[144]. Repeated skin exposure can cause irritation and dry skin due to defatting[141], and lead to irritant dermatitis[142].

Ethanol can produce subjective irritation, irritant contact dermatitis and non-immunologic urticaria[145-148]. The prevalence of occurrences is low. The cause is often occupational exposure, with the possibility of cross-reaction with other primary alcohols and aldehydes[144,145].

Ethanol-induced bronchial asthma (a trigger of or worsening of asthma by ethanol consumption) is so far only observed in Asians. It has been connected to increasing acetaldehyde levels in the blood often seen in Asians with a genetically determined variation in ethanol metabolism[149,150]. Hooper[151] reported a reduction in forced expiratory volume (FEV_1) by 20-40% in two patients with mild asthma inhaling nebulised ethanol (10%, 100µl and 20%, 250µl).

6.2 Toxicity due to acute and short-term exposure

Acute ethanol intoxication is related to the Blood Alcohol Concentration (BAC). The BAC is proportional to the air concentration of ethanol and to the rate of ventilation, and inversely proportional to the weight of the subject[51,53,139,140] (see Table 6.1).

In an effort to assess the effects of low doses and gender, 16 volunteers were exposed to low doses via inhalation. In the first series subjects were exposed for 4 hrs to constant ethanol air concentrations of 150 mg/m^3 (80 ppm), 750 mg/m^3 (400 ppm) or 1500 mg/m^3 (800 ppm) in an inhalation chamber. The second test series also lasted 4 hrs, but the concentration changed every 60 minutes, either increasing from 190 mg/m^3 (100 ppm) to 3,610 mg/m^3 (1900 ppm), or decreasing from 3,610 mg/m^3 to 190 mg/m^3. The hourly changing levels were either 190/3,610/190/3,610 mg/m^3 or 3,610/190/3,610/190 mg/m^3. Subjects were tested during and after the experiments for changes in physical wellbeing or discomfort, reaction time and mental performance, in accordance with the Swedish Performance Evaluation System. A 4-hr exposure to 1,900 mg/m^3 (1,000 ppm) had no adverse systemic or local effect, whereas increasing the concentration from 190 to 3,610 mg/m^3 caused temporary irritation[52,136]. No significant exposure-related effects were evident in the psychological performance variables in either study. There were no significant gender specific differences[52].

It is unsure whether ethanol alone causes the symptoms observed at concentrations of ~2622-6346 mg/m^3 (~1380-3340 ppm) by Loewy and von der Heide[138]. The study was published in 1918, and several more recent studies report no effects in this range[141]. Likewise, although the study performed by Lester and Greenberg[53] is widely quoted, the results will not be taken in to consideration as the reported endogenous BAC (9-27 mg/l) is much higher compared to the observations of Sprung *et al.*[19]. For the reasons indicated above, others[1,4,54] have also questioned the validity of the results of Loewy and von der Heide[138] and Lester and Greenberg[53].

Some of the adverse effects of ethanol have been ascribed to its metabolites, mainly acetaldehyde. It is uncertain whether vasodilation of blood vessels in the skin, lower blood pressure, higher heart rate, nausea and headache, actions normally denoted 'alcohol sensitivity', are caused in part or completely by increased acetaldehyde levels in the blood[108].

The central nervous system (CNS) can be affected at BACs from 200-300 mg/l, according to the DFG[1], while another source sets the lower limit for CNS effects like reduced reaction time at BAC 500 mg/l for non-tolerant individuals[12]. The majority will be clearly influenced at 600-700 mg/l, and a BAC of 1.5-3 g/l is characterised by moderate intoxication with an increasing muscular incoordination; slowed reaction time; sensory loss and slurred speech. A BAC increasing from 3 to 5 g/l causes double vision, vomiting and stupor, leading to coma, respiratory depression, hypotension and hypothermia; with a lethal BAC >4 g/l (4‰) when death occurs by respiratory/circulatory failure[1,12]. However, highly concentrated ethanol vapours need to be inhaled to reach detectable blood ethanol concentrations (~tens of mg/l); To compare, oral ethanol consumption can result in a maximal BAC of up to 1000 mg/l in social drinking (see also chapter 5).

6.3 Case reports

There have been reports of percutaneous intoxication in young individuals with damaged skin and pre-term infants with poorly keratinised skin[152]. A 63-year-old man had suffered more than 40 years from erythema from sites of topical application of ethanol and palpitations and general flushing from oral intake of ethanol. He reacted to patch testing of ethanol (83%, 10%) at 15 minutes with transient erythema, and eczema at 2 and 3 days. Due to frequent blood collection, the patient was exposed to ethanol on cotton wool routinely sealed tightly over the point of vene-puncture. The author presumed that ethanol had, over time, progressed from causing non-immunologic urticaria to inducing hypersensitivity[147]. However, the observed patch test reactions may also be explained by a non-immunologic mechanism.

6.4 Summary of short-term effects after inhalatory exposure to ethanol

Based on the available data in table 6.1, DECOS derives a NOAEL of 1900 mg/m^3 for acute and/or short-term exposure. DECOS concludes that inhalatory exposure for one hour to concentrations at or below 1,900 mg/m^3 ethanol (1000 ppm) will probably not cause local irritation or systemic effects. Concentrations

above 3000 mg/m^3 (for 30 minutes) might result in minimal effects as transient cough, dry throat, tickling in the nose etc. A sudden increase in ethanol concentration (from 0 or 190 to 3,610 mg/m^3) may cause temporary irritation, whereas a concentration of 17,000 mg/m^3 ethanol is described as 'intolerable' for unacclimatized persons.

In concentrated form, ethanol is very irritating to the eyes. Non-occlusive, repeated dermal exposure to 95% ethanol does not cause skin irritation, but may cause dry skin due to defatting. Occlusive contact, in contrast, may induce erythema and induration (thickening/hardening of the skin). Also irritant contact dermatitis and non-immunologic urticaria have been reported after ethanol exposure. Based on available human experience, ethanol is not considered a skin or respiratory allergen.

6.5 Long-term effects based on epidemiological studies

6.5.1 Introduction

Selection of the literature

No human data are available on the effects of long-term inhalatory exposure to ethanol.

Human data on the effects of chronic consumption of alcohol beverages, however, are numerous. As most of the long-term health effects of oral ethanol exposure are attributed to systemic exposure via the blood stream, which is also the destination of inhaled ethanol, studies on orally consumed ethanol are also suitable to assess potential health effects of ethanol exposure by inhalation as far as these effects can be attributed to systemic exposure.

As the number of studies available on long-term health effects of oral alcohol consumption is enormous, only a selection of all publications will be discussed in this section. The following approach was chosen to evaluate the relation between oral alcohol consumption and long-term health effects. Evaluations by four (inter)national bodies are chosen as starting point for the following reasons: (1) they were written by a number of internationally respected scientists, (2) the description and evaluation of the epidemiological studies was, with some exceptions, adequate and balanced, and (3) they were relatively recent. The selected reviews are those by the Inter-Departmental Working Group on Sensible Drinking of the UK Department of Health[3], by the International Life Science Institute (ILSI)[2], and, for reprotoxic effects only, by the Health Council of the Netherlands[6].

For the UK report a substantial amount of evidence was reviewed by the Group, for some health effects (cancer and reprotoxic effects) by a specific committee. The aim of the review was to establish levels of intake associated with a beneficial effect and those associated with adverse effects on health. The references were also evaluated for methodological quality and efforts were made to achieve consistency in the evaluation by external reviews and consultation of external experts.

For the ILSI report, published in 1999, nine panels were composed of scientists from different disciplines and asked to address the relationship between alcohol consumption and one of the following areas: moderate drinking, assessment of intake, genetics, body weight, the cardiovascular system, pregnancy, breast cancer, bone, and the central nervous system. Also included as appendices to this book are two not updated chapters from the first edition (from 1992), Alcohol and Liver Diseases and Cancers of the Digestive Tract and Larynx. Some topics involving the neuropathological consequences of alcohol use, such as the psychiatric effects of alcohol abuse and injuries from drinking and driving, were not addressed. A disadvantage of the ILSI report is that the panels worked independently from each other and did not always apply the same criteria for evaluating the epidemiologic evidence for different health effects.

In addition, the committee used the review by Anderson *et al.*[153], as this review, although concise, followed a well-structured and transparent approach for the selection and evaluation of 156 papers derived from 131 studies. Articles were included in this latter review if they contained: (a) a quantitative measure of individual alcohol consumption; and (b) a measure of individual risk outcome related to consumption.

To assess dose-response differences between men and women, the review by Bradley *et al.* was used[154].

As the focus of the current report is to retrieve the levels of exposure to ethanol that may cause adverse health effects, the reviews were screened for information on dose-response associations. Where appropriate, the original publications were consulted. The selected reviews were updated with the results from recent studies, provided that these were sufficiently large to achieve statistical stability and investigated a dose-response association between alcohol consumption and health outcome. Estimates of dose-response associations will be mainly based on cohort studies, if available, as case-control studies on self-reported alcohol consumption may be subject to recall and selection bias.

Single studies, also those that can be considered as adequate studies, are subject to statistical fluctuation and possible small biases. Therefore, the “weight of

the evidence" is optimally represented through (good) meta-analyses and – preferably – pooling studies. While a meta-analysis summarises the published results of presumably all eligible studies, a pooling study conducts a (re)analysis of the combined original data from a number of studies. In general, pooling studies have greater possibilities to estimate dose-response associations; a drawback is, however, that usually not all eligible datasets can be obtained.

If a man uses 10 m^3 of breath per work shift of 8 hours and the pulmonary intake (at an absorption efficiency) is 60% (see chapter 5), it can be calculated that after inhalatory exposure to 1900 mg/m^3 (1000 ppm) ethanol the total amount of internal ethanol increases 11,4 g. This corresponds to drinking a little over 1 standard glass of an alcoholic beverage, assuming 10 g per glass. Therefore, the focus is on the health effects that may occur after daily consumption between zero and 2-3 glasses per day, and not on those effects that are known to occur in heavy drinkers or alcohol abusers.

Light to moderate levels of alcohol consumption have been associated with reduced risks of several diseases, in particular coronary heart disease (CHD), but also peripheral arterial disease, ischaemic stroke, and gallstones[2,3,153]. Since DECOS focuses on prevention of the adverse effects, these beneficial effects are not taken into account.

Exposure assessment

For the purpose of the current report, only studies are reviewed that quantitated alcohol consumption. Studies using classifications, such as drinkers versus non-drinkers or light, moderate, and heavy drinkers without indication of the amount consumed, were excluded. In the reviewed epidemiological studies, oral alcohol consumption was usually measured by means of a self-administered questionnaire (sometimes interview), in which information was collected on the current (or recent past) frequency of consumption and the type and amount of beverages consumed weekly. Only few studies collected information on the lifetime drinking history or, for that matter, information on past drinking. The implicit assumption with measuring current (or recent past) alcohol consumption is that it is representative for the drinking habits since adulthood. In reality drinking habits are, of course, not exactly similar over lifetime, but the change over time is only very gradual. For example, Goldbohm *et al.* conducted a reproducibility study on a food frequency questionnaire repeated annually during five years[155]. The correlation for the test-retest reproducibility of alcohol intake was 0.90, while the cor-

relation coefficient decreased to 0.82 after a five-year interval. This is still a very high correlation, indicating that alcohol drinking is a rather stable habit. This is, of course, not true for subjects, usually high consumers, who stopped completely because of medical advice or other reasons and could therefore be classified as past drinkers. This latter group can bias the dose-response association if they cannot be distinguished from the never-drinkers. In contrast, replacing lifetime alcohol consumption with current consumption or recent past consumption results in slight attenuation (underestimation of the dose-response association). The validity of self-reported alcohol consumption has been subject of much discussion. Generally, the finding is that alcohol consumption is under-reported, in particular among high consumers[156]. Underreporting would result in overestimation of the health effects for a given amount of alcohol[157]. Since we cannot assume that the degree of underreporting is similar for all alcohol consumers, it is difficult to infer a true dose-response association from an observed dose-response association.

In most studies, alcohol consumption is expressed as the average amount consumed per day or week, leaving the pattern of alcohol consumption out of consideration. To make the average amount of alcohol consumed comparable between the different studies the intake is recalculated to grams of ethanol per day [g/day], where necessary. Internationally, different standard sizes are used for the amount of ethanol in one glass or drink (e.g., 12 g in the USA; 10 g in the Netherlands) and the amount of ethanol in one unit (8 g in the UK). In this report, recalculated intake levels are put in square brackets [ ]. Intake levels originating from the publications are put between round brackets ( ). It should, however, be kept in mind that oral alcohol intake is a discrete parameter (a multiple of units) rather than a continuous parameter. Apart from the total dose per week or day, the drinking pattern might also be of great importance. Some effects are thought to be associated to the total dose (AUC) while others are thought to be associated with the blood-alcohol concentration, which is dependent on the consumption pattern. Unfortunately, information on the drinking pattern, e.g. binge drinking versus regular daily drinking was often not collected in the studies. If available, information on the influence of the drinking pattern on each effect will be discussed.

Finally, for the same level of alcohol consumption, the internal dose is approximately 50% higher for women than for men, due to differences in body weight and amount of body water per kilogram of bodyweight (see paragraph 5.2.2). This should be taken into account when comparing the results from the epidemiological studies among women and men. Methods other than question-

naires or interviews to assess alcohol intake were not encountered in the epidemiological studies.

Other methodological considerations

Epidemiological studies on health effects of exposure to oral alcohol may be subject to selection bias, occurring if cases with a particular disease are not comparable to those without the disease, and information bias. Both types of bias are more difficult to exclude in case-control studies, in which subjects are recruited and exposure is assessed after they become a case, than in prospective cohort studies, in which cases arise after assessment of exposure. For this reason, we consider cohort studies superior to case-control studies and base dose-response associations mainly on cohort studies.

An epidemiological study may also be biased due to confounding by another risk factor for the health outcome than the exposure in question (alcohol). To be an actual confounder, such a risk factor must be associated with the exposure of interest. Depending on the health effect, different sets of confounders are of importance to take into account in the design and data analysis of a study. For example, in studies on the effect of exposure to ethanol on birth weight and cardiovascular disease, consideration of and, if necessary, adjustment for smoking is very important. Without such consideration, a study is worthless since in general people who drink (more) alcohol also tend to smoke (more) and this could result in an incorrect attribution of the effects of smoking to alcohol consumption. In general, recent cohort studies (published after 1990) take confounding factors much better into account and use appropriate multivariate adjustment strategies. Several methodological issues that were still hampering proper evaluation of a (causal) dose-response association ten years ago, have now been settled[3].

6.5.2 All cause mortality

The UK Inter-departmental Working Group on Sensible drinking confirmed that the relationship between all-cause mortality and alcohol consumption follows a J-shaped curve. Non-drinkers have higher all-cause mortality than light and moderate drinkers, and heavy drinkers have even higher all-cause mortality than either group. The Group attempted to establish a dose of alcohol at which the relative risk of death increases steadily and significantly, but favoured a band of minimal mortality associated with consumption between 7 and 28 units per week [8-32 g/day] for men. The Group emphasised, however, that this conclusion does not imply that this level of alcohol consumption is entirely risk free. The Inter-

departmental Group concluded that drinking alcohol confers a significant health benefit in terms of reduced CHD mortality and morbidity on men aged over 40 and postmenopausal women. In terms of all causes of mortality and morbidity, the benefit can be largely gained by drinking as little as 1 unit/day [8g/d] on a regular basis. Consumption above 2 units/day [16 g/d] does not confer any major additional health benefit. Men who drink >3 or 4 units/day [>24-32g/day] and women who drink >2 or 3 units/day [16-24 g/day] had an increasing statistically significant risk of illness and death from a number of conditions, including haemorrhagic stroke, some cancers, accidents, and hypertension. The relevant recommendations of the Group are the following:

- "Men: Regular consumption of between 3 and 4 units a day [24-32 g/day] by men of all ages will not accrue significant health risk; consistently drinking 4 or more units a day [>32 g/day] is not advised as a sensible drinking level because of the progressive health risk it carries.
- Women (non pregnant): Regular consumption of between 2 and 3 units a day [16-24 g/day] by women of all ages will not accrue significant health risk; consistently drinking 3 or more units a day [>24 g/day] is not advised as a sensible drinking level because of the progressive health risk it carries."

In a meta-analysis, published in 1996, of 16 cohort studies on alcohol consumption and all-cause mortality, also a J-shape curve was observed[158]. The cohort studies were conducted in the USA (10), UK (2), Puerto Rico (1), Finland (2), and Sweden (1); in four of the studies, ex-drinkers were excluded. The lowest mortality was achieved at a consumption of 0-9 g per day for women and 10-19 g per day for men. From these minimum risk levels, the risk increased faster for women than for men at the same dose levels. For women, the RR was significantly increased at a consumption level of 20-29 g/day compared to abstainers (RR 1.13, 95% CI, 1.10-1.16), whereas for men the RR was significantly increased at a consumption level of 40-49 g/day (RR 1.06, 95% CI 1.03-1.10). The meta-analysis also included a larger number of non-identified and not further described studies (cohort and case-control) that looked at other alcohol-related endpoints. At consumption levels considered as "responsible" (*i.e.* <20 g/day for women and < 40 g/day for men), the relative risks for alcohol-related cancers and liver cirrhosis compared to abstainers were statistically significantly increased, whereas those for ischaemic heart disease, stroke and cholelithiasis were significantly decreased[158].

An important prospective cohort study not yet included in the latter review nor in the meta-analysis, was the study by Thun *et al.* (American Cancer Society Study

II) among 490,000 US men and women (mean age 56 years; range 30 to 104), who reported their alcohol and tobacco use in 1982[159]. During the next nine years of follow-up, 46,000 of them died. In this study, histories of current and past alcohol and tobacco consumption were assessed in detail, subjects with missing data on amount or duration of alcohol or tobacco use as well as past drinkers were excluded, and the analyses were adjusted for age, race, education, smoking, body-mass index and several other potential confounders. Total mortality appeared to be lowest at a consumption of one alcoholic drink per day [12 g/day] for men as well as women, but in women the rate of increase of the risk at a higher consumption level was larger than in men. Despite the lowest mortality at one drink per day, some causes of death were already slightly more prevalent at this consumption level as compared to non drinkers: liver cirrhosis and alcohol-related cancers (but not colorectal) in men and liver cirrhosis and breast cancer in women. For liver cirrhosis, the increased risks for one drink per day were not statistically significant.

Several authors stressed that the J-shaped curve trading off beneficial effects against adverse effects of alcohol on mortality is to be expected only in populations where cardiovascular disease mortality is high and, for the same reason, among the middle-aged only. Available evidence indicates that minimal mortality in young people does occur among the non drinkers[160].

6.5.3 Cardiovascular effects

The ILSI report, reviewed the available scientific (epidemiologic) evidence (197 references) for the following conditions: coronary heart disease (CHD), lipids, haemostasis, atherosclerosis, blood pressure, insulin sensitivity, and (different types) of stroke[161].

