Health Council of the Netherlands

Benzene

Health-based recommended occupational exposure limit

2014/03

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Benzene

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Benzene*
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Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan benzeen.

Dit advies maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige adieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. De conclusies van het genoemde advies zijn opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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

to:

the Minister of Social Affairs and Employment

No. 2014/03, The Hague, February 21, 2014

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 and health (services) research..." (Section 22, Health Act).

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (Commissie GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht waaraan mensen blootgesteld worden tijdens de beroepsuitoefening. De gezondheidskundige advieswaarden vormen vervolgens de basis voor grenswaarden, vast te stellen door de minister