Coronary heart disease

The ILSI report reviewed the evidence from case-control and cohort studies separately. More than 50 cohort studies have been published up to 1998 (the date of the report). Twelve of them, which were all large to very large, provided detailed information on the quantity of alcohol consumed, and were able to adjust for age and smoking (besides other variables), were included in a table; several others were also discussed in the text. The twelve studies were conducted in Japan (2x), China (1x), USA (6x), UK (1x), Denmark (1x), and France (1x). Length of follow-up varied from 2 to 19 years, but was for most of the studies between 7 and 15 years. In most of the studies, the endpoint was CHD mortality, with exception

of the studies by Klatsky *et al.* among almost 129,000 participants in a Health Maintenance Organisation (Kaiser Permanente)[162], a relatively small study (n = 6788 men and women) by Rehm *et al.*[163], and the study by Camargo *et al.*[164], all conducted in the USA, and the Japanese study by Kitamura *et al.*[165]. These latter studies had incident CHD (or myocardial infarction) as endpoint. The authors of the review concluded that most of the studies reviewed showed remarkable consistency, even across diverse populations[161]. There appears to be a U-shape relationship between alcohol dose and CHD. The level at which the right side of the U for fatal and non-fetal CHD begins to increase could not be identified exactly and appears to be somewhere between 2 and 6 drinks/day [20-60 g/day][161]. For women, less data are available than for men. However, the cohort studies conducted among women in the USA by Rehm *et al.* , Thun *et al.* (American Cancer Society Study II) , Fuchs *et al.* (Nurses' Health Study), and Klatsky *et al.* (Kaiser Permanente Study) all showed protective effects of alcohol at consumption levels below 48 g/day[159,162,163,166]. Heavy drinkers (> 80 g/day) have an increased risk of CHD. Although vascular disease takes a long time to develop it is not known how long alcohol needs to be consumed in order to have an effect on reducing the manifestation of vascular disease[3,161].

Part of the protective effect of alcohol against coronary disease is believed to be a long-term effect mediated through increased levels of high density lipoprotein (HDL) cholesterol and decreased levels of low density lipoprotein (LDL) and therefore inhibiting the formation of coronary artery atheroma[3,160,161]. Another mechanism of the protective effect of alcohol against coronary heart disease may be due to effects on blood clotting, a short-term effect that appears to last only about 24 hours after alcohol intake[3,160,161].

Regular consumption of small amounts of alcohol is more protective than the same amount taken in larger doses less frequently[161]. This may be due to both harmful effects of sporadic heavy drinking and the short-term nature of some protective effects, particularly those involving blood clotting[3].

Hypertension

Epidemiological studies provide strong support for the view that alcohol use is an important factor in raising the blood pressure and that a causal association exists between the use of ≥30-60 g/d and blood pressure elevation in men and women[161]. Several large cohort studies have suggested that the risk of hypertension is increased in women who drink as few as 2 drinks a day [20 g/day] with recent intake being more important than average intake[154]. A single episode of

heavy drinking can lead to an increase in blood pressure for the succeeding 3 to 4 days[160]. In some studies the relationship between alcohol intake and blood pressure has been reported to be linear; in others, a threshold effect was apparent at around two or three standard drinks per day [20-30 g/day][160]. Assuming a linear relationship with no threshold an additional drink a day (10 g) would increase both systolic and diastolic blood pressures by 1-2 mm Hg[3,153]. A generally accepted clinical view would be that for men the rise in blood pressure produced by 4 units a day [32 g/day] (about 6 mmHg systolic blood pressure and 4 mmHg diastolic) would give rise for concern [3].

Stroke

Most epidemiological studies suggest that regular light to moderate alcohol intake (2-4 units a day) [16-32 g/day] probably reduces the risk of ischeamic stroke. However, regular alcohol consumption of more than 40 g/day and binge drinking may increase the risk of haemorrhagic stroke (due to cerebral or subarachnoid haemorrhage). Binge drinking or acute intoxication might also be associated with an increased risk of ischaemic stroke. Heavy alcohol consumption is associated with both ischaemic and hemorrhagic stroke[3,161]. Positive as well as U-shaped dose-response relationships between alcohol consumption and all strokes were reported by Anderson. When data were analysed according to type of stroke, only subarachnoid haemorrhage showed a consistent dose-response relationship[153]. The UK Inter-Departmental Working Group did not consider it possible to draw a definitive conclusion regarding the relationship of alcohol to all forms of stroke, as consumption levels, pre-existing hypertension, age and sex are all significant variables[3]. However, guidelines adopted in the USA and UK suggest that individuals may achieve some benefit if daily consumption is limited to 2-3 drinks [10-30 g/day][161].

6.5.4 Type 2 Diabetes Mellitus

The results of several epidemiological studies have indicated that the risk of developing Type 2 (non-insulin-dependent) diabetes mellitus is lower in moderate drinkers (15-40 g/day) than in abstainers[3,160].

6.5.5 Gallstones

Several epidemiological studies indicate that the consumption of alcoholic beverages is associated with a reduced risk of cholesterol gallstones[160]. It appears

that an alcoholic consumption of up to 40 g/day in men and up to at least 15 g/day in women may inhibit the formation of cholesterol type gallstones[3].

6.5.6 *Liver toxicity*

There is no doubt that alcohol *abuse* results in liver damage and ultimately liver cirrhosis and death due to cirrhosis. Reversible conditions, such as steatosis (fatty liver) and alcoholic hepatitis, precede the occurrence of irreversible cirrhosis and are presumably causally related to it[167]. The question at what level of alcohol consumption liver damage may occur is much more difficult to answer. First, liver damage as such can hardly be assessed in epidemiological studies, except in a screening setting. For this reason, most studies focused on the most serious manifestations of liver damage, *i.e.*, cirrhosis as cause of death and diagnosis at hospitalisation. Secondly, the majority of studies were case-control studies for which the retrospective assessment of alcohol consumption and the possibility of selection bias posed a problem, as explained previously. Prospective cohort studies on alcohol consumption and liver cirrhosis were, however, conducted in a few instances. The older cohort studies had very limited statistical power due to the relatively small study sizes[168]. However, several well-conducted, well-analysed, and large cohort studies were published more recently[159,169,170]. An overview of these studies is presented in Table 6.2. The American studies were conducted among participants in the Kaiser Permanente Medical Care Program[169] and by the American Cancer Society[159]. The Danish study was a pooling study of several cohort studies from Copenhagen, among which the Copenhagen City Heart Study (follow-up 21 years) contributed more than half of the person years; this latter study was also analysed before[170]. The study by Klatsky *et al.* was rather small, but excluded past drinkers and studied death due to liver cirrhosis. The study by Thun *et al.* is the largest conducted to date and also included death due to alcoholic psychosis and dependence as endpoint (15% of all cases), together with liver cirrhosis. Past drinkers and subjects with missing data on the quantity of alcohol consumed were excluded. Becker *et al.* not only studied death, but also hospitalisation due to liver cirrhosis. They were not able to exclude past drinkers, however, and attempted to deal with this problem by taking subject who drank between 1 and 7 drinks per week as reference group.

Table 6.2 Characteristics of large cohort studies on alcohol consumption and liver disease (cirrhosis).

Reference	Country	Year	Follow-up (y)	Size	Endpoint (ICD)[a]	Number of cases		Past drinkers
						Men	Women	
169	USA	1992	10	128,934	571	51	29	excluded
159	USA	1997	3-9	490,000	571,291,303	303	143	excluded
170	Denmark	2002	7-21	30,630	571.09[b]	212	80	included

[a] International Classification of Diseases: 571, liver cirrhosis; 291, alcoholic psychosis; 303, alcoholic dependence.
[b] Death or discharge from hospital with alcohol induced cirrhosis according to ICD (ICD-8 code 571.09).

Table 6.3 Relative risks for liver cirrhosis according to level of alcohol consumption (adjusted for amongst others age, smoking, Body Mass Index and education level etc.).

Ref.	Sex	Non	<6g/d	<12g/d	12g/d	18g/d	30g/d	48g/d	>48g/d	>72g/d	Past
169	Men	1.0 (Ref)				3.5**[a]		4.9**		25.0***	9.9***
	Women	1.0 (Ref)				3.0*		7.7***		14.3*	5.0*
159	Men	1.0 (Ref)	1.2 (0.7-2.0)		1.2 (0.7-2.2)		2.6 (1.6-4.0)		7.5 (4.9-11.4)		-
	Women	1.0 (Ref)	0.9 (0.5-1.4)		1.5 (0.8-2.8)		2.1 (1.3-3.4)		4.8 (2.9-7.9)		-
170	Men	7.8 (3.4-18)	1.0 (Ref)				2.3 (1.2-4.6)	10.4 (5.4-20)		20.4[b] (11-39)	-
	Women	1.3 (0.5-3.4)	1.2 (0.5-2.6)				5.3 (2.6-11)	10.8 (4.3-27)		14.1[b] (4.5-45)	-

[a] * p<0.05; ** p<0.01; *** p<0.001
[b] for >60g/d

The results of the three cohort studies are presented in Table 6.3. All Relative Risks (RRs) in the table above 1.5 were statistically significant. Despite the differences between the studies in the categorisation of alcohol consumption, it was possible to compare the relative risks for different levels of alcohol consumption across the studies (Table 6.3). In fact, the magnitude of the RRs appeared to be quite comparable. A consumption of twelve grams of alcohol per day did not seem to increase the risk of liver cirrhosis to a very large extent, but above that level an increase of the risk was apparent. The study by Klatsky *et al.* differed from the other studies in observing a substantially increased risk at a level of 18 g per day, whereas the other two found less increased risks at the higher consumption level of 30 g per day. While it is plausible that all three studies overestimate the RRs somewhat due to underreporting of their alcohol consumption, it is likely that such an effect was stronger in the study by Klatsy *et al.*, as the participants of the study were members of an health insurance organisation and their data on alcohol consumption were obtained in the context of a health examina-

tion. In the study by Becker *et al.* the high RR in the male non-drinker category supports the notion that the non-drinker group apparently contained past (heavy) drinkers, who contaminated the non-drinker group, in particular among men. It is not clear from any of the studies that women run a consistently higher risk than men for a given level of alcohol consumption.

In conclusion, liver cirrhosis is the irreversible and very serious (usually fatal) end stage of a disease process of the liver, which can be adequately assessed in epidemiological studies. Less serious damage to the liver (e.g. fatty liver and alcoholic hepatitis) can only be assessed in a screening setting and we have no knowledge of such studies. Two effects have to be balanced in evaluating the effect of alcohol consumption on liver toxicity: on one hand liver damage may have been underestimated as only the most serious endpoint was assessed, but at the other hand underreporting of alcohol consumption may have resulted in overestimation of the dose-response association. The net result is unknown, but from a more practical approach, it seems sensible to conclude that a consumption level up to 12 g per day is relatively safe, whereas higher doses may carry a risk of liver damage for some people. Based on the epidemiological data of good quality, there is no reason to set different limits for women than for men.

6.5.7 *Cancer*

Numerous epidemiological studies were conducted on the association between (oral) alcohol consumption and the risk of a large number of cancers. For several cancers a likely causal association has been established. These cancers are:

- Oral cavity, pharynx, and larynx cancer
- Oesophageal cancer, notably the squamous cell type
- Hepatocellular cancer
- Colorectal cancer
- Breast cancer.

Cancers at the first two sites show a rather strong association with alcohol consumption, in particular in combination with cigarette smoking. The last three types of cancer show weaker, but quite consistent associations with alcohol consumption[171].

Whereas it seems plausible that the upper aerodigestive tract cancers are caused by direct contact between the alcohol and the epithelium lining the tract in the presence of strong carcinogens from cigarette smoke, cancers at the other sites can only be caused through a systemic effect of alcohol. Hence, the aerodi-

gestive tract cancers may possibly be caused by combined exposure to several substances and not by ethanol itself, and may further be specific for the oral route of exposure. For these reasons, the effect of alcohol in causing aerodigestive tract cancers is not relevant for the evaluation of cancers due to inhalation of ethanol and therefore not further discussed.

In the area of alcohol consumption and risk of cancer, a considerable number of meta-analyses and several pooling studies were conducted. This review will be mainly based on these studies, supplemented with single studies when these are more recent than a meta-analysis or shed more light on a particular characteristic.

In 1988, IARC concluded that adequate evidence in humans exists to show that drinking of ethanol is causally related to malignant liver tumours[172]. Furthermore, IARC was of the opinion that malignant tumors of the oral cavity, pharynx, larynx and oesophagus are causally related to the consumption of ethanol as well. With respect to breast cancer, IARC was of the opinion that "The modest elevation in relative risk that has been observed is potentially important because of the high incidence of breast cancer in many countries. Although the available data indicate a positive association between drinking of alcoholic beverages and breast cancer in women, a firm conclusion about a causal relationship cannot be made at present[*]."

Hepatocellular cancer

There is little doubt that liver cancer can be caused by alcohol consumption, besides other, stronger factors (Hepatitis B and C infection, aflatoxin exposure), which modify each other's effects[173,174]. A number of studies were conducted, many of them from Asia. Most of these studies focussed on hepatitis infection and aflatoxin exposure and not primarily on alcohol consumption. The studies that focussed on alcohol, did mostly not take into account the association with the other three, much stronger risk factors. One of the few studies that assessed in a prospective design the antigen status of hepatitis B and antibody status of hepatitis C viruses and, at the same time, alcohol consumption and cigarette smoking, found some evidence of an independent effect of alcohol, but a much stronger effect in the presence of hepatitis infection[175]. However, this study was very

* In 2003, IARC placed alcohol beverages on the *Priority List of agents and exposures to consider in future IARC Monographs* because of new information concerning additional cancer sites (*breast*, liver, colorectal cancer) and better knowledge of mechanisms of action.

small. In general, the committee has to conclude that far too little information is available to assess a dose-response association for alcohol, independent from the other risk factors. Moreover, although there is still some debate, the majority of cases of liver cancer occur as a consequence of liver cirrhosis[3]. For this reason, combined with the lack of accurate data, the committee considers it not useful to try to assess quantitatively the effect of alcohol on liver cancer, as liver cirrhosis probably is the more sensitive, serious health outcome.

Colorectal cancer

There is considerable evidence on the association between alcohol consumption and the risk of colon and rectal cancer. Often, a positive association is observed, although there are inconsistencies between the studies. One, not very recent, meta-analysis by Longnecker *et al.*[176] is available in the literature. Furthermore, a pooling study is to be published, which covered eight large cohort studies, none of them included in the meta-analysis, *i.e.* the Alpha-Tocopherol Beta-carotene Cancer Prevention Study (men), the Canadian National Breast Screening Study (women), the Health Professionals' Follow-up Study (men), the Iowa Women's Health Study (women), the Netherlands Cohort Study (men and women), the New York State Cohort (men and women), the Nurses' Health Study (women), and the Swedish Mammography Cohort (women). The 1990 meta-analysis including 27 studies observed in a linear association a RR of 1.10 (95% confidence interval (CI), 1.05-1.14) for two drinks per day (equivalent to approximately 24 g/day)[176]. The included cohort studies (n=5), however, observed a RR of 1.32 (CI, 1.16-151) for the same amount of alcohol. The association did not vary according to gender and site within the large bowel. The pooling study, including almost half a million subjects and 4687 cases of colorectal cancer, observed an increased risk from a consumption of 30 g/day[177]. For the categories of 30-45 g/day and > 45g/day compared with non-drinkers, confounder-adjusted RRs of 1.16 (95%CI: 0.99-1.37) and 1.42 (95%CI 1.17-1.73) were observed respectively. The dose-response association was also assessed by applying spline regression. Spline regression is a method to model a dose-response association without prior assumptions about the shape of the curve. The spline regression curve appeared to be slightly J-shaped: alcohol consumers drinking less than 12 g/day had a RR slightly below 1. No differences were observed between man and women. Not all of the pooled cohorts were able to separate past-drinkers from non-drinkers. However, for the studies that could, a subanalysis showed that all RRs were a little higher, but the conclusion about the dose-response association

was not changed. The pooling study did not observe heterogeneity for gender nor for type of alcoholic beverage and it is therefore concluded that alcohol itself has the effect on colorectal cancer risk.

A case-control study nested in a cohort study by Murata *et al.*[178] in Japan, which was not included in the Pooling study, observed odds ratios (comparable to relative risks) of 3.5, 1.9, and 3.2 for alcohol intakes of 27, 54, and 81 g alcohol respectively as compared to abstainers.

The Health Professionals' Study demonstrated that subgroups with a methyl-group-deficient diet (*i.e.* low in folate and methionine) may experience a stronger effect from alcohol. To date, an insufficient number of studies were conducted to substantiate this effect[179].

Breast cancer

The evidence on alcohol consumption and breast cancer in women is very adequately summarised in a recent meta-analysis[180], a study based on the pooled data from six prospective cohort studies[181] and a very recent pooling study including 53 cohort and case-control studies, covering 58515 breast cancer cases and 95067 controls and representing 80% of all data available on this subject world-wide[182].

The study pooling the six cohort studies, included 322 647 women from the US (three studies), Canada, Sweden and the Netherlands. 4335 cases of invasive breast cancer were detected among them[181]. In all studies, alcohol consumption was assessed at baseline (*i.e.* before the occurrence of the cancer) by a self-administered food frequency questionnaire. The dose-response association was assessed by categorising alcohol consumption and also by applying spline regression. The relative risk (RR) appeared to be linearly related to alcohol consumption up to 60 g per day. Above this level, the relative risk did not appear to increase further, although this conclusion was based on limited data. Between a mean daily consumption of 0 and 60 g per day, the relative risk appeared to increase linearly with 9% for each 10 g of alcohol (RR, 1.09; CI, 1.04-1.13). The heterogeneity between the six studies was not statistically significant. No differences in effects were observed between types of alcoholic beverages consumed.

The meta-analysis considered 74 publications, but included only 42 in the analysis. The major reasons for excluding studies were the lack of quantifiable alcohol consumption data or duplicate data[180]. The analysis comprised 13 cohort studies (including those comprised in the Pooling Study[181]), the remainder were case-control studies. Altogether 41,477 cases of breast cancer were included. The

relative risk increased monotonically with alcohol consumption; 12 g alcohol per day corresponded with a RR of 1.10 (CI, 1.06-1.14). The 13 cohort studies found the same pooled estimate. Older studies (published before 1990) observed a slightly higher pooled RR (1.12) than more recent ones (1.08) and studies within the United States a lower pooled RR (1.07) than those that were conducted outside the United States, *i.e.* mainly Europe (RR, 1.13). No difference was observed for post- and premenopausal breast cancer. No differences were observed for different types of alcoholic beverages.

The most recent and largest pooling study also observed an almost linear dose-response association and estimated an (confounder-adjusted) increased risk of 7.1% overall for every 10 g alcohol consumption[182]. For the pooled case-control studies this figure was 7.4% (population controls) and 7.3% (hospital controls). For the pooled cohort studies, an increased risk of 5.0% was observed for every 10 g alcohol. This estimate was lower than the one made in the other pooling study of cohorts (9%)[181], although the included cohorts were the same in both studies with exception of the addition to the most recent pooling study of two large studies (American Cancer Society, USA and Million Women Study, UK), which even showed a stronger dose-response association than the other cohort studies. Although it is not clear what caused the different estimates, it may be due to different analytic methodology.

Almost no information is available on the association between drinking pattern and breast cancer relative risk. In other words, in the studies referred to above, for a given amount of mean daily alcohol consumption a relatively low regular consumption was not distinguished from less frequent, but high consumption. The authors of another meta-analysis on alcohol and breast cancer, who observed that the relative risk was higher in Mediterranean countries than in Northern Europe and North-America for the same amount of alcohol, suggested that this effect might be attributed to a daily drinking pattern as opposed to a more binge-drinking pattern, respectively[171]. This effect can, however, also be attributed to the fact that all these studies in Mediterranean countries (mainly Italy), were hospital-based case-control studies. The pooling study, however, did not confirm that the studies from Mediterranean countries found higher risks[182].

Furthermore, there are only a few data concerning the relation between drinking alcohol and the risk for breast cancer in men. The risk for male breast cancer is inconsistently reported. Male breast cancer is rare. A recent European population-based case-control study[183] found that the relative risk of breast cancer in men is comparable to that in women for alcohol intakes below 60 grams per day. However, these data needs to be confirmed in other studies.

Summarising the data on breast cancer, the following can be concluded. Due to the large number of available studies on alcohol and risk of breast cancer in women, which were summarised in high quality pooling and meta-analysis studies, it is possible to establish a RR for women with a narrow confidence interval per unit of oral alcohol intake from a dose-response curve that is apparently linear up to 30 g/day or higher; at high intake the relative risk seems to level off or even decrease[180,184]. Some authors doubt whether such a weak association at the lower range of exposure can be considered causal[185], although the consistency of the association certainly supports causality.

Differences in susceptibility

Several subgroups of people may be more susceptible for the cancer-enhancing effect of alcohol than the population at large. The first group constitutes those with a polymorphism, a genotype with (a) variant allele(s) that influences the metabolism of ethanol or its metabolites. The last decade research has been conducted to see which polymorphisms are of substantial importance. The most frequently investigated in relation to alcohol metabolism is the alcohol dehydrogenase 3 polymorphism. Alcohol dehydrogenase 3 (ADH3) metabolises alcohol to acetaldehyde, a carcinogen. Two studies on breast cancer found discordant results[186,187]; only the study by Freudenheim *et al.*[186] observed an increased risk for subjects with the ADH3(1-1) genotype. The same was true for two studies on head and neck cancer: Harty *et al.* observed an increased risk for subjects with the ADH3(1-1) genotype[188], whereas Olshan *et al.* did not[189]. A study on upper aerodigestive tract cancer did not observe a difference between the different genotypes[190]. Aldehyde dehydrogenase 2 polymorphisms were also studied to some extent. It is obviously too early to draw any firm conclusions with respect to the importance of alcohol metabolising polymorphisms in the susceptibility of humans to the carcinogenic effect of alcohol.

Other susceptible subgroups may be those with a bad nutritional status, such as observed by Giovannucci *et al.*[179]. In such subgroups, the effect of alcohol may be increased several-fold, but more research is needed to substantiate this effect.

Carcinogenicity of acetaldehyde

In 1999, IARC concluded that there is *inadequate evidence in humans for the carcinogenicity* of acetaldehyde but there is *sufficient evidence in experimental*

animals for the carcinogenicity of acetaldehyde. Acetaldehyde causes gene mutations in bacteria and gene mutations, sister chromatid exchanges, micronuclei and aneuploidy in cultured mammalian cells, without metabolic activation[191]. Based on the evaluation of IARC, the committee concludes that acetaldehyde is a genotoxic carcinogen in experimental animals.

Conclusions regarding carcinogenicity

For several human cancers for which there is sufficient evidence that they are caused by exposure to ethanol, it is possible to derive a dose-response association. Oral cavity cancer, pharynx and larynx cancer and oesophageal cancer are assumed to be less relevant after inhalatory exposure. Alcohol consumption can cause liver cancer as well, and the committee is of the opinion that liver cirrhoses precede the liver cancer.

For colorectal cancer, a clear dose response relationship is observed and assumed to be the same for both genders (Table 6.4). In addition, an increased risk for breast cancer caused by drinking alcohol has been demonstrated in women. No data concerning the development of breast cancer in man are available.

Another issue might be important with respect to exposure to alcohol via inhalation. The aetiology of oesophagus and larynx cancer has shown very clearly how alcohol, probably as co-carcinogen, interacts with carcinogens to cause cancer. It is not implausible that inhaled alcohol causes the same sort of interaction, but then in the lungs. This may have an especially large impact on smokers, but also non-smokers are continuously exposed to small amounts of carcinogens from the environment. It is, however, very difficult to estimate the effect of this exposure.

Table 6.4 Best estimate of the relative risk (RR) per 10 g/day oral alcohol intake for several types of cancer caused by a systemic effect of alcohol.

Type of cancer	Shape	RR
Colorectal	J-shape, threshold	Threshold at ca.20 g/day, then ca. 1.12 per increment of 10 g/day[177]
Breast	Linear, no apparent threshold	1.07 per increment of 10 g/day[182] 1.09 per increment of 10 g/day[181]

6.5.8 *Reproduction toxicity*

There are no data available on the effects on human reproduction after inhalation of ethanol. However, based on epidemiological data on the effects of ethanol consumption in the form of alcoholic beverages, the Health Council's Committee for Compounds Toxic to Reproduction recommended classification of ethanol in category 1 for its effects on fertility (*substances known to impair fertility in humans*) as well as for its effects on the development of offspring (*substances known to cause developmental toxicity in humans*), according to the Directive 93/21/EEC[5].

For the description of the effects of ethanol on fertility, development and lactation, DECOS used a recent review of a committee of the Health Council of the Netherlands (2004[6]), hereafter referred to as the Committee on Alcohol consumption and reproduction as a basis. In this report the effects of moderate alcohol consumption on human fertility, development and lactation were evaluated. The conclusions of this committee are summarised below. For detailed information on individual studies, DECOS refers to the original report.

Fertility

In 2004, the Committee on Alcohol consumption and reproduction evaluated the effects of moderate alcohol consumption on male and female fertility. Based on a study by Jensen *et al.*[192] (female fertility), a study by Tolstrup *et al.*[193] (female fertility), by Eggert *et al.*[194] (female fertility), and by Hassan *et al.*[195] (male fertility), the committee concluded that a dose-effect relationship for the consumption of alcohol and effects on male and female fertility exists. The committee is of the opinion that there are indications that consumption of less than 10 gram of ethanol per day might decrease female fertility (eg time to pregnancy). For effects on male fertility, only one study is available in which effects on fertility are described. Based on this study of Hassan *et al.*[195], the committee concluded that there are indications that consumption of less than 10 gram of ethanol per day might decrease the male fertility as well.

Spontaneous abortion and foetal death

The Committee on Alcohol consumption and reproduction evaluated the effects of moderate alcohol consumption before (by man and women) and during preg-

nancy on spontaneous abortion and foetal death. The committee concluded that it is possible that drinking alcoholic beverages might increase the incidence of spontaneous abortion and foetal death. Several human studies by Armstrong *et al.* Harlap *et al.*, Kesmodel *et al.*, Windham *et al.*, Kline *et al.* and Rasch *et al.* demonstrated this effect in pregnant women drinking an average of 10 grams of ethanol per day or more[196-203]. Drinking less than 10 grams of ethanol per day also revealed these effects in some studies. However, these data are less consistent.

Pre-term delivery and length of gestation

Consumption of alcoholic beverages during pregnancy might increase the incidence of preterm delivery (birth before 37 weeks of pregnancy) and decrease the length of the gestation. This was concluded by the Committee on Alcohol consumption and reproduction. Several cohort studies by Kesmodel *et al.*, Lundsberg *et al.*, Sulaiman *et al.* and Parazzine *et al.* show an increased incidence of pre-term delivery and shorter gestational length after drinking more than 17 gram of ethanol[204-207]. It has been suggested that this effect is predominantly related to consumption of alcohol in the second and third trimester of pregnancy[205,206,208]. The cohort study by Lundsberg *et al.* also found effects on pre-term delivery and gestation length after drinking 1 to 8 grams of ethanol per day[206]. This is, however, not confirmed in the other studies.

FAS and anomalies

The definition of Foetal Alcohol Syndrome (FAS), which was first described more than 20 years ago[209], has been repeatedly discussed. One of the points of discussion is whether or not the diagnosis should include the criteria chronic alcoholism of the mother. However, as supported by several evaluations, the full spectrum of physical and mental handicaps of FAS is only seen in offspring of female heavy drinkers. Whether the individual anomalies characterising FAS may be the result of a lower exposure to alcohol is not clear. The diagnosis of FAS is made if signs of the following three categories are present: growth retardation, central nervous system involvement (neurological abnormalities, development delay, intellectual impairment, low head circumference (lowest tertile)) and facial dysmorphology (short palpebral fissures, elongated midface, flattened maxilla). Further, non-specific malformations in heart, limbs and kidney may

occur. When only some of the above signs are present, the term Foetal Alcohol Effect (FAE) is used[210].

The Committee on Alcohol consumption and reproduction concluded that the risk for FAS increased after longterm consumption of 90 grams (or more) of ethanol per day. There are no convincing indications that consumption levels lower than 60 grams ethanol per day might increase the incidence of individual anomalies.

Birth weight, size and growth

To the most studied reprotoxic effects of ethanol belong the effects on birth weight, size, and growth. These effects also belong to the spectrum of adverse effects characteristic to the FAS.

The Committee on Alcohol consumption and reproduction concluded that the human data concerning the effects of drinking alcohol on foetal growth are not consistent. The studies of Whitehead *et al.*, Passaro *et al.*, Ogston *et al.* and Marbury *et al.* suggest a relation between alcohol consumption and decreased foetal growth and birth weight[211-214]. The Committee of Alcohol consumption and reproduction is of the opinion that there are no clear indications that drinking 10-20 grams of ethanol per day might affect foetal growth and birth weight. There are, however, sufficient indications that drinking more than 20 grams of ethanol per day might increase the risk for this effect.

Foetal behaviour

In 2004, the Committee on Alcohol consumption and reproduction concluded that acute oral exposure to 12-30 grams of ethanol per day in the third trimester of pregnancy suppresses the human foetal breathing movements[215-218]. This effect is observed during the first two hours after consumption. In addition, there are indications in one study of Little *et al.* that the consumption of 1-10 grams of ethanol per day might affect the foetal startle behaviour[219,220].

Neurobehavioral effects

The Committee on Alcohol consumption and reproduction concluded that the exposure to alcohol in utero, has an adverse effect on the mental development of the offspring. A meta-analysis performed by Testa *et al.* confirmed that prenatal exposure to more than 12 grams of ethanol per day will result in a lower mental

development index in one year old children[221]. This effect was not observed in younger and older children. Two large cohort studies (from Seatle and from Detroit) showed that maternal alcohol consumption (5 grams per day or more) during pregnancy has an adverse effect on the behaviour of 6-7 year old children[222-239]. Therefore, the Committee on Alcohol consumption and reproduction concluded that it is not sufficiently investigated whether consumption of 1-10 grams of ethanol per day will influence the neurobehaviour of children but effects are confirmed at higher consumption levels.

Lactation

In their report, the Committee on Alcohol consumption and reproduction described several controlled human studies of Mennella *et al.*[240-243]. Based on these studies, the committee concluded that maternal consumption of 15-20 grams of ethanol, results in a reduction (21-23%) of breast milk consumption by babies during the next three hours. Effects at lower alcohol consumptions are not described. In addition, Mennella *et al.* studied effects of breast milk containing ethanol on the rapid eye movement of infants[244,245]. The Committee on Alcohol consumption and reproduction concluded that the normal sleeping pattern can be disturbed after drinking breast milk of mothers who consumed 15-20 grams of ethanol.

The former committee concluded that there are no convincing data that maternal alcohol consumption has an adverse effect on the neurobehaviour of children after breast feeding[246]. The effects of lower consumption levels have not been studied.

Overall conclusion with respect to alcohol consumption and reproduction

The Committee on Alcohol consumption and reproduction concludes that there is evidence that an intake of less than 10 grams of ethanol (one alcoholic consumption) per day has an adverse effect on reproduction (decreased male and female fertility, increased incidence of spontaneous abortion, foetal death, preterm delivery, decreased length of gestation).

6.5.9 Conclusion regarding the effects after longterm exposure

A very precise threshold for adverse affects of alcohol consumption on longterm health effects is difficult to establish due to variation between study results but also for methodological reasons. As far as the methodological reasons are con-

cerned, DECOS acknowledges that underreporting overestimates the quantitative dose-response associations, but this effect is likely to be lower in the low exposure range of interest to this report.

In non-pregnant women, the most critical adverse effect of exposure to ethanol is the increased lifetime risk of breast cancer (7-10 per 1000 women) at every 10 g of ethanol consumption per day. Other adverse health effects in women as well as men may occur at levels starting from 10 to 20 g oral ethanol consumption per day. These adverse health effects comprise increased risk on colon cancer, liver cirrhosis and hypertension. With respect to the effects on reproduction, the Health Council's Committee on Alcohol consumption and reproduction concluded that the available data show that after consuming less that 10 grams of ethanol per day, adverse effects on human fertility and development might be expected.

6.6 Summary and evaluation

Only a couple of human studies on short-term dermal and inhalatory exposure are available. Based on the available studies DECOS is of the opinion that inhalation for one hour of concentrations less than 1900 mg/m^3 ethanol (1000 ppm) will probably not cause local or systemic effects after acute and/or short-term exposure. However, a sudden increase in ethanol concentration may cause temporary irritation, whereas a concentration of 17,000 mg/m^3 ethanol is described as 'intolerable' for unacclimatized persons.

Concentrated ethanol solution is very irritating to the eyes. Non-occlusive, repeated dermal exposure to 95% ethanol does not cause skin irritation, but may cause dry skin due to defatting. Occlusive contact, in contrast, may induce erythema and induration (thickening/hardening of the skin). Also irritant contact dermatitis and non-immunologic urticaria have been reported after ethanol exposure. Based on available human experience, ethanol is not considered a skin or respiratory allergen.

Most human data on ethanol concern effects as a result of the consumption of alcoholic beverages. In the epidemiological studies on carcinogenic as well as non-carcinogenic effects, oral alcohol consumption was usually measured by means of a self-administered questionnaire or interview, in which information was collected on the current (or recent past) frequency of consumption and the type and amount of beverages consumed weekly. Most studies did not collect information on the lifetime drinking history, implicitly assuming that the measured consumption is representative for the drinking habits in the relevant time period for the development of the effect.

Generally, drinking habits are not exactly similar over lifetime, but the change over time is only very gradual. However, this is not true for subjects who stopped completely because of medical advice, pregnancy or other reasons. These subjects can bias the dose-response association if they are included with the non-drinkers.

The validity of self-reported alcohol consumption has been subject of much discussion. Generally, the finding is that alcohol consumption is under-reported, in particular among high consumers. The ranking of subjects, important for assessing a dose-response association, might be less affected.

In most epidemiological studies only a rough distinction is made between light, moderate, and heavy alcohol consumption. In many epidemiological studies the alcohol consumption is expressed as the average amount of alcohol consumed per day or week, leaving the pattern of alcohol consumption out of consideration.

Several epidemiological studies reported that the dose-effect curve for ethanol appears to be U- or J-shaped, indicating beneficial effects, like a reduced risk of coronary heart disease, at low exposures. The most critical non-carcinogenic effects in humans appear to be liver cirrhosis and effects on offspring (birth weight). With respect to carcinogenicity the most relevant types of cancers appear to be breast and colorectal cancer.

Liver cirrhosis is the irreversible and very serious (usually fatal) end stage of a disease process of the liver, which can be adequately assessed in epidemiological studies. Two effects have to be balanced in evaluating the effect of alcohol consumption on liver toxicity: on the one hand liver damage may have been underestimated as only the most serious endpoint was assessed, but on the other hand underreporting of alcohol consumption may have resulted in overestimation of the dose-response association. The net result is unknown, but from a more practical approach, it seems sensible to conclude that a consumption level up to one unit (approximately 10-12 g of ethanol) per day is relatively safe, whereas higher doses may cause a risk of liver damage for some people. Based on the epidemiological data for liver cirrhoses of good quality, there is no reason to set different limits for women than for men.

For the human cancers that are likely to be caused directly by systemic exposure to alcohol (colorectal and breast cancer) it is possible to derive a dose-response association. For colorectal cancer the dose response relationship is assumed to be

the same for both genders and a threshold of 20 g/day is suggested. The dose response association for breast cancer has been found in women. For every 10 grams of daily ethanol consumption the excess breast cancer risk is calculated to increase by 7-10 per 1000 women. There are only limited data available concerning the risk for breast cancer in men.

The Committee on Alcohol consumption and reproduction is of the opinion that drinking small amounts of ethanol (less than 10 g/day) might decrease fertility in both men and women. Effects on development have been observed after maternal consumption of 1-10 grams per day as well. High levels of alcohol consumption have been found to cause Foetal Alcohol Syndrome (FAS). The epidemiological studies strongly suggest that the facial malformation characteristic of FAS result from exposure to high levels of ethanol during the first trimester, but reducing alcohol consumption in the second trimester may reduce the adverse effects. Whether the individual anomalies characterising FAS may also result from a lower exposure to alcohol is not clear.

Chapter 7

Effects in experimental animals

7.1 Irritation and sensitisation

95-99% ethanol was not irritating to the skin of rabbits in tests performed according to OECD guideline 404 (a 4-h semi-occlusive exposure to 0.5 ml)[1,143]. A 24-h occlusive test induced mild irritation on rabbit skin[142].

96% ethanol induced some irritation on the rabbit eye in a Draize test[142]. In a test performed according to OECD 405 guidelines for acute eye irritation/corrosion, ethanol was shown to be strongly irritating[142].

The allergenic potential of ethanol was assessed in a Magnusson-Kligman maximising test with guinea pigs. A 75% solution did not induce or trigger allergenic skin reaction[142]. In the mouse ear sensitisation assay performed on BALB/c mice, ethanol did not induce any adverse effects[12].

In two inhalation studies on mice the results of an Alarie test for sensory irritants gave RD_{50} values of 25,983 mg/m^3 (13,675 ppm) and 51,897 mg/m^3 (27,314 ppm), respectively[12].

7.2 Toxicity due to acute exposure

Ethanol is of low acute toxicity via inhalation, and via the oral and dermal routes of exposure. The lowest lethal concentration reported is 24,130 mg/m^3 (12,700 ppm), during a 22-h exposure of rats[12], while rats exposed for 3.75 hours sur-

vived 85,500 mg/m^3 (45,000 ppm). The lowest reported deadly dose in mice is 55,100 mg/m^3 (29,000 ppm) after 7 hours of exposure, whereas the LC_{50}-value was 39,900 mg/m^3 (21,000 ppm) after 4 hours[1]. Aspiration of 0.2 ml of 100% ethanol caused death in 5 out of 10 rats tested, whereas 0.2 ml of 70% ethanol in water caused death in 1 of 10 rats[12]. The symptoms of acute poisoning range, with increasing concentration, from irritation of the mucous membranes and excitability through ataxia, lack of co-ordination and hyperactivity; to lethargy, rapid, shallow breathing and finally death by respiratory failure in rat, guinea pig and mouse[12,141,247]. The severity of the symptoms increased with increasing exposure time, and reduced the concentration necessary to provoke a specific response. A recent test performed on male Wistar rats demonstrated at which concentration levels these symptoms can be expected[248]. Animals were exposed for 12 hours to concentrations of 29, 37, 43, 51 and 56 g ethanol/m^3 air, respectively (15,263; 19,474; 22,632; 26,842 and 29,474 ppm). No intoxication was observed in rats exposed to the lowest concentration, while mild lethargy and incoordination was induced after 3 hours exposure to 56 g/m^3 (BAC 960±50 mg/l). After 6 hours, when BAC was 2290±140 mg/l, rats displayed substantial lethargy and incoordination, and 9 hours exposure (BAC 3390±200 mg/l) resulted in severe lethargy and incoordination, loss of muscle tonus and severe loss of righting reflex. At 12 hours (BAC 4150±260 mg/l), three of six rats displayed no corneal reflex, five of six had no righting reflex. One study reported increases in motor effects on male CD-1 mice as a function of concentration and exposure time, with an EC_{50} of 94,517 mg/m^3 (49,746 ppm) for 10 min, 61,589 mg/m^3 (32,415 ppm) for 30 min and 57,564 mg/m^3 (30,297 ppm) for 60 min[247]. Male Charles-River-CD rats inhaling 15,200 mg/m^3 (8,000 ppm) or more for more than 4 hours showed changes in behavioural tests, while 7,600 mg/m^3 (4,000 ppm) had no effect. Exposure to 7,828 mg/m^3 (4,120 ppm) resulted in a decreased avoidance performance after 4 hours[249]. The lowest adverse effect level found in literature is a study by Ghosh *et al.* on behavioural effects of ethanol inhalation in F344-rats. The animals were trained either to press a lever to receive a sucrose solution (liquid-reinforced schedule) or to press a lever giving a pleasant electrical stimulation (self-stimulation). The behavioural depression was significant at 384 mg/m^3 (202 ppm) and 756 mg/m^3 (398 ppm) after 45 min of exposure. There were no effects during a 5-h exposure to 266 mg/m^3 (140 ppm). A high degree of tolerance developed from day 3 forth during a 2-h daily exposure to 391 mg/m^3 (206 ppm) for 5 days. Likewise, there were no significant changes in the reinforcement rate of rats exposed to 194 mg/m^3 (102 ppm) or 384 mg/m^3 (202 ppm) for 80 min. The self-stimulation tests did not result in signifi-

cant changes in the reinforcement rate. The blood ethanol concentration was determined to be approximately 393 mg/l (given as 393 μg/ml) after exposure to 1140 mg/m^3 (600 ppm), and 545 mg/l (given as 545 μg/ml) after exposure to 2,280 mg/m^3 (1,200 ppm)[250].

The LD_{50} for single-dose oral toxicity in the rat also varies substantially (6.2-17.8 g/kg), with the age of the animals (Table 6.7). The oral LD_{50} for guinea pig (5.6 g/kg) and dog (5.5-6.6 g/kg) are lower than for the rat.

The lowest lethal dermal dose for rabbits is reported to be 20 g/kg bw[1].

In conclusion, an acute exposure with occlusion to 95% ethanol caused mild irritation in rabbits. 96% ethanol is mildly irritating to the eyes of the rabbit. The lowest lethal dose by inhalation is 55,100 mg/m^3 (29,000 ppm) in mice (7-h exposure) and 24,130 mg/m^3 (12,700 ppm) in rats (22 hours). In one study behavioural depression occurred in rats inhaling 384 mg/m^3 (202 ppm) for 45 minutes, although the rats developed tolerance after two days in a similar exposure scenario, and there was no effect on the reinforcement rate by exposure to 384 mg/m^3 (202 ppm) for 80 minutes. The lowest reported lethal dermal dose for rabbits is 20 g/kg bw.

Table 7.1 Single-dose oral toxicity values for ethanol in animals (adapted from[12]).

Species	LD_{50} values (g/kg bw)
Rat	6.2[a]
	11.5[b]
	13.7
	17.8[c]
Mouse	8.3
	9.5
Guinea pig	5.6
Rabbit	9.9
Dog	5.5-6.6[d]

[a] 14 days old
[b] older adults
[c] young adults
[d] lethal dose

7.3 Toxicity due to short-term exposure

7.3.1 Inhalation studies

A 14-day inhalation study with two groups of 9 male Sprague Dawley rats was carried out. The test group was continuously exposed whole body to 25,000 mg/m^3 ethanol (13,250 ppm). Blood samples were taken at 10 AM on days 4, 6, 8, 10, 12, 13 and 14. Stable, constant blood ethanol levels were obtained over the 14-day period (ca. 1690 mg/l; range 1200-1990 mg/l). Controls were exposed to clean air. No changes in body weight gain were observed. Haematological observations revealed a decrease in the relative proportion of granulocytes and a concomitant increase in the relative proportion of lymphocytes, although the total white blood cell count had not changed. No changes were observed in erythrocyte count or haemoglobin content. When compared to controls, decreases in cellularity were observed in the spleen, thymus and bone marrow. In bone marrow, the number of erythroid progenitor cells was reduced whereas the granulocyte-macrophage progenitor cells were unaffected. Splenic lymphocytes did not show significant differences in the ability to proliferate when stimulated by non-specific mitogens[251].

In a 5-week inhalation study, two groups of 6 male Sprague Dawley rats each were continuously exposed to either ethanol or room air. The ethanol concentration was not measured but was maintained at a high level (*i.e.* a concentration that resulted in ataxia). After an initial increase in blood alcohol levels until day 12, blood alcohol levels remained between 2000-3000 mg/l. Ethanol exposure resulted in decreased body weight gain and increased the activities of catalase and Cu/Zn-superoxide dismutase in the lung but not in the liver. Although hepatic glutathione and vitamin E were reduced there was no increase in malondialdehyde content in either liver or lung. The elevation of pulmonary antioxidant enzyme activities could indicate that lung is a target for ethanol-induced oxidative stress. But since no loss of pulmonary GSH or vitamin E and no increase in malondialdehyde formation was observed, it appeared that ethanol exposure did not produce a significant degree of oxidative stress[252]. Other endpoints were not examined.

In a 90-day inhalation study 15 male and female rats (Sprague Dawley and Long Evans), 15 male and female guinea pigs, 3 male New Zealand rabbits, 3 male squirrel monkeys and 2 male Beagle dogs were continuously exposed whole-body to 86 mg/m^3 ethanol (46 ppm) for 90 days. No clinical signs of toxicity were observed. Haematology and histopathological examination of liver,

kidneys, lungs, heart, spleen did not reveal any treatment-related changes in these species. Also, histopathology of brain, spinal cord and adrenals of monkeys and dogs was negative[253]. In the latter study it was noted by the committee that although control animals had been used, no information was provided on these animals.

Overall, since in these studies with repeated inhalation exposure only one concentration of ethanol was tested and only specific endpoints were studied, these studies are not appropriate to establish health-based occupational exposure limits. From these data, however, it was concluded that at the low concentration of 86 mg/m^3 no changes were observed, whereas at the high levels used in the other two studies (*i.e.* resulting in blood alcohol concentrations > 1700 mg/l), only slight toxicity was observed.

7.3.2 *Oral studies*

Ethanol (1.5 ml of 47.5% ethanol per 100 g body weight; corresponding to ca. 7 g/kg bw) given daily to rats (not specified) by gastric intubation for 11 days revealed a significant increase in the hepatic concentration of esterified fatty acids when compared to controls given isovolumetric amounts of water. Animals fed for 30 days a liquid diet with 33% of the calories as ethanol also revealed an increase in the hepatic concentrations of total esterified fatty acids and triglycerides when compared to pair-fed controls receiving the same diet except that ethanol was isocalorically substituted by carbohydrate. Liver histology of the ethanol-fed animals revealed marked fatty infiltration[254]. In several other animal studies, repeated intake of ethanol has resulted in damage of almost all organ systems, with the liver as the main target organ[1].

In a study on the effects of ethanol at the brain level, a group of 10 male Wistar rats was given ethanol in drinking water (3% v/v) for 8 weeks, resulting in a mean intake of 4 g/kg bw/day. A similar control group received an equivalent diet in which ethanol was substituted by an equicaloric amount of sucrose. Ethanol treatment ameliorated emotional reactivity and improved learning. The concomitant measure of neurochemical parameters indicated changes in receptor (dopamine) and post-receptor (protein kinase C) mechanisms. The authors concluded that a dose of 4 g ethanol/kg bw/day did not produce dependence and relieved stress in otherwise normally behaving rats[255].

Ethanol appears to affect several organs after oral administration with the liver as main target organ. Increased hepatic concentrations of fatty acid and trig-

lycerids were observed after a 30-day administration of a liquid diet containing 33% of the calories as ethanol.

7.4 Toxicity due to long-term exposure and carcinogenicity

7.4.1 Inhalation studies

No studies have been found.

7.4.2 Oral studies

The IARC[172] concluded that there was inadequate evidence for the carcinogenicity of ethanol in animals.

To determine the effects on life-span of daily consumption of alcohol throughout adulthood, three groups of 100 male C57BL/10J mice (individually housed) were given 3.5% ethanol from week 14-19, and next 3.5, 7.5 or 12% v/v ethanol in distilled water as the only source of drinking fluid for the rest of their lives. Two control groups of 100 mice each (one group singly housed and the other housed five to a cage) received distilled water ad libitum. Based on the fluid intake the authors calculated an average ethanol intake of ca. 2.8, 7.3 or 11.1 g/kg bw/day, respectively. Blood samples were taken when mice were 27 weeks old and at two 3-month periods thereafter. Four samples were taken at different times of the day so that 12 samples per mouse were obtained. Mean blood alcohol levels were 66, 142 and 268 mg/kg blood (more or less equal to mg/l blood), respectively. There was no difference between the survival rates of the 3.5% alcohol and water-drinking singly housed controls. The 7.5% alcohol group had a significantly longer lifespan when compared to the single-housed controls. Of the singly housed animals 72-89 animals per group were examined histopathologically; of the cage housed animals only 55 due to cannibalism. Incidence, kind and degree of non-neoplastic hepatic lesions did not differ among the groups. In addition, no treatment-related changes were observed in brain, lungs, heart, pancreas, spleen, kidneys, duodenum or testes. The incidence and kind of neoplastic lesions in the individual groups did not indicate ethanol carcinogenicity[256].

In 1996, a 2-year oral carcinogenicity study with ethanol (administered in drinking water) in mice was started by NTP[257]. Male and female B6C3F_1 mice were exposed to ethanol (92.6% ethanol, 7.4% water) in drinking water for 4 weeks or 2 years. Groups of four male and four female mice were exposed to 0%, 2.5%, or

5% ethanol in drinking water ad libitum for 4 weeks. Concentrations of 2.5% and 5% ethanol resulted in average daily consumption of approximately 85 and 170 mg ethanol for males and 70 and 130 mg for females. In the 2-year study, groups of 48 male and 48 female mice were exposed to 0%, 2.5%, or 5% ethanol in drinking water ad libitum. Concentrations of 2.5% and 5% ethanol resulted in average daily consumption of approximately 100 and 180 mg ethanol for males and 80 and 155 mg for females. The incidences of hepatocellular neoplasms occurred with positive trends in males exposed to increasing concentrations of ethanol (hepatocellular adenoma: 0% ethanol, 7/46; 2.5% ethanol, 12/47; 5% ethanol, 19/48; hepatocellular adenoma or carcinoma: 12/46, 16/47, 25/48). Under the conditions of this 2-year drinking water study, NTP concluded the design of their study was inadequate to determine the carcinogenic activity of ethanol in male and female mice[257].

Sprague Dawley rats (50 rats/sex/group) were fed 1 or 3% ethanol or an equicaloric amount of glucose in a semi-synthetic liquid diet for 104 weeks. Based on dietary intake it was estimated that these doses corresponded to ca. 1 or 3 g/kg bw/day. From week 104-120 animals were kept on a liquid control diet. Survival was similar in all groups. Body weights were reduced from week 13 in males and from week 69 in females of the high dose group. No changes in liver and kidney weights were observed. Histopathological examination revealed more non-neoplastic changes in ethanol-treated animals when compared to controls. In males these changes included liver and bile duct injury and inflammatory reactions of the pancreas. In females, inflammatory reactions were found in the clitoral gland, and hyperplasia in the adrenals. Rats of both sexes showed hyperplasia of the thyroid gland and peripheral nerve degeneration. For male rats, no neoplastic lesions were observed that could be related to ethanol exposure. For females, an increase in mammary gland tumours was seen for females receiving the low ethanol containing diet, but not in animals of the high ethanol containing diet group. In some tumour frequency comparisons the opposite, namely a decrease in the rate of incidence, was obtained. Overall, the total tumour incidence was significantly reduced in animals of the high dose group. Based on these results the authors concluded that there was no carcinogenic activity of ethanol after long-term oral administration[258].

To study influence of ethanol on vinyl chloride-induced hepatocarcinogenicity, a (control) group of 80 male Sprague Dawley rats received drinking water with 5% ethanol v/v. Based on an average intake of 15 ml a day and an average body weight of 350 g a dose of ca. 2 g ethanol/kg bw/day was estimated. Another

control group of 80 rats received water. There was no control group to control for the increase of ethanol-derived calories. Survival rate after 18 months was similar, *i.e.* 73 and 70% for the ethanol and control group, respectively. In the ethanol group, at the end of the study, the incidence of hepatic carcinomas was 8, the incidence of hyperplastic nodules was 29, whereas in the control group, these figures were 1 and 10, respectively. The incidences of endocrine tumours were 26/79 and 8/80 (pituitary), 14/79 and 0/80 (adrenals), 14/79 and 0/80 (pancreas; not further specified), and 3/79 and 0/80 (testes), for the ethanol and control group, respectively. In total, 91 tumours were diagnosed in the ethanol group, of which 44% were classified as malignant. In the control group 16 tumours were observed of which 5 were malignant[259].

The committee noticed that in some of the experiments, tumor incidences were increased, despite the fact that the MTD has not been reached. Therefore the committee is of the opinion that, the results from these experiments were not conclusive: Tumour incidences were increased in male Sprague Dawley rats upon chronic intake of ethanol-containing drinking water, but not in male and female rats of the same strain upon oral intake of ethanol through a liquid diet. A drinking water study in mice was also negative. NTP concluded that from a 2-year drinking water study that there the design of their study was inadequate to determine the carcinogenic activity of ethanol in male and female mice.

7.5 Genotoxicity

7.5.1 In vitro

See for a summary of *in vitro* genotoxicity tests table 7.2.

Bacterial mutation assays

Ethanol was negative in several Salmonella (*S. typhimurium*) strains i.e TA97, TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of metabolic activation[172]. In another study ethanol was tested using strains TA97 and TA102. Ethanol, with metabolic activation, induced a reproducible increase in revertants over controls but this was less than 2-fold and only observed at a very high concentration of 160 mg/plate[260]. The authors, however, concluded that this result might not necessarily point to genotoxic activity of ethanol but could also have been caused by impurities present. The same

investigators had earlier shown that ethanol was negative in TA98, TA100, TA1535, TA1537, TA1538[260].

A 10 min incubation of *E. coli* strain CHY832 (temperature-sensitive phenotype RK+) with 180 mg ethanol per ml without metabolic activation resulted in an increased forward mutation frequency, *i.e.* an increase in temperature-insensitive bacteria. However, surviving rate was only 15% (Hay84). A differential DNA repair tests with derivates of *E. coli* using ethanol (1720 mmol/l) was negative[261].

Table 7.2 Summary of *in vitro* genotoxicicity tests.

Test	Concentration(s) used	Without(-) and with(+) metabolic activation	Results	Reference(s)
Ames: TA97, TA98, TA100, TA1535, TA1538	Not indicated	-/+	Negative	172,260
Ames TA97, TA102	Up to 160 mg/plate	+	Equivocal[a]	260
Ames	79 mg/plate	-/+	Negative	262
E. coli CHY832	180 mg/ml	-	Equivocal[b]	263
S. cerevisiae	Not indicated	Not indicated	Positive[c]	172
A. nidulans	40 mg/ml	Not indicated	Positive[d]	172
Micronucleus V79 cells	40 mg/ml	Not indicated	Negative	172
CA[e] Human lympho's	4-8 mg/ml	-	Negative	172
CA Human lympho's	1.16-3.48 mg/ml	-	Positive	264
CA Human lympho's	8 mg/ml	-/+	Negative	262
CA CHO cells	8 mg/ml	Not indicated	Negative	172
CA CHL cells	8 mg/ml	-/+	Negative	262
L5178Y Mouse Lymphoma TK	Up to 36 mg/ml	-	Negative	265
L5178Y Mouse Lymphoma TK	8 mg/ml	-/+	Negative	262
SCE Human lympho's	0.8-16 mg/ml	-	Negative	172
SCE Human lympho's	0.8 mg/ml	+	Negative	172
SCE CHO cells	0.8-8 mg/ml	-	Negative	172
SCE CHO cells	4-32 mg/ml	+	Positive[f]	266
SCE CHO cells	4-32 mg/ml	-	Equivocal[g]	266
SCE Mouse kidney fibroblasts	0.8 mg/ml	-	Negative	172
DNA damage Human lympho's	0.07-4.6 mg/ml	Not indicated	Negative	267
DNA damage Rat hepatocytes	8 mg/ml	Not indicated	Negative	172

[a] less than 2-fold increase (see text)
[b] surviving rate was only 15% (see text)
[c] positive at 30°C but negative at 4°C
[d] aneuploidy
[e] CA= chromosome aberrations
[f] increase already at 4 mg/ml; maximal increase of 3-times control value at 16 mg/ml
[g] increase only 1.5-fold

Ethanol has been used as a vehicle for some test compounds in the Ames test. Historical data showed no evidence of mutagenicity with or without S9 for solvent controls with a typical ethanol concentration of 100 μl (79 mg)/plate[262].

Mutation in yeast

Using *S. cerevisiae*, ethanol was mutagenic, albeit at an incubation temperature of 30°C only. At 4°C the test was negative. Using *A. nidulans*, a concentration of 5% ethanol (40 mg/ml) resulted in aneuploidy (non-disjunction, mitotic recombination). No forward mutations were induced[172].

Micronucleus assay

There were no increases in micronuclei in V79 cells following a 1 h incubation period with 5% ethanol (40 mg/ml)[172].

Chromosome aberration tests

A total of 13 investigations using human lymphocytes were described by IARC[172]. An incubation period of 24-48 hours of 0.5-1% ethanol (4-8 mg/ml) did not induce chromosome aberrations. No metabolic activation was used.

There were no increases in chromosome aberrations in Chinese Hamster Ovary (CHO) cells upon a 30 min incubation period with 1% ethanol (8 mg/ml)[172].

Human blood lymphocytes were incubated with 1.16, 2.32 or 3.48 mg/ml ethanol for 50 h. A dose-related increase in chromosome structural aberrations was observed in the absence of metabolic activation. Chromatid and chromosome gaps and breaks were recorded separately[264].

Ethanol has been used as a vehicle in chromosome aberration assays using human lymphocytes and CHL cells. Historical data demonstrated a lack of chromosome aberration induction both with and without metabolic activation at a concentration of 8 mg/ml ethanol (174 mM)[262].

Cell mutation assays

Ethanol was negative in the L5178Y Mouse Lymphoma TK assay at the highest tested concentration of 4.5% (36 mg/ml)[265]. It is, however, noted that ethanol was tested without metabolic activation.

Historical data demonstrated a lack of mutagenic activity both with and without metabolic activation in the mouse lymphoma cell mutation assay at a concentration of 8 mg/ml (174 mM) ethanol[262].

Sister chromatid exchanges (SCE)

A total of 13 investigations using human lymphocytes were described by IARC[172]. An incubation period of 48-72 hours of ethanol concentrations of 0.1-2% (0.8-16 mg/ml) did not reveal increases in SCEs. No metabolic activation was used. The only study in which metabolic activation was used did not show an increase in SCE upon application of 0.1% ethanol (0.8 mg/ml).

Ethanol was investigated on its potential to induce Sister Chromatid Exchanges (SCE) in CHO cells by several investigators. No metabolic activation was used. The following test protocols were negative: 0.5 h with 1% ethanol (8 mg/ml), 3 h with ca. 0.6% (4.8 mg/ml), 28 h with 1% (8 mg/ml) or 8 days with 0.1% (0.8 mg/ml) daily. Also a 44 h treatment of mouse kidney fibroblasts with 0.1% ethanol (0.8 mg/ml) did not induce SCEs[172].

In contrast, incubation of CHO cells with 0.5, 1, 2 or 4% ethanol (4, 8, 16 or 32 mg/ml) using metabolic activation, resulted in an increase in SCE already at the lowest concentration. The maximal increase of three times the control value was reached at a concentration of 2%. There was no cytotoxicity. Without metabolic activation, the SCE rate increased only 1.5-fold. Results were similar for both technical (96%) ethanol and absolute (100%) ethanol[266].

DNA damage

A 3 h incubation of primary rat hepatocytes with 1% ethanol (8 mg/ml) did not reveal DNA damage[172].

A 1 h incubation of human lymphocytes with 1.56-100 mM ethanol (0.07-4.6 mg/ml) did not reveal DNA single- or double-strand breaks. No cytotoxicity was observed[267].

7.5.2 In vivo experiments

Somatic cells

See for a summary of *in vivo* genotoxicicity tests in somatic cells Table 7.3.

Table 7.3 Summary of *in vivo* genotoxicicity tests in somatic cells.

Test	Species	Concentration(s) used	Results	Ref(s)
Micronuclei bone marrow	Swiss mice	Ca 50 g/kg bw	Negative	172
Micronuclei bone marrow	Ddy-mice	0.05 ml[a]	Negative	172
Micronuclei bone marrow	Parkes albino mice	0.62-1.86 g/kg bw	Equivocal[b]	264
Micronuclei bone marrow	Wistar rats	4 or 8 g/kg bw	Negative	268
Micronuclei bone marrow	CD rats	12-16 g/kg bw	Positive	269
Micronuclei bone marrow	CD rats	6 g/kg bw plus 3 g/kg bw[c]	Negative	269
Micronuclei bone marrow	Mice	2 g/kg bw	Negative	262
Micronuclei hepatocytes	Wistar rats	4 or 8 g/kg bw	Negative	268
CA[d] bone marrow	Wistar rats	4 or 8 g/kg bw	Negative	268
CA bone marrow	Chinese hamsters	10% or 10%/15%/20%	Negative	172
CA blood lympho's	Wistar rats	4 or 8 g/kg	Negative	268
CA blood lympho's	Chinese hamsters	10%	Negative	172
SCE bone marrow	CBA mice	12 or 25 g/kg bw	Equivocal[e]	270
SCE bone marrow	NIH mice	0.3-2.4 g/kg bw	Equivocal[f]	271
SCE bone marrow	Chinese hamsters	10% or 10%/15%/20%	Negative	172
SCE bone marrow	Wistar rat	4 or 8 g/kg bw	Negative	268
SCE blood lympho's	Wistar rat	4 or 8 g/kg bw	Equivocal[g]	268
SCE Foetal liver cells	ICR mice	Up to 8 g/kg bw	Positive[h]	272
SCE homogenized embryo cells	ICR mice	2 or 4 g/kg bw	Equivocal[i]	273
DNA adducts liver	C57Bl/6 mice	12 g/kg bw	Equivocal[j]	274

[a] subcutaneous application of 0.05 ml of 95% ethanol
[b] no dose-response relationship; increases at the two lowest levels only (see text)
[c] two administrations only (see text)
[d] CA= chromosome aberrations
[e] increases were statistically significant but only ca. 1.5 times the control value (see text)
[f] increases were dose-related but only ca. 1.4 times the control value at the highest dose (see text)
[g] a 1.5 times increase was obtained in both treatment groups (see text)
[h] a 2-times increase was obtained at the highest level tested only (8 g/kg bw)
[i] a 1.5 times increase was obtained at 4 g/kg bw; no increase at 2 g/kg bw
[j] the average concentration of DNA adducts was 3 times above level of detection

Micronucleus assay

Ethanol (40% v/v; ca. 50 g ethanol/kg bw/day) given to Swiss mice in drinking water for 26 days did not result in an increase in micronuclei in polychromatic erythrocytes in bone marrow. This was also true upon subcutaneous application of 0.05 ml of a 95% ethanol solution to male ddy-mice[172].

Three groups of 10-week old Parkes albino mice consisting of 4 males and 4 females each were injected intraperitoneally on 2 consecutive days with 0.3 ml of 20, 40 or 60% ethanol, corresponding to 0.62, 1.24 or 1.86 g/kg bw. A control group with the same number of animals received no treatment. Six hours after the

last treatment, animals were sacrificed and examined for micronuclei in both polychromatic and normochromatic erythrocytes in bone marrow. A number of 1000 cells per animal were examined. The test groups had higher frequencies of micronuclei than controls, but a dose-response relationship was not obtained (*i.e.* statistical significance was only reached at the two lower doses). The maximum increase was about two times the control value[264]. It is, however, noted that the study is of limited relevance, since the control group mean micronucleus frequencies of 4.63 and 5.60% in normochromatic and polychromatic erythrocytes, respectively, should be considered very high.

Adult male Wistar rats were given 10% v/v (4 rats/group) or 20% v/v (1 rat/group) ethanol (ca. 4 or 8 g/kg bw/day) as their only liquid supply for 3 or 6 weeks. Control rats (2/group) received tap water. At the end of the treatment period, rats were sacrificed and examined for micronuclei in hepatocytes and erythrocytes in bone marrow. There were no increases in the number of micronuclei[268]. These results are of limited value, however, in view of the very limited number of animals used in this study.

Male CD rats (10) were given a nutritionally adequate liquid diet containing 36% as ethanol (ca. 12-16 g/kg bw/day) for 6 weeks. A control group of 10 rats was pair-fed a liquid diet containing 36% carbohydrate. Blood alcohol concentrations measured were about 1500 mg/l. Alcohol feeding resulted in a body weight gain reduction of 15% and in a slight, though significantly, increased frequency of micronuclei in bone marrow erythrocytes. This was associated with bone marrow hypoplasia and erythrocyte macrocytosis[269]. By contrast, acute ethanol administration (6 g/kg bw) by gastric intubation (10% solution) the day before sacrifice (4 pm) followed by 3 g/kg bw at 9 am six hours before sacrifice to four rats produced no changes in bone marrow cell population, mitotic index or percentage of cells with micronuclei. According to the authors these results suggest that prolonged alcohol consumption is required to produce the bone marrow changes[269].

Ethanol at its maximum tolerated dose level (2 g/kg bw) was used as the vehicle in standard mouse micronucleus assays using the oral route. No increase in micronucleus frequency above expected levels was observed[262].

Chromosome aberration assay

Adult male Wistar rats were given 10% v/v (4 rats/group) or 20% v/v (1 rat/group) ethanol (ca. 4 or 8 g/kg bw/day) as their only liquid supply for 3 or 6 weeks. Control rats (2/group) received tap water. At the end of the treatment period, rats were sacrificed and examined for chromosomal aberrations in bone marrow and blood lymphocytes. There were no increases in the number of chromosomal aberrations[268]. These results are of limited value, however, in view of the very limited number of animals used in this study.

In Chinese hamsters the incidence of chromosomal aberrations in bone marrow cells was not increased following intake of ethanol (10% in drinking water) for 9 weeks, nor was the incidence of chromosomal aberrations in lymphocytes following oral intake for 46 weeks. The incidence of chromosomal aberrations in bone marrow cells was neither increased following intake of ethanol (10% in drinking water the first week, followed by 15% in the second and third week, and by 20% in the 4th-12th week)[172].

Sister Chromatid Exchange (SCE)

Groups of male CBA mice were given 10 or 20% (v/v) ethanol (ca. 12 or 25 g ethanol /kg bw/day) as their only liquid supply for 3 to 16 weeks. Control animals received tap water. After 3, 5, 12 or 16 weeks (10% groups), or after 12 or 16 weeks (20% groups), animals were sacrificed and bone marrow was examined. Significant increases in SCE were found at both dose levels and at all sacrifice points; the maximum increase was ca. 1.5 times the control value[270]. The results of this study, however, are of limited value in view of the limited number of animals used (2-4 per group per time point; with one exception of 6 per group) and the limited number of examined metaphases (minimally 15 but not more than ca. 30 per animal).

Upon intraperitoneal application of 50% ethanol (0.3, 0.6, 1.2 or 2.4 g/kg bw) to male NIH mice, a slight but dose-related increase in the number of SCEs was found in bone marrow cells. Thirty cells (in second division) were counted per mouse, but the number of mice per dose level was not indicated. At the highest dose of 2.4 g/kg bw a 1.4 times increase in SCEs was observed when compared to controls. The average generation time (AGT) was not changed[271].

ICR mice were force-fed with ethanol at different doses, administration rates and durations during pregnancy. The highest cumulative dosis was 8 g ethanol/kg bw. The maximal blood alcohol level was 450 mg/l. BrdU was administered sub-

cutaneously in the animals between days 16-18 of gestation. Twenty-one hours later, the animals were killed, the foetuses removed and the foetal liver cells were examined for SCEs. Per dosis group 2-3 pregnant animals were treated; per litter 50 metaphases were examined. A dose-related increase in SCEs up to two-times the control value was found in ethanol-fed mice. Doses up to 4 g/kg bw did not result in increased numbers of SCEs[272].

A single application of a 10% ethanol solution (4 g/kg bw) to four pregnant ICR mice on day 10 of gestation resulted in a 1.5 times higher SCE frequency in litter-pooled embryo chromosomes when compared to controls. This dose resulted in a blood alcohol level of 2250 mg/l. A dose of 2 g/kg was inactive[273].

Adult male Wistar rats were given 10% v/v (4 rats/group) or 20% v/v (1 rat/group) ethanol (ca. 4 or 8 g/kg bw/day) as their only liquid supply for 3 or 6 weeks. Control rats (2/group) received tap water. At the end of the treatment period, rats were sacrificed and examined for SCEs in bone marrow and blood lymphocytes. The SCE frequency in blood lymphocytes was 1.5 times higher in both treatment groups following 3 and 6 weeks of treatment when compared to controls[268]. These results are of limited value, however, in view of the very limited number of animals used in this study.

In Chinese hamsters the incidence of SCEs in bone marrow cells was not increased following intake of ethanol (10% in drinking water the first week, followed by 15% in the second and third week, and by 20% in the 4th-12th week). Also, no increases in SCEs were observed upon oral intake (10% in drinking water) for 46 weeks[172].

DNA adduct formation

A group of 7 male C57BL/6 mice was given ethanol in drinking water (10% v/v) ad libitum for 5 weeks (ca. 12 g/ethanol/kg bw/day). At the end of the treatment period, animals were killed, livers were excised and the DNA isolated for measurement of DNA adducts using the 32P-postlabelling method. The average concentration of the adducts was 1.5 ± 0.8 adducts per 10^8 nucleotides. No adducts were found in untreated controls; the level of detection was 5 adducts per 10^9 nucleotides[274].

Germinal cells

See for a summary of *in vivo* genotoxicicity tests in germinal cells Table 7.4.

Table 7.4 Summary of *in vivo* genotoxicicity tests in germinal cells.

Test	Species	Concentration(s) used	Results	Ref(s)
CA[a] spermatogonia	Wistar rats	10% in drinking water	Negative	172
CA testicular cells	Sprague Dawley rats	7 to 20% in drinking water	Negative	276
Aneuploidy testicular cells	CBA/CA mice	Ca. 5 g/kg bw	Equivocal[b]	275
Aneuploidy sperma cells[c]	Chinese hamsters	1.5 ml of 12.5%	Negative	172
Aneuploidy eggs of mated females	(C57BLxCBA) F1 mice	Ca. 4-6 g/kg bw	Positive[d]	277
Aneuploidy eggs of mated females	(C57BLxCBA) F1 mice	Ca. 5 g/kg bw	Positive[e]	277
Aneuploidy Eggs, embryos and foetuses	CFLP mice	Ca. 7.5 g/kg bw	Positive	278
Aneuploidy embryos	(C3HxC57) F1 mice	Ca. 5 g/kg bw	Positive	279
Dominant lethal assay	CBA mice	Ca. 1.24 and 1.86 g/kg bw	Positive	264,280
Dominat lethal assay	CFLP mice	Up to 0.6 g/kg bw	Negative	281
Dominant lethal assay	Long Evans rats	Ca. 10 g/kg bw	Positive	282
Dominant lethal assay	Wistar rats	Ca. 15 g/kg bw	Negative[f]	283
Dominant lethal assay	Wistar rats	Increasing concentrations up to ca. 15 g/kg bw	Negative[f]	283
Dominant lethal assay[g]	Sprague Dawley rats	Ca. 10-15 g/kg bw	Positive	284
Dominant lethal assay[h]	Rats (not further specified)	Ca. 2.5 g/kg bw	Equivocal[i]	285
Dominant lethal mutations in egg cells	(C3HxC57) F1 mice	Ca. 5 g/kg bw	Positive	279

[a] CA = chromosome aberrations (not further specified)
[b] although overall frequency of aneuploidy was significantly higher in test groups, a limited number of measurements was carried out at each sacrifice point (see text)
[c] spermatogonia and spermatids type I and II
[d] however, no dose-response relationship was obtained
[e] in this study it was shown that the non-disjunction was female-derived (see text)
[f] limitations in reporting (see text)
[g] each male was mated with two females only (see text)
[h] each male was allowed to mate with an unknown number of females only once (see text)
[i] the test group consisted of a limited number of females only

Non-disjunction and aneuploidy

Male CBA/CA mice were administered ca. 0.8 ml of 12.5 or 15% ethanol in PBS (ca. 5 g/kg bw) by gastric intubation. Blood alcohol levels of 4,000-6,000 mg/kg (~4,000-6,000 mg/l) were obtained. Controls received PBS only. Most test animals showed signs of CNS depression some 15-20 min after gastric instillation; complete recovery occurred within several hours. The animals were sacrificed 2, 3, 4, 5 or 6 h after treatment and the testes were removed and cell counts of second meiotic metaphase divisions were scored. The overall frequency of aneup-

loidy was 2.99 and 4.15% for the 12.5 and 15% groups, respectively. The aneuploidy level of controls (*i.e.* 0.50%) was significantly lower. When the results of the test groups were pooled, a decrease in aneuploidy was seen with time[275]. It is, however, noted that only 7 to 10 animals per group were used, resulting in only 1 or 2 animals per sacrifice point and the maximum number of counted cells at each sacrifice point was only 190.

No increase in chromosome aberrations was observed in spermatogonia of male Wistar rats that had received 10% ethanol in drinking water[172]. A drinking water study in which Sprague Dawley rats received ethanol over 36 weeks, gradually increased from 7% until 20% (w/v), also did not reveal differences in chromosomal aberrations in testicular cells[276]. It is, however, noted that although the control and test group consisted of about 30 animals each, the number of analysable mitoses was limited and very variable.

No aneuploidy was observed in spermatogonia and spermatids type I and II of male Chinese hamsters upon administration of 1.5 ml of 12.5% ethanol[172].

Recently mated 6-8 weeks old (C57BLxCBA) F1 mice were given orally 1 ml of either a 10%, 12.5% or 15% solution of ethanol (ca. 4-6 g/kg bw) in distilled water 13.5 h after superovulation (ca. 1.5-2.5 h after the predicted time of ovulation). Controls received distilled water. The females were sacrificed 6-7 h later and the eggs were examined for the presence of pronuclei. In the test animals, the percentage of aneuploidic cells was between 5.9 and 18.9% but no dose-response relationship was obtained. No aneuploidy was observed in controls. Since from this test it was not clear whether the origin of the aneuploidy was male- or female-derived, a second series of experiments was carried out. (C57BLxCBA)F1 mice were mated with males that were homozygous for the T6 translocation in which a reciprocal change occurs between segments of chromosomes 14 and 15 with the resultant production of a larger and a small translocation product (T6 marker chromosome). The female mice were given orally 1 ml of a 12.5% solution of ethanol (ca. 5 g/kg bw) in distilled water 13.5 h after superovulation (ca. 1.5-2.5 h after the predicted time of ovulation). The females were sacrificed 6-7 h later and the eggs were examined for the presence of pronuclei. The results of this study showed that the percentage of aneuploidic cells was 17.9% and that the non-disjunction was female-derived[277].

Eight to twelve week-old female CFLP mice, after induction of a superovulation, were mated with naive males. Females that had mated were given 1.5 ml of a 12.5% ethanol solution (ca. 7.5 g/kg/bw) via gastric intubation 4, 13.5 or 17 h after the superovulation. Blood alcohol levels reached levels up to 2600-2800

mg/l. Controls received distilled water 13.5 h after the superovulation. The females were sacrificed 20-21 h after the superovulation (first cleavage), on the 3rd day (morula stage) or on gestation day 10 or 11. The incidence of aneuploidy was significantly increased (approximately 19% and 13.5%) 20-21 h after the superovulation or the 3rd day, respectively, with a similar number of monosomic and trisomic conceptuses. In addition, about 2% of all conceptuses examined were triploid. From animals sacrificed on gestation day 10 or 11, only the morphological abnormal or developmentally retarded embryos were determined. Eight embryos of a total of 16 were either aneuploid or triploid, whereas in the control group only one out of 11 examined proved to be aneuploid[278].

Female 10-12 weeks old (C3H x C57)F1 mice were, after induction of a superovulation, mated with untreated males. Females that showed a vaginal plug (19 in total) were administered orally with 1 ml 12.5% ethanol (ca. 5 g/kg bw) ca. 1.5 to 2.5 h after ovulation. A control group (13) received distilled water. About 9-10 hours later, the animals received 2.5 mg colchicin/kg bw intraperitoneally. Fifteen hours after colchicine treatment cytogenetic analysis was performed. In total 105 embryos were examined in the test group, whereas 59 were examined in the control group. The percentage of embryos with a normal chromosome number (40) was not different between test and control group. Two embryos with 41 chromosomes were observed in the test group (none in controls), and 11 embryos with 39 chromosomes were found (2 in controls). It was concluded by the authors that ethanol treatment resulted in aneuploidy[279].

Dominant lethal assay

In a dominant lethal assay, groups of 10-week old male CBA mice were given 0.1 ml of a 40% ethanol solution (ca. 1.24 g/kg bw) once a day for 3 consecutive days. Controls received no alcohol treatment. After treatment the test males were allowed to mate with virgin females at 7-day intervals until the 6th week. Pregnant females were allowed to produce their litters, size, sex ratio and morphological abnormalities of the litters were recorded. The frequency of fertile matings was not different amongst the groups. A significantly reduced litter size was observed produced from matings that took place 14-17 days after cessation of treatment. In two repeat experiments, 40 and 60% ethanol solutions were used (ca. 1.24 and 1.86 g/kg bw). Males were allowed to mate at 4-day intervals. Pregnant females were not allowed to produce their litters but were sacrificed 13-15 days after conception. The number of corpora lutea, dead and live implants were examined. There was a significant increase in the number of dead implants (con-

comitant with a decrease in the number of life implants) in females mated on days 4-13 after treatment with both dose levels. Male group size was 6-13[264,280].

In a dominant lethal assay (carried out in three laboratories as a ring study) 10-week old male CFLP mice (15/group) were given 10 or 40% ethanol (2 ml/kg bw/dag) for 5 consecutive days. In a preliminary study, the high dose of 0.6 g ethanol/kg bw/day was concluded to be the MTD (maximum tolerated dose). Next, each male was caged sequentially with 2 untreated virgin females (10-12 weeks old) each week for 8 consecutive weeks. All females were killed and examined 18 days after first being caged with the males. The dominant lethal mutation rate was not increased in the three tests. The few statistically significant changes observed were not consistent and did not point to dominant lethal mutations[281].

To determine dominant lethal mutations in egg cells, 10-12 weeks old (C3H x C57)F1 female mice were administered orally 1 ml 12.5% ethanol (ca. 5 g ethanol/kg bw) or distilled water 1, 1.5 or 2 h after mating. The females were sacrificed on day 12 (1 and 1.5 h postmating groups) or day 17 (1 or 2 h post-mating groups) of gestation. The incidence of late death was found to be significantly increased when ethanol was administered 2 h following a 30-min mating period, but not when the interval was shorter. Foetuses classified as having died late were those that had developed at least some eye pigment (*i.e.* had died on day 11 postconception or later). Measurements of early death were not sensitive enough (because of the high control frequency) to show an effect of ethanol treatment. A level of significance of <0.05 could only be reached when group size was higher than 40[279].

In a dominant lethal assay, a group of 10 male Long Evans rats was given 20% v/v ethanol in the drinking water for 60 days (ca. 10 g/kg bw/day). Controls received distilled water. Each male was then allowed to mate with three virgin female rats once per week for three consecutive weeks. The females were killed on day 20 of gestation, and the offspring were examined for fetal growth, skeletal ossification, and soft-tissue anomalies. Total embryonic deaths (resorptions and preimplantation loss) were increased by ethanol, while implantations and litter size were significantly decreased. Foetuses fathered by alcoholic male rats were malformed: 55% had soft-tissue anomalies (microcephalus, microphthalmia, cranial fissure, and hydronephrosis). Litter weight and average pups weight were also reduced by paternal ethanol consumption ([282]; see also section 6.2.6).

In another dominant lethal assay, a group of male Wistar rats (number not indicated) received 30% ethanol in drinking water (ca. 15 g ethanol/kg bw) for 4 days. Immediately after the last treatment the males were allowed to mate with 2-

3 virgin female rats once per week for 8 consecutive weeks. No changes were observed in the number of corpora lutea nor in the number of dead, live and total implantations when compared to an untreated control group (number not indicated). Also administration of ethanol to male Wistar rats (numbers not indicated) for 35 days, at concentrations increasing from 15 to 20% or from 15 to 30%, did not show dominant lethal mutations[283]. In every group and at each mating point 26-30 female animals were used, which indicates that the number of males per group should have been minimally 9-10.

A group of 6 male Sprague Dawley rats received a liquid diet containing 6% ethanol during one week followed by a liquid diet containing 10% ethanol for another 4 weeks. Based on the daily liquid diet intake, a dose of 10-15 g ethanol/kg bw/day was estimated. Mean blood alcohol levels were low (0.5 mg%, *i.e.* 5 mg/l) but it is noted that these levels were measured only 12h after the last alcohol exposure. A control group of 6 male rats received the liquid diet with an isocaloric amount of sucrose substituted for the alcohol. After 15 days each male was placed with 2 females during the hours of darkness; during this time diet and drinking water were not available. The males were separated from the females during light hours. Successful matings and the first day of pregnancy were determined by the presence of vaginal plugs and of spermatozoa in the vaginal smears. All pregnancies were terminated on gestational day 20. Notable differences detected were: a higher incidence of early abortions, a smaller mean litter size, an increased foetal weight, an increase in crown-rump length, and a decrease in placental index (relating foetal weight to placental weight). According to the authors, the increase in foetal weight and crown-rump length were explained by the smaller litter sizes. Taking this into account, the mean growth index indicated smaller offspring from treated than from untreated males, although the difference was not statistically significant ([284]; see also section 6.2.6).

A group of male rats was administered 2 ml 40% ethanol solution (ca. 2.5 g/kg bw). Two weeks later they were allowed to mate with virgin females. A significant increase in pre-and post-implantation losses were observed in treated males when compared to controls. The control group contained 34 pregnant females whereas the test group consisted of 11 pregnant females only. When male rats were allowed to mate 6 weeks after ethanol administration, no differences were observed in pre- and post-implantation losses ([285] (in Russian; no English translation available) cited in[1]). However, due to several omissions in methodology the results of this study are difficult to judge[1].

The Committee on the Evaluation of the carcinogenicity of chemical substances concluded that a lot of information is available regarding the genotoxic properties of ethanol. In a minority of the *in vivo* and *in vitro* genotoxicity tests (other than aneuploidy), ethanol was positive. Most genotoxicity tests were negative after treatment with ethanol. Therefore, based on the genotoxicity tests they conclude that there is only limited evidence for a genotoxic potential of ethanol. However, acetaldehyde, a genotoxic compound, is one of the major metabolites of ethanol and can be detected in the human body after ingestion of ethanol. As a role for acetaldehyde in the genotoxicity of ethanol cannot be excluded, the committee cannot neglect the few positive results in the genotoxicity test (see Annex E).

7.6 Reproduction toxicity

7.6.1 Fertility

Inhalation studies

Whole body exposure of male Sprague Dawley rats (18 rats/group) to 0, 19,000 or 30,400 mg/m^3 ethanol (10,000 or 16,000 ppm, respectively) 7 h/day for 6 weeks did not result in changes in male fertility upon mating with untreated females. No changes were observed in body weights, food and water intake of these males. Although blood alcohol levels were not measured in these studies, levels of 30 and 500 mg/l ethanol were reported to have been found in previous studies upon exposure to 19,000 or 30,400 mg/m^3, respectively[286,287]. It is, however, not indicated at which time blood samples were taken.

Oral studies

In a dominant lethal assay (carried out in three laboratories as a ring study) 10 week-old male CFLP mice (15/group) were given 10 or 40% ethanol (2 ml/kg bw/day) for 5 consecutive days. In a preliminary study, the high dose of 0.6 g ethanol/kg bw/day was concluded to be a MTD (maximum tolerated dose). Next, each male was caged sequentially with 2 untreated virgin females (10-12 weeks old) each week for 8 consecutive weeks. Ethanol did not influence mating behaviour and success ([281]; see also section 6.2.5). It is, however, noted that a dose of 0.6 g/kg bw/day, which was considered a MTD, was low in comparison with all other studies reported.

A group of 6 male Sprague Dawley rats received a liquid diet containing 6% ethanol during one week followed by a liquid diet containing 10% ethanol for another 4 weeks. Based on the daily liquid diet intake, a dose of 10-15 g ethanol/kg bw/day was estimated. A control group of 6 male rats received the liquid diet with an isocaloric amount of sucrose substituted for the alcohol. After 15 days each male was placed with 2 females during the hours of darkness. Successful matings and the first day of pregnancy were determined by the presence of vaginal plugs and of spermatozoa in the vaginal smears. On day 36, all males were killed and blood samples collected. Obvious signs of intoxication such as weight loss, withdrawal symptoms (when liquid diet was not available), decreased serum glucose and serum testosterone levels as well as reduced reproductive performance were observed in treated males[284].

A group of 10 male Long Evans rats was given 20% v/v ethanol in the drinking water for 60 days (ca. 10 g/kg bw/day). Controls received distilled water. Each male was then allowed to mate with three virgin female rats once per week for three consecutive weeks. The males were necropsied after the third mating. Ethanol caused mean testicular weight reductions and gross testicular atrophy in 1 of 10 males. Five matings of alcoholic males proved infertile. No recovery in reproductive function was evident over the 21-day post-ethanol mating period[282].

Other studies into the fertility effects of ethanol following oral intake have shown that oral intake of high ethanol doses (> 2000 mg/kg day) by female rats and mice before and during pregnancy had no influence on mating behaviour. In males, repeated oral intake of high ethanol doses resulted in tissue changes of reproductive organs of both rats and mice.Plasma testosteron and luteinising hormone levels were reduced in male rats, whereas changes in oestrus cycle and ovary function were observed in female mice, rats, rabbits and monkeys[1].

Overall, exposure of rats by inhalation up to concentrations of 30,400 mg/m^3 ethanol (16,000 ppm), resulting in blood alcohol levels of about 500 mg/l, did not result in changes in male fertility. Oral intake of high amounts of ethanol (ca. 10 g ethanol/kg bw/day or higher), in contrast, resulted in decreased reproductive performance, decreased serum testosterone levels, decreased testicular weight and testicular atrophy in rats. An oral study in male mice at levels up to 0.6 g ethanol/kg bw/day) did not influence mating behaviour and success. Other studies into the fertility effects of ethanol have shown that oral intake of high ethanol doses (> 2 g/kg bw/day) before and during pregnancy had no influence on mating behaviour of females.

7.6.2 *Developmental toxicity*

Inhalation studies

Groups of pregnant ICR mice (10 mice/group) were continuously exposed whole body to 0 or 15,000 mg/m^3 (7950 ppm) ethanol on gestation days 7-9 or on gestation days 7-12. In satellite, non-pregnant animals, blood was sampled on days 1, 3 and 6 of exposure. Blood alcohol levels of 26-32 mg/l were found. Dams were sacrificed on day 18 of gestation. There were no significant differences in skeletal malformations or variations. The number of resorptions and dead foetuses was increased, and the number of life foetuses was decreased in the 6-day-exposure group when compared to controls. No changes were observed in foetal and placenta weight[288]. It is noted that blood alcohol levels were very low, and no explanation for these low levels could be found. In the same paper, however, an intraperitoneal injection of 4 g/kg bw ethanol was reported to result in a blood alcohol level of ca. 5500 mg/l 5 min after injection which decreased to ca. 2300 mg/l at 180 min, indicating that the measurement method seemed to be correct. However, since no data were reported on maternal toxicity, these data cannot be properly used to evaluate developmental toxicity.

Whole body exposure of pregnant female Sprague Dawley rats (15 rats/group) to 0, 10,000, 16,000 or 20,000 ppm ethanol (19,000, 30,400 or 38,000 mg/m^3, respectively) for 7 h/day throughout gestation days 1-19 did not result in changes in the offspring (sacrificed on day 20), *i.e.* no skeletal and visceral defects were observed and no changes in the number of implantations, resorptions and foetal body weight were seen. Dams exposed to 20,000 ppm were narcotised by the end of exposure and maternal weight gain and food intake were reduced during the first week of exposure. Reduced weight gain during the first week was also observed in dams exposed to 16,000 ppm. Blood alcohol levels were measured in 3 non-pregnant animals per group exposed for 1, 10 or 19 days. About 5 min after exposure cessation, animals were removed from the exposure chamber and blood was taken from the vena cava. Blood alcohol levels were between 10-44, 330-1100 and 900-2540 mg/l, for the low, mid, and high concentration, respectively[289].

Male Sprague Dawley rats (18 rats/group) were exposed whole body to 0, 10,000 or 16,000 ppm ethanol (19,000 or 30,400 mg/m3, respectively) 7 h/day for 6 weeks and after a two day non-exposure were mated with untreated females. Also pregnant female Sprague Dawley rats (15 rats/group) were

exposed to the same levels for 7 h/day throughout gestation (days 1-20). No changes were observed in body weights, and food and water intake of these males and females. The offspring of both paternally and maternally exposed rats did not show changes in number, body weight gain or in neuromotor coordination, activity or learning ability at the age of 16-58 days[286]. There were, however, slight changes in neurochemical parameters observed at the age of 21 days[287]. Since these changes were not concentration-related and were often not consistent across brain regions, it is difficult to conclude about their toxicological significance.

Oral studies

Exposure of pregnant C57BL/6J mice to 5.4 g ethanol/100 ml diet (30% of the calories were ethanol derived) during gestation days 5-11 resulted in delayed sexual maturation as measured by the time of vaginal opening in female offspring, when compared to a normal fed control group and a pair-fed sucrose control group. Histopathological examination of the vaginas of some of the animals revealed heterotropic vaginal epithelium[290].

C57Bl/6J mice were fed a liquid diet in which 17, 25 or 30% of the calories were derived from ethanol during gestation days 5-10. [Based on the mean liquid intake and the mean body weight data, these dose levels correspond to ca. 15, 25 or 25 g/kg bw/day, respectively. It is noted that liquid diet intake of the 30% group was about 20% lower than that of the 25% group, which finally resulted in about the same intake per kg bw]. Control mice were fed normal diet or pair-fed identical diets except that sucrose substituted isocalorically for ethanol. Blood samples were collected from satellite pregnant animals, also on the liquid diet, each morning at 8.00-9.00 AM on gestational days 6-11. Blood alcohol reached levels up to ca. 20, 260 and 380 mg% (200, 2600 and 3800 mg/l), for the 17, 25 and 30% groups, respectively. No changes in maternal body weights were observed (no further data on maternal toxicity were reported). At term (gestation day 19), foetuses were removed and examined. The incidence of foetal resorptions and congenital malformations increased in a dose-related manner in animals of the 25 and 30% groups. Anomalies included skeletal, neurological, urogenital, and cardiovascular systems. Animals of the 17% group did not show any more anomalous offspring than its pair-fed control group[291].

Pregnant C57Bl/6J mice were exposed by gastric intubation to 25% ethanol administered in two doses of 2.9 g/kg four hours apart on gestation days 7 or 10, or in a single dose of 5.8 g/kg on gestation days 8 or 9. Mice were also exposed

to 25% ethanol by intraperitoneal injection (either two doses of 2.9 g/kg four hours on gestational days 7, 8, 9, or 10, or six hours apart on gestational days 7 or 10). Blood samples were taken from the first group treated by gastric intubation, and from both intraperitoneal groups 30 min after (each) administration and at hourly intervals for the next 8-12 hours. Upon gastric intubation, 30 min after the first administration a highest blood alcohol level of ca. 3000 mg/l was obtained; 30 min after the 2nd administration a highest level of ca. 4000 mg/l was reached. Upon intraperitoneal administration, maternal blood levels reached values of ca. 4000 and 6000 mg/l, respectively. Mice were sacrificed on day 18 of gestation. The incidence of malformations varied according to the stage of embryonic development at the time of exposure, the route of administration, and the amount of alcohol and the time period over which it was administered. Oral doses of alcohol were teratogenic although less so than the same dose given intraperitoneally, and two intraperitoneal doses four hours apart produced significantly more malformations than the same two doses six hours apart[292].

Maternal mice were fed a liquid diet containing 20% ethanol-derived calories during pregnancy, pregnancy and lactation, or lactation. Ad libitum-fed and pair-fed control groups, fed a control liquid diet, were included. Blood samples were taken on day 12 of pregnancy and day 11 of lactation at 8 PM (2 hours after lights off). On day 12 of pregnancy average blood alcohol levels were 290-530 mg/l whereas on day 11 of lactation levels were about 2480-2950 mg/l. Ethanol exposure reduced the number of splenic lymphocytes and altered the phenotypic development of these cells of offspring reared by these animals. The greatest effect occurred when females consumed ethanol during the period of lactation. The authors suggested that direct exposure of the nursing offspring to ethanol via the breast milk was responsible[293].

Ethanol containing liquid diet (30% of daily caloric intake) was administered to pregnant albino rats (strain not specified) on gestation days 1-10. The control group received isocaloric sucrose-liquid diet. At term the animals were sacrificed. Blood alcohol concentrations were reported to be ca. 190 mg% (1900 mg/l) [no further details were given]. Foetal changes consisted of prenatal growth retardation, resorption and still births, cleft palate, hydrocephaly and lowered brain weights, hydronephrosis, and limb and joint defects[294]. Since no data were reported on maternal toxicity, these data cannot be properly used to evaluate developmental toxicity.

In their evaluation, the Committee on Alcohol consumption and Reproduction described studies of Bonthius *et al.*, Kelly *et al.*, Pierce *et al.* and West *et al.*[295-301]. These animal studies showed that prenatal exposure to ethanol during the trimester of pregnancy which is comparable to the third trimester of human pregnancy, might result in microencephaly or decreased brain weight. This effect seems to be best correlated to the maximal alcohol concentration in blood (BAC_{max}) and not to the total exposure (AUC).

7.7 Summary and evaluation

The lowest lethal dose by inhalation is 55,100 mg/m^3 (29,000 ppm) in mice (7-h exposure) and 24,130 mg/m^3 (12,700 ppm) in rats (22 hours). In one study behavioural depression occurred in rats inhaling 384 mg/m^3 (202 ppm) for 45 minutes, although the rats developed tolerance after two days in a similar exposure scenario, and there was no effect on the reinforcement rate by exposure to 384 mg/m^3 (202 ppm) for 80 minutes. The lowest reported lethal dermal dose for rabbits is 20 g/kg bw. In rabbits acute occluded exposure to 95% ethanol caused mild irritation. 96% ethanol is mildly irritating to the eyes of the rabbit.

In the studies with repeated inhalation exposure, only one concentration of ethanol was tested and only specific endpoints were studied, rendering these studies not appropriate to establish health-based occupational exposure limits. From these data, however, it was concluded that at the low concentration of 86 mg/m^3 no changes were observed, whereas at the high levels used in the other two studies (*i.e.* resulting in blood alcohol concentrations > 1700 mg/l), only slight toxicity was observed.

After repeated oral administration, ethanol appears to affect all organs with the liver as main target organ. Increased hepatic concentrations of fatty acid and triglycerids were observed after a 30-day administration of a liquid diet containing 33% of the calories as ethanol.

No long-term inhalation studies have been found and the results from long-term oral exposure studies were inconclusive. Overall, the results from these experiments were not conclusive: Tumour incidences were increased in male Sprague Dawley rats upon chronic intake of ethanol-containing drinking water, but not in male and female rats of the same strain upon oral intake of ethanol through a liquid diet. A drinking water study in mice was also negative. Because in all three studies the MTD (maximal tolerated dose) was not reached, these studies are inadequate to evaluate the carcinogenic potential of ethanol.

Ethanol is probably not mutagenic in bacteria. In most in vitro test with mammalian cells, ethanol was negative as well. Only a minority of the in vivo micronucleus tests is positive. There is, however, some evidence that ethanol induces SCE in vivo. Further, ethanol induces aneuploidy in male and female mouse germ cells. Finally, ethanol was positive in several, but not all, dominant lethal assays in the rat and mouse.

The Committee on the Evaluation of the carcinogenicity of chemical substances concluded that a lot of information is available regarding the genotoxic properties of ethanol. In a minority of the *in vivo* and *in vitro* genotoxicity tests (other than aneuploidy), ethanol was positive. Most genotoxicity tests were negative after treatment with ethanol. Therefore, based on the genotoxicity tests they conclude that there is only limited evidence for a genotoxic potential of ethanol. However, acetaldehyde, a genotoxic compound, is one of the major metabolites of ethanol and (allthough in limited studies) low concentrations of aceetaldehyde has been detected in the human body after ingestion of ethanol. As a role for acetaldehyde in the genotoxicity of ethanol cannot be excluded, the committee cannot neglect the few positive results in the genotoxicity test.

Inhalation exposure of rats to concentrations up to 30,400 mg/m^3 ethanol (16,000 ppm) resulting in blood alcohol levels of about 500 mg/l, did not result in changes in male fertility. Oral intake of high amounts of ethanol (ca. 10 g ethanol/kg bw/day or higher), in contrast, resulted in decreased reproductive performance, decreased serum testosterone levels, decreased testicular weight and testicular atrophy in rats. An oral study in male mice at levels up to 0.6 g ethanol/kg bw/day) did not influence mating behaviour and success. Other studies into the fertility effects of ethanol have shown that oral intake of high ethanol doses (> 2 g/kg bw/day) before and during pregnancy had no influence on mating behaviour of females.

Exposure of female rats by inhalation during pregnancy up to concentrations of 20,000 ppm (38,000 mg/m^3) ethanol, resulting in blood alcohol levels up to about 2500 mg/l, did not result in developmental toxicity although maternal toxicity was observed. Exposure of male rats to concentrations up to 16,000 ppm (30,400 mg/m^3) did not show changes in paternal offspring. Teratogenic effects, however, were observed in rats and mice following oral intake of large amounts of ethanol during pregnancy, resulting in blood alcohol levels of about 2000-6000 mg/l. No teratogenic effects were observed in mice after oral intake of ethanol resulting in blood alcohol levels of about 200 mg/l. Several animal

studies showed that prenatal exposure to ethanol, might result in microencephaly or decreased brain weight. This effect seemed to be best correlated to the maximal alcohol concentration in blood (BAC_{max}) and not to the total exposure (AUC).

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

No guidelines were found for the general population covering the inhalation or dermal exposure to ethanol.

In 1995, a UK report of an inter-departmental working group on sensible drinking[3] recommended a maximum oral intake of 3 units (8 grams of ethanol/ unit) per day for women, with 1-2 units giving the maximum health advantage. For men, the corresponding recommendations were drinking not more than 4 units/day to avoid adverse health effects, with 1-2 units/day as the optimal amount.

The WHO acknowledged epidemiological reports which "have found evidence of a protective effect of levels of drinking as low as one drink per week" (no defined unit concentration), but advised that any beneficial health effects should be sought by other means due to the overwhelming number of adverse effects[302]. The 2001, WHO Global Status Report on Alcohol[302] stated that practically no organ in the body is immune from alcohol related harm. Intake can lead to ethanol toxicity, alcoholic liver cirrhosis, alcoholic cardiomyopathy, alcoholic polyneuropathy etc. Although large-scale epidemiology studies have found evidence of a protective effect of as little as one drink per week, this is relevant only in populations where low levels of drinking are the norm. WHO has therefore no recommended intake threshold.

8.2 Working population

International occupational exposure standards in mg/m^3 (ppm) as Time-Weighted Averages for 8-hr exposure (TWA) are summarised in Table 8.1.

Table 8.1 International occupational exposure standards for ethanol.

Country/ organisation	Occupational exposure limit		Time-weighted average	Type of xposure limit	Note	Year of adaptation	reference
	mg/m^3	ppm					
The Netherlands -ministry	1000	500	8 h	Administrative force			303
Germany							
-DFG MAK- committee	960 4800	500 2500	8 h 30 min, max. two times/shift		Category 5[a] Group C Group 2	Set 1998 STEL[b] set 2001	304
United Kingdom							
-HSE	1900	1000	8 h				305
USA							
-ACGIH	1880	1000	8 h	TLV		Revised 1996	8
-OSHA	1900	1000	8 h	PEL			306
-NIOSH	1900	1000	10 h	REL			307
Sweden	1000 1900	500 1000	8 h 15 min			Revised in 1993	308
Denmark	1900	1000	8 h				309

[a] Carcinogen effect: category 5
Reproduction toxic effect: group 2
Genotoxic effect: group C

[b] STEL: Short Term Exposure Limit

In the Netherlands, the Health Council's committee Compounds toxic to reproduction classified ethanol in category 1 (according to EU guidelines) for its effects on fertility and development (*Substances known to impair fertility in humans, substances known to cause developmental toxicity in humans*). For its effects on lactation, the committee recommended to label ethanol with R64 (*may cause harm to breastfed babies*).

The Deutsche Forschungsgemeinschaft (DFG) re-evaluated ethanol in 1998[1]. It concluded that the critical effects of occupational exposure are an increased risk of certain forms of cancer. It is stressed in the assessment that there are so far no studies linking a possible carcinogen effect and occupational exposure by inhalation. However, it has been shown in humans that the chronic oral intake of ethanol increases the risk of tumours in the mouth, pharynx, larynx, oesophagus,

liver and probably also in mammary glands and the colon, presumably via acetaldehyde and other genotoxic, reactive metabolites of ethanol. Assuming linear kinetics, the amount of metabolites will increase proportionally with the intake of ethanol with no apparent threshold limit of carcinogen effect. The occupational exposure should therefore be kept to a minimum, to ensure that the internal exposure due to lifetime endogenous ethanol exposure will not be significantly increased. The Area Under the Curve (AUC) parameter (time versus BAC) can be employed to assess whether a given inhalatory exposure pattern will cause a significant increase in the BAC (compared to the endogenous BAC) and consequently in the carcinogenic risk. On this basis, ethanol was assigned to category 5: the carcinogenic potential is so low that the MAK value (500 ppm) will not represent an unacceptable risk level, as the resulting occupational exposure will lie within the standard deviation of the endogenous level.

For acute exposure ethanol was classified to category II,2 in 2001, limiting the acute exposure to 2500 ppm, 30 min, 2 times per shift. This is due to the short half-life and rapid resorption. Due to genotoxic effects ethanol is considered a group 2 substance (the substance is shown to be genotoxic in studies performed on mammals). Adverse reproduction effects are seen in humans and animals only at high concentrations of exposure, justifying a classification in group C (no need for concern at exposure levels at/lower than the MAK level). No threshold was found for teratogenic effects, but the German MAK-committee concluded that effects were observed at an intake in excess of 30 g/day for pregnant women, after careful assessment of the available data. It was not deemed necessary to give ethanol a skin-notation.

The scientific basis for Swedish occupational standards is set by the Swedish National Institute of Occupational Health. It concluded that the critical effects on occupational exposure to ethanol vapour is irritation of mucous membranes. The assessment stressed that there were no inhalation studies on the mutagenic/genotoxic and carcinogenic effects of ethanol, but registered that IARC considered that there was “sufficient evidence” that acetaldehyde is carcinogenic to experimental animals, and that drinks containing alcohol are carcinogenic to humans. Some animal inhalation studies have shown teratogenic effects, while repeated intake of ethanol by mothers during gestation has also been shown to damage human foetuses[141].

IARC[172] considered there was sufficient evidence to classify the major metabolite of ethanol, acetaldehyde, as a carcinogen in experimental animals. However,

there was inadequate evidence for the carcinogenicity of ethanol in experimental animals. IARC found sufficient evidence for the carcinogenicity of alcoholic beverages in humans (Group 1), and a causal relation between consumption of alcoholic beverages and the occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus and liver. In most of the evaluated studies ethanol was administered orally.

ACGIH[307] recommended a TLV-TWA of 1000 ppm, based on the lack of eye and upper respiratory tract irritation at levels below 5000 ppm and on long industrial hygiene experience with human exposures to ethanol. The designation A4, Not Classifiable as a Human Carcinogen, was assigned due to the lack of animal data addressing the carcinogenicity of ethanol. No STEL will be recommended until sufficient toxicological data and industrial hygiene experience is available. Data used in the assessment show that the acute toxicity is low for animals and humans, although low concentrations will be irritating to the upper respiratory tract and eyes. This effect was deemed more important in setting the limit than secondary toxic effects of absorbed ethanol[310].

In the OSHA (Occupational Safety & Health Administration) classification system of health effects, ethanol was listed as HE14 (marked irritation in the eye, nose, throat and on the skin), HE8 (nervous system disturbance: narcosis) and HE5 (reproduction hazard: reproductive impairment)[16].

Chapter 9

Hazard assessment

9.1 Assessment of health hazard

In this report DECOS evaluates the health effects of inhalatory and dermal occupational exposure to ethanol. The committee acknowledges that drinking alcoholic beverages may be a more important source of ethanol exposure for workers. However, DECOS leaves this outside work exposure out of consideration for the assessment of the health effects after occupational exposure to ethanol.

Furthermore, several epidemiological studies reported that for the overall mortality, the dose-effect curve for long-term exposure to ethanol appears to be U- or J-shaped, indicating beneficial effects. At low exposure levels, these beneficial effects are for instance a reduced risk of coronary heart disease. DECOS does also not take these beneficial effects of ethanol into account for a quantitative health assessment after occupational exposure.

9.1.1 Health effects after single of short-term exposure

Only a few human studies on effects of short-term inhalatory exposure to ethanol are available. DECOS is of the opinion that these data indicate that inhalatory exposure for one hour to concentrations below 1900 mg/m^3 ethanol (1000 ppm) will not cause local or systemic effects. Therefore, DECOS considers 1900

mg/m^3 as a NOAEL for short term exposure (15 minutes). Exposure to concentrations 3000 mg/m^3 for 30 minutes might result in minimal effects as transient cough, dry throat, tickling in the nose etc. A sudden increase in ethanol concentration (from 0 mg/m^3 to levels up to ~4000 mg/m^3) may cause temporary irritation, whereas a concentration of 17000 mg/m^3 ethanol is described as 'intolerable' for unacclimatized persons.

9.1.2 *Health effects after repeated exposure*

There are no human data available concerning the effects of ethanol after long-term *inhalatory* exposure. Most data on the effects of long-term exposure to ethanol concern the oral consumption of alcoholic beverages. These epidemiological studies have several limitations.

- The alcohol consumption is expressed as average amount per day, leaving the pattern of alcohol consumption out of consideration (*binge drinking vs regular consumption*).
- The studies may be subject to recall bias, selection bias and information bias. For example, the validity of self-reported alcohol consumption has been a subject of much discussion. The general finding is that the alcohol consumption is underreported.
- Confounding factors seem to be smoking, age and other lifestyle factors.

The above mentioned limitations will have influenced the results in the epidemiological studies in different directions. All together, DECOS is of the opinion the effects of these limitations on the dose-effect relationship will probably counteract each other.

Furthermore, the kinetics after *drinking* alcoholic beverages differs from the kinetics after *inhalatory exposure* to ethanol. On the one hand, more than 90% of the orally ingested ethanol is absorbed with a high rate. After oral absorption, the blood ethanol concentration (BAC) peaks during the first hour and decreases with a short half life (at low ethanol exposure) thereafter. On the other hand, there are only limited data available concerning the absorption of ethanol after inhalatory exposure. Absorption of ethanol by the lungs is relatively low, ~60%. Physical activity will increase the inhalatory absorption. Inhalatory exposure to 1900 mg/m^3 results in a maximal blood alcohol concentration 10 to 100 times lower than the maximal blood ethanol concentration after drinking one alcoholic beverage. For the risk assessment of inhalatory exposure to ethanol, DECOS is therefore of the opinion that studies concerning oral exposure will overestimate

those effects which are related to the maximal blood ethanol level (*eg* several reproduction toxic effects[295-301]).

However, for genotoxic carcinogens (compounds without a NOAEL), DECOS generally assumes that the total internal exposure, ie. the Area Under the Curve (AUC defined as BAC times the exposure time) is a more relevant health exposure estimate. The AUC after drinking one glass of beer (containing ~11 g ethanol) is in the same order of magnitude as the AUC after eight hour inhalatory exposure to 1900 mg/m^3.[311] Therefore, DECOS is of the opinion that the oral carcinogenicity studies have limitations but can be used for the risk assessment of inhalatory exposure to ethanol.

The blood ethanol concentration will depend on several factors, like gender, age and genetic predisposition. Genetic predisposition plays a role because many isoenzymes and polymorphisms exist in ethanol metabolising enzymes, which results in different metabolic rates between racial and ethnic groups. Habitual alcoholic beverage consumption is important because ethanol exposure leads to induction of metabolizing enzymes. Overall, many different ethanol metabolizing enzyme systems exist which result in a large ethanol elimination capacity. A healthy subject is considered to eliminate between maximally 6 and 9 g of pure ethanol per hour.

In 1988, IARC concluded that there is sufficient evidence in humans showing that drinking alcoholic beverages is causally related to different types of cancer, such as malignant liver tumors and malignant tumors of the oral cavity, pharynx, larynx and oesophagus[172].

More recently, a meta-analysis of Ellisson et al, 2001[180] and pooled studies of Smith Warner *et al.*, 1998 and Beral *et al.*, 2002[181,182] showed that there is sufficient evidence in humans for an almost linear dose response association for ethanol and breast cancer. The risk for breast cancer linearly increases with 7-10% (RR 1.1) for every 10 grams of ethanol consumed. The risk for breast cancer in men after alcohol consumption is insufficiently investigated. A recent case control study[183] found a relative risk of breast cancer in men which is comparable to that in women.

The Committee on the Evaluation of carcinogenic compounds of the Health Council of the Netherlands concluded that it can not be excluded that acetaldehyde, a major (genotoxic) metabolite of ethanol, plays a role in the genotoxicity of ethanol, due to the few positive results in the genotoxicity tests (see Annex E). The Committee on the Evaluation of carcinogenic compounds is therefore of the opinion that ethanol is carcinogenic to humans and should be considered as a

(stochastic) genotoxic agent (as a worst case approach). In addition, the committee concludes that ethanol is known to be carcinogenic to humans (comparable with EU-category 1).

Several epidemiological studies[159,169,170] showed that drinking alcoholic beverages causes liver cirrhosis even at low alcohol concentrations (>18-30 g/day). DECOS concludes that 10 gram of ethanol per day seems to be a threshold for liver damage and assumes that these effects will be less relevant after inhalatory exposure as the first path metabolism plays no role after inhalatory exposure to ethanol.

The Committee on Alcohol consumption and reproduction of the Health Council of the Netherlands concluded that there is evidence that an intake of less than 10 grams of ethanol (one alcoholic consumption) per day may have an adverse effect on reproduction (decreased male and female fertility, increased incidence of spontaneous abortion, foetal death, preterm delivery, decreased length of gestation).

Table 9.1 Effects of long-term oral exposure to low doses of ethanol in humans.

Effect	NOAEL	Remark	Reference
Breast cancer (women)	RR = 1.07 per increment of 10 g/day	No threshold found	182
Breast cancer (women)	RR = 1.09 per increment of 10 g/day	No threshold found	181
Reproduction toxicity	< 10 g/day	Committee on Alcohol consumption and reproduction	6
Liver cirrhoses (men and women)	12 g/day	Above 18-30 g/day RR is increased significantly	159,169,170
Colorectal cancer (men and women)	20 g/day	At higher exposure, RR = 1.12 per increment of 10 g/day	177

9.2 Quantitative hazard assessment

9.2.1 Recommendation of an HBROEL, twa 15 min (Short-term Exposure Limit, STEL)

Concerning the recommendation of a STEL, DECOS is of the opinion that the available human data are limited but indicate that short-term inhalation of concentrations up to 1900 mg/m^3 ethanol (1000 ppm) for one hour will not cause local or systemic effects. Inhalatory exposure for 30 minutes to concentrations higher that 3000 mg/m^3 will cause minimal effects such as transient cough and dry throat, whereas exposure to 17000 mg/m^3 is described as intolerable for non-acclimized persons. Therefore, the committee is of the opinion that a short term

exposure limit of 1900 mg/m^3 (twa 15 minutes) will be low enough to protect workers against effects of short-term exposure.

9.2.2 *Recommendation of a Health Based Calculated Occupational Cancer Risk Value (HBC-OCRV)*

DECOS considers the development of breast cancer in women after exposure to ethanol as the critical effect. Based on the advice of the Health Council's Committee on the Evaluation of carcinogenic substances that a genotoxic mechanism cannot be excluded, DECOS recommends calculating of so called health based calculated occupational cancer risk values (HBC-OCRV) for ethanol. For the establishment of the HBC-OCRV's, the committee generally uses a linear extrapolation method, as described in the committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens'[312]. The linear model to calculate occupational cancer risks is used as a default worst case model, unless scientific data would indicate that using this model is not appropriate.

From the meta-analysis of Ellisson *et al.*, 2001, and the pooled studies of Smith Warner *et al.*, 1998 and Beral *et al.*, 2002, DECOS concludes that drinking a glass of alcoholic beverage (~10 gram ethanol) per day would increase the risk for breast cancer linearly with 7-10% (RR 1.1).

From these data, DECOS estimates the following HBR-OCRV* (see Annex D):

- 4×10^{-5} for 40 years of occupational exposure to 13 mg/m^3
- 4×10^{-3} for 40 years of occupational exposure to 1300 mg/m^3.

Ethanol is present in the human body without drinking alcoholic beverages as well. The endogenous ethanol concentration in human blood amounts to 0.27 (± 0.17) mg/l, corresponding to an AUC for 80 years of 21.6 (± 13.6) (mg/l)×year.

DECOS calculates that occupational exposure to 150, 750 or 1500 mg ethanol/m^3 for 40 years (8 hours/day, 5 days/week, 48 weeks/year) might result in AUCs of 2.0, 7.5, 19.2 (mg/l)×year, respectively (see Annex D). From these data, the committee estimates that an occupational exposure of 13 mg/m^3 (corresponding to a extra cancer risk of 4×10^{-5}) results in an AUC of approximately 0.2 (mg/l)×year.

The committee concludes that the additional AUC (0.2 mg/l per year) as a result of occupational exposure to 13 mg/m^3 is negligible to the AUC from the lifetime endogenous ethanol concentration in blood (~22 mg/l×year). Conse-

* Assuming a lung retention of 60% and that 10 m^3 air is inhaled per 8-hour working day.

quently, the committee considers the relevance of the calculation of an HBC-OCRV corresponding to a risk of 4×10^{-5} doubtful.

Therefore, DECOS recommends an HBC-OCRV corresponding to a breast cancer risk in women of 4 additional death per 1000 deaths (4×10^{-3}) for 40 years of occupational exposure to 1300 mg/m^3.

The Committee on Alcohol consumption and reproduction concluded that first signs of developmental toxicity and effects on fertility may become manifest after drinking one alcoholic consumption per day or less (<10 gram ethanol per day). However, DECOS is of the opinion that the maximal blood alcohol concentration is probably the relevant exposure parameter for these toxic effects. The BAC_{max} after one (oral) drink is approximately 10-100 times higher than the BAC_{max} after inhalatory exposure to 1300 mg/m^3. Therefore, the committee is of the opinion that at an exposure level of 1300 mg/m^3, developmental toxicity and effects on fertility will probably not occur.

9.3 Skin notation

From the available data, DECOS is of the opinion that dermal exposure might occur in the occupational setting. Therefore, the DECOS estimates whether a skin notation might be appropriate for ethanol (see Annex F).

A skin notation is considered when exposure of two hands and forearms (*i.e.* 2000 cm^2 of the skin) to ethanol during one hour leads to an additional uptake of 10% of the maximal inhalatory uptake as a results of exposure to the HBC-OCRV of 1300 mg/m^3 during an 8-hour working day.

From the calculation in Annex F, DECOS concludes (as a worst case approach) that a skin notation should be applied for ethanol.

Risk assessment of ethanol used as an desinfectant

For the specified occupational situation that a (hospital) worker desinfects both hands and underarms by washing with ethanol once, DECOS estimates (worst case) that under these (non-occlusive) conditions the dermal uptake will be 30 mg ethanol (see Annex F)*. Consequently, when hands will be desinfected with ethanol 20 times per day, this results in an dermal uptake of approximately 600 mg/day. This is less than 10% of the uptake caused by an 8 hours inhalatory ethanol exposure to the HBCOCRV of 1300 mg/m^3 (7800 mg/day).

* Washing hands only (400-800 cm^2 in stead of 2000 cm^2) will decrease the dermal uptake.

9.4 Groups at extra risk

Ethanol is known to induce the cytochrome P450IIE1 enzyme. This enzyme is a major catalyst of a number of substances of low molecular weight, among which are suspected carcinogens[313,314]. In specific cases, non-toxic doses of those substances may give rise to adverse effects due to increased bioactivation. Hence, workers who have co-exposure to ethanol and other chemicals that are activated by P450IIE1, may have a higher risk for adverse effects induced by these chemicals. This aspect was beyond the scope of the present report and is therefore not further discussed.

Furthermore, as presented in chapter 5, polymorphism of enzymes relevant for the biotransformation of ethanol exists. The results are difficult to interpret in a quantitative way for possible consequences for standard setting. Some effects related to ethanol exposure may also be related to the parent compound (ethanol itself) or a metabolite (*e.g.* acetaldehyde). The final result of a polymorphism may be completely the opposite, depending on whether the parent compound or a metabolite is responsible for the effects under consideration.

9.5 Health based calculated occupational cancer risk value and short-term exposure limit

DECOS recommends a short-term exposure limit (STEL) of 1900 mg/m^3, twa 15 minutes.

Based on an advice of the Committee on the evaluation fo carcinogenic compounds, DECOS concludes that ethanol is known to be carcinogenic to humans (comparble with EU category 1) and should be considered a genotoxic agent.

In addition, DECOS derives an HBC-OCRV corresponding to a breast cancer risk in women of 4 additional death cases per 1000 (4×10^{-3}) death cases for 40 years of occupational exposure to 1300 mg/m^3.

Finally, DECOS recommends a skin notation.

9.6 Recommendations for research

- Mechanistic research to discriminate between the effects induced by ethanol and its metabolite acetaldehyde: is ethanol really a genotoxic agent?
- Modelling as a tool for extrapolation of oral toxicity data to inhalation.
- Appropriate inhalatory repeated dose experiments in animals.

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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

- GJ Mulder, *chairman*
  emeritus professor of toxicology, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- LJNGM Bloemen
  epidemiologist; Exponent Inc., Terneuzen
- PJ Boogaard
  toxicologist; SHELL International BV, The Hague
- PJ Borm
  toxicologist; Centre of Expertise in Life Sciences, Hogeschool Zuyd, Heerlen
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  professor of risk assessment in occupational epidemiology; IRAS, University of Utrecht, Utrecht
- TM Pal
  occupational physician; Dutch Centre for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen.

- T Smid
  occupational hygienist/epidemiologist; KLM Health Safety & Environment, Schiphol; and, professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Dow Chemical Company, the Netherlands
- RA Woutersen
  toxicologic pathologist; TNO Quality of Life, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *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 draft

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

- Th Daha, Werkgroep Infectie Preventie, Leiden
- H Cappelleveen, College voor Toelating Bestrijdingsmidden, Wageningen
- J van Raaij, Diosynth, Oss
- E Cremer, EAG, CEFIC, Belgium
- R Zumwalde, National Institute for Occupational Safety and Health

Annex **D**

Derivation of HBC-OCRV* and comparison with the endogenous ethanol levels in blood

D.1 Derivation of the Health based calculated occupational cancer risk values (HBC-OCRV)

For the establishment of the HBC-OCRV's, the committee generally uses a linear extrapolation method, as described in the committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens'[312]. The linear model to calculate occupational cancer risks is used as a default worst case model, unless scientific data would indicate that using this model is not appropriate.

From the studies of Smith-Warner *et al.* (1998)[181], Beral *et al.* (2002)[182] and Ellisson *et al.* (2001)[180], DECOS concluded that drinking one glass of alcohol beverage (~10 gram ethanol) per day will increase the risk for breast cancer in women with 7-10%. This corresponds approximately to a RR of 1.1.

The dose response relationship will increase linearly per unit dose of ethanol up to 30 g/day, resulting in the following equation:

$$RR = a \times exposure\ (g/day) + 1$$

Because the RR is 1.1 at an exposure (dose) of 10 gram of ethanol, it can be calculated that a=0.01, resulting in the following dose-respons relationship:

* health based calculated occupational cancer risk values

RR = 0.01 x exposure (g/day) + 1

Furthermore, the excess lifetime cancer risk depends on the background rate of breast cancer (in women). In the Netherlands, 12.5 per 250 death cases (per year) in the female population are from breast cancer (CBS 2003, Statline).

One extra death (12.5 +1) per 250 deaths due to breast cancer (corresponding to an additional risk of 4×10^{-3}) as a result of drinking one alcoholic beverage will therefore correspond to a RR of 1.08 ((12.5+1)/12.5).

From the dose-response relationship above, the committee calculates that a RR of 1.08 corresponds to an exposure of 8000 mg per day*.

In addition, DECOS assumes that for genotoxic carcinogens the AUC (blood alcohol concentration times exposure time) is the exposure estimate associated with the health effect. Furthermore, from the study of Campbell *et al.*[51], DECOS concluded that the AUC after drinking one glass of alcohol (~10000 mg ethanol) is comparable with the AUC after inhalatory exposure to 1900 mg/m^3 for 8 hours. Therefore, DECOS is of the opinion that it is relevant to extrapolate an oral uptake of 8000 mg ethanol per day (resulting in one extra death due to breast cancer per 250 death cases), to an inhalatory exposure to ethanol during 8 hours.

Assuming that 10 m^3 air is inhaled per working day (8 hours) and the lung retention is 60%, DECOS calculates that an oral dose of 8000 mg per day corresponds to an inhalatory dose of 1300 mg/m^3 (8000 mg/(10*0.6)). Moreover, DECOS assumes that both exposures will result in comparable AUC's.

In conclusion, DECOS calculates the following HBR-OCRVs:

- 4×10^{-5} for 40 years of occupational exposure to 13 mg/m^3
- 4×10^{-3} for 40 years of occupational exposure to 1300 mg/m^3.

D.2 Comparison of the HBC-OCRV with the endogens ethanol levels in blood

Ethanol is present in the human body of non-drinkers as well. Sprung *et al.*[19], measured a blood alcohol concentration (BAC) for endogenous ethanol of 0.27 mg/l (± 0.17 mg/l). This correspond to an AUC (Area under the curve, ie BAC × exposure time for 80 years, 24 hours/day) of 21.6 ±13.6 (mg/l)×year (0.27 mg/l × 80 year).

* Assuming that the RR for breast cancer in women is comparable to the RR for death caused by breast cancer.

Exposure	(*measured*) BAC	(*calculated*) AUC for 80 years, 24 hours/day, 7 days/ week, 52 weeks/year (BAC × 80 year × $^{24}/_{24}$ × $^{7}/_{7}$ × $^{52}/_{52}$)
endogenous	0.27 mg/l	21.6 (mg/l) × year
1 glass (6 g)	80 mg/l (BAC_{max})	-

Furthermore, Seeber *et al.*[52] exposed 12 men and 12 women to ethanol (150, 750, 1500 mg/m^3) during 4 hours and determined the corresponding maximal blood alcohol concentrations (BAC_{max}). The following ethanol concentrations were measured in the human blood: 0.23 mg/l, 0.85 mg/l, 2,18 mg/l, respectively.

Using these BAC's, the committee calculates the corresponding AUCs* for 40 years.

Exposure for 4 hours	(*measured*) BAC	(*calculated*) AUC for 40 years, 8 hours/day, 5 days/week, 48 weeks/year (BAC × 40 year × $^{8}/_{24}$ × $^{5}/_{7}$ × $^{48}/_{52}$)
150 mg/m^3	0.23 mg/l	2.0 (mg/l) × year
750 mg/m^3	0.85 mg/l	7.5 (mg/l) × year
1500 mg/m^3	2.18 mg/l	19.2 (mg/l) × year

From this table, DECOS estimates that an occupational exposure of 13 mg/m^3 (corresponding to a extra cancer risk of 4×10^{-5}) results in an AUC of approximately 0.2 (mg/l)×year. An occupational exposure to 1300 mg/m^3 (corresponding to an extra cancer risk of 4×10^{-3}) results in an AUC of approximately 19 (mg/l)×year.

* Assuming a constant blood alcohol concentration during 8 hours and 40 years of occupational exposure

Annex **E**

Advice of the Committee on the evaluation of the carcinogenicity of chemical substances

concerning the genotoxic properties of ethanol (September 2004)

E.1 Scope

The Committee on the evaluation of the carcinogenicity of chemical substances (further referred to as 'the committee') of the Health Council of the Netherlands has been asked by the Dutch Expert Committee on Occupational Standards (DECOS) to advise on two questions.

- Does the committee agree with DECOS' conclusion that sufficient evidence in humans is available to conclude that oral exposure to ethanol increases the incidence of breast cancer in women?
- What is the committee's conclusion regarding the genotoxic properties of ethanol.

E.2 Does oral exposure to ethanol cause an increased incidence of breast cancer in women?

Carcinogenicity

In 1988, IARC concluded that adequate evidence in humans exists to show that drinking of ethanol is causally related to malignant liver tumours. Furthermore, IARC was of the opinion that malignant tumors of the oral cavity, pharynx, larynx and oesophagus are causally related to the consumption of ethanol as well. With respect to breast cancer, IARC was of the opinion that "The modest eleva-

tion in relative risk that has been observed is potentially important because of the high incidence of breast cancer in many countries. Although the available data indicate a positive association between drinking of alcoholic beverages and breast cancer in women, a firm conclusion about a causal relationship cannot be made at present*." Numerous studies have been performed on the effects of drinking alcohol on breast cancer since then. However, several methodological problems (lack of reporting of other breast cancer risk factor, difficulties in assessing the levels of consumed alcohol etc) complicate interpretation of many of these studies. The positive correlation found in some studies, could not be confirmed in others. Therefore, the committee based her conclusions predominantly on the meta-analysis of Ellison *et al.* (2001)[13], and the (pooled) studies of SmithWarner *et al.* (1998)[31] and Beral *et al.* (2002)[5] regarding the effects of drinking alcohol and breast cancer.

The committee is of the opinion that these reports[13, 31, 5] summarize the data on the relation between drinking of alcohol and breast cancer adequately. The committee concludes that there is sufficient evidence that alcohol consumption elevates the relative risk for breast cancer in women linearly with 7 to 10% per each 10 gram ethanol (one drink contains about 10 grams).

In addition, in experimental animals the (breast) tumor incidences are increased after oral exposure to ethanol[34, 29].

Mechanisms of action

The association between alcohol intake and the occurrence of breast cancer has been studied extensively in man. However, only limited information is available concerning the possible mechanism of action for this effect. Although progress has been made in understanding potential mechanisms, it is yet not clear how alcohol intake may increase breast cancer risk. Some studies implicate a role for endogenous hormones, while others suggested a role for genetic polymorphisms.

Endogenous hormones

- Alternation in *estrogen* levels has been thought to modulate the breast cancer risk in women. Several studies show a positive correlation between alcohol intake and increased (plasma or urinary) estrogen levels[18]. However, this

* In 2003, IARC placed alcohol beverages on the *Priority List of agents and exposures to consider in future IARC Monographs* because of new information concerning additional cancer sites (*breast*, liver, colorectal cancer) and better knowledge of mechanisms of action.

association was not confirmed by others (cited from Smith-Warner[31]). Moreover, a few studies demonstrated that women with alcoholism have higher estrogen levels than moderate alcohol consumers and non drinkers[17].

- Alternation in *prolactin* levels has been associated with breast cancer risk in experimental animals[36, 6]. However, the hypothesis that prolactin might play a role in increasing the breast cancer risk is not supported convincingly by human data and more research should be performed[10]. Moreover, data regarding the effect of the intake of alcohol beverages on the prolactin levels are difficult to interpret. The influence of alcohol uptake on the prolactin levels seems highly dependent on, amongst other factors, the age and sexual maternity of the women and the mode of alcohol administration (acute or chronic ingestion).
- *Examples of other endogenous hormones* which have been hypothesized to play a role in breast cancer development are androgens, thyroid hormones and estriol. However, the available data are not convincing and more research is needed to elucidate a possible relationship between these hormones, ethanol ingestion and breast cancer.
- There is evidence linking alcohol consumption to *p53-mutations (tumor suppressor gene) in tumors* and considerable evidence linking alcohol consumption to *p53-mutations with risk to breast cancer*[16].

Genetic polymorphisms

Several studies have suggested a role for genetic polymorphisms in the alcohol/breast cancer association. *Glutathione S-transferases (GSTs), Cytochrome P450 2E1 (CYP2E1) and aldehyde dehydrogenase-2 (ALDH)* are considered to be important for detoxifying many carcinogenic compounds. Data suggest that the breast cancer risk is elevated among those who carry susceptible GST genotypes (GSTM1A, GSTT1-null) (Zheng *et al.*, 2003)[35]. In addition, premenopausal women with certain genotypes of ALDH drinking ethanol had higher risk for breast cancer than other women[15]. Moreover, genotypes of CYP2E1 have been identified which are less inducible than others[25, 28].

Conclusion

The committee is of the opinion that the available data provide potential explanations for a relationship between ethanol intake and breast cancer but do no allow any definite conclusions. To elucidate the role of ethanol ingestion in the increased breast cancer development, more research is needed.

E.3 Is ethanol a genotoxic carcinogen?

Introduction

In the previous paragraphs, the committee discussed different postulated mechanisms for the development of breast cancer as a result of drinking alcohol. In the following paragraphs, the committee describes the results of several genotoxicity tests and evaluates the genotoxic properties of ethanol. In addition, the possible role of one of the metabolites of ethanol, ie acetaldehyde, will be discussed.

In their report 'Evaluation of the carcinogenicity of chemical substances' (1996/26), the committee distinguished the following categories of carcinogens on the basis of their mechanism of action:

1 Genotoxic carcinogens
   - Stochastic genotoxic carcinogens
   - Non-stochastic genotoxic carcinogens
2 Non-genotoxic carcinogens.

Evaluation of the genotoxicity tests of ethanol

The available genotoxicity tests are summarized in table 6.8 (*in vitro* genotoxicity tests), table 6.9 (*in vivo* genotoxicity tests in somatic cells) and table 6.10 (*in vivo* genotoxicity tests in germinal cells) of the DECOS document.

In vitro genotoxicity tests

The available *in vitro* genotoxicity tests (see table 7.2) are performed in the presence and in the absence of metabolic activation. Most of these *in vitro* tests gave negative results for (a) gene mutations in bacteria[11, 21], (b) micronuclei[21] and chromosome aberrations (CA) in human lymphocytes and Chinese Hamster Ovary (CHO) cells[21, 30], (c) Sister Chromatid Exchange (SCE)[21], and (d) DNA damage[9, 21]. However, a few of the *in vitro* tests were positive for ethanol. Gene mutations in yeast[21], induction of chromosome aberrations (CA) in human blood lymphocytes[3] and sister chromatid exchange in CHO cells[12] were observed after treatment with ethanol.

The committee noticed that the *in vitro* tests, showing negative results for genotoxicity, were predominantly performed in the absence of a metabolic activation system[2, 3, 12, 20, 21]. Based on these studies the committee concludes that in

most studies ethanol is not mutagenic without the presence of a metabolic system. Only in the study of Badr *et al.*[3], positive results were found in human lymphocytes without metabolic activation*.

In vivo genotoxicity tests

Ethanol predominantly gave negative results in the *in vivo* genotoxicity tests in somatic cells (see table 7.3). The micronucleus assay was negative in cells of Swiss mice[21], Ddy-mice[21], Wistar rats (268)[32] and CD rats[4] given ethanol (orally). In addition, tests for chromosome aberrations (CA) and Sister chromatid exchanges (SCE) in somatic cells of Wistar rats[32] and Chinese hamsters were predominantly negative as well[21]. In germinal cells (see table 7.4), ethanol did not induce CA in spermatogonia and testicular cells[19, 21].

But again, in a minority of the *in vivo* tests in somatic cells (micronuclei bone marrow[3, 4], sister chromatid exchange in foetal liver cells[1 **]), ethanol was found to be positive. Moreover, an increase of aneuploidy was detected in most germinal cells tested[23, 24, 33]. In spermatogonia and spermatids of male Chinese hamster[21], aneuploidy was absent. Furthermore, four (out of seven) dominant lethal assays (279, 280, 282, 284)[3, 26, 27, 33] were positive.

Evaluation of the genotoxic potential of ethanol by Phillips and Jenkinson[30]

In 2001, Philips and Jenkinson reviewed the genotoxic potential of ethanol. They concluded that there is clear evidence that ethanol is not a bacterial or mammalian cell mutagen, but *in vitro* assays for chromosome aberration, although mostly negative, have generally not included exogenous metabolic activation. Reported tests for chromosome aberration induction *in vivo* are all negative and only a minority of the micronucleus tests are positive. Conflicting results have been reported for dominant lethal assay. There is some evidence that ethanol induces SCE *in vivo* and can also act as a aneugen at high doses. The authors noted many *in vivo* studies used very high doses and sometimes for long periods. The results in these studies, with excessive exposure to ethanol, may show some degree of genotoxicity. However, they concluded that there is no convincing evi-

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* The committee points out that for the metabolic activation of ethanol, both a microsomal ethanol oxidizing system (MEOS), catalase and alcohol dehydrogenase are necessary. Therefore, the addition of an S9-preparation is less relevant because this metabolic system might not contain (sufficient) alcohol dehydrogenase.

** Only under special circumstances

dence that ethanol is a genotoxic hazard at exposure level which are obtainable by either inhalation or dermal exposure in the workplace.

Conclusion

The committee is of the opinion that ethanol was predominantly negative in bacteria. In most *in vitro* tests with mammalian cells, ethanol was negative as well.

The committee agrees with Phillips *et al.*[30] that, except for aneuploidy, most *in vivo* tests (in somatic and germinal cells) were negative. However, positive results were also observed (mainly dominant lethal assays);

Finally, ethanol clearly induces aneuploidy in germinal cells[23, 24, 33] of exposed animals. For aneuploidy, the committee assumes a non-stochastic genotoxic mechanism.

Acetaldehyde

Ethanol is metabolized in several steps. First, it is oxidized to acetaldehyde and subsequently converted to acetate.

In 1999, IARC concluded that there is inadequate evidence in humans for the carcinogenicity of acetaldehyde and there is sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde[22]. Acetaldehyde causes gene mutations in bacteria and gene mutations, sister chromatid exchanges, micronuclei and aneuploidy in cultured mammalian cells, without metabolic activation.

Based on the evaluation of IARC, the committee concludes that acetaldehyde is a genotoxic carcinogen in experimental animals.

Limited studies are performed in which acetaldehyde concentrations are determined in human blood or amniotic fluid after ethanol exposure. O'Brien *et al.*[7] studied six pregnant women who ingested ethanol once in the 16-18 week of pregnancy. In four of the six women acetaldehyde was detected in the venous blood samples. Other studies did not find acetaldehyde concentrations in human blood samples. The limited amount of information concerning the presence of acetaldehyde in human blood is probably a result of the fact that acetaldehyde levels are extremely difficult to detect in human blood after exposure to ethanol[14].

Moreover, Castro *et al.*[8] showed that a cytosolic and microsomal fraction of breast tissue of female Sprague Dawley rats has the ability to bioactivate ethanol to acetaldehyde and free radicals. No studies are available concerning levels of

acetaldehyde determined in different human tissues (e.g. breast) after ethanol intake.

Conclusion

The committee concludes that there is a positive association between alcohol intake and breast cancer risk (and cancer of the oral cavity, pharynx, larynx, oesophagus and liver). Several mechanisms of action have been suggested.

On the one hand, a role for the endogenous hormones has been proposed. However, information concerning these mechanisms is limited and uncertainty remains whether there may be a threshold dose below which alcohol intake does not elevate the breast cancer risk in women. Moreover, the committee emphasizes that based on the present available data, such a threshold cannot be identified.

On the other hand, a lot of information is available regarding the genotoxic properties of ethanol. In a minority of the *in vivo* and *in vitro* genotoxicity tests (other than aneuploidy), ethanol was positive. Most genotoxicity tests were negative after treatment with ethanol (although some of them were not performed correctly). Therefore, based on the genotoxicity tests the committee concludes that there is only limited evidence for a genotoxic potential of ethanol. However, acetaldehyde, a genotoxic compound, is one of the major metabolites of ethanol and can be detected in the human body after ingestion of ethanol. As a role for acetaldehyde in the genotoxicity of ethanol cannot be excluded, the committee cannot neglect the few positive results in the genotoxicity test.

In conclusion, the committee is of the opinion that ethanol should be considered as a (stochastic) genotoxic carcinogen.

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Annex F

Skin notation

Skin notation

To decide wether a skin notation should be assigned to a compound, DECOS uses ECETOC document No. 31 as a guideline.

ECETOC Document No. 31[315]

A skin notation should be applied when the amount absorbed by both arms and forearms in 1 hour (continiously) could amount to more than 10% of the amount that can be absorbed via the lungs on exposure to the proposed OEL for 8 hours, provided that this OEL is set on the basis of systemic toxicity.

For chemicals where there is considerable industrial experience, current best practices and reliable information on health effects from them should be taken into consideration in preference to or along with the theoretical approach.

Ethanol

For assessing whether a skin notation may be appropriate for ethanol, DECOS uses the data from Beskitt and Sun [75]. A worst case estimate of the penetration rate was 0.7 mg ethanol/cm^2/h, calculated from an experiment performed under occlusive conditions. This flux was obtained with a 25% (v/v) aqueous solution. However, experience learns that although theoretically the flux will linearly

increase with increasing concentration (the k_p will remain constant), this will not be the case in practice. Since the flux of 0.7 mg/cm^2/h is obtained under occlusion, it might overestimate the dermal uptake under occupational conditions (which are without occlusion).

A one-hour absorption by two hands and forearms (2000 cm^2) of ethanol results in a total dermal uptake of (2000 cm^2 × 0.7 mg/cm^2/h =) 1400 mg/h. On the other hand, a working day inhalatory exposure to an HBC-OCRV of 1300 mg/m^3, will result in an internal uptake of 7800 mg per day, assuming a worker inhales 10 m^3 air during an 8 hour working day and a pulmonary retention of 60%. From this DECOS calculates that one hour absorption by two hands and forearms contributes to 18% (1400/7800*100% = 18%) of the internal exposure after absorption via the lungs*.

Therefore, DECOS recommends (as a worst case approach) a skin notation.

Use of ethanol as an antiseptic agent

In several occupational settings, dermal ethanol exposure will mostly occur as droplets on the skin under non-occlusive conditions and by washing hands with ethanol as antiseptic agent. The penetration rate of ethanol under these conditions is probably at least one order of magnitude lower than under occlusion due to the high evaporation rate of ethanol. The half-life of evaporation was estimated to be 12 seconds [74]. This means that within 75 seconds more than 99% of the applied dose will be evaporated.

DECOS estimates that during a period of 75 seconds, a total dose of approximately 30 mg (0.7 * 2000 *75/3600) may penetrate into the skin (arms and forehands). Therefore, DECOS assumes that disinfection of both hands (and forearms) once with ethanol could result in a dermal uptake of 30 mg ethanol (worst case approach).

* Desinfecting only the hands (and not the forearms) with ethanol (420-800 cm^2) will lower the amount absorbed via the skin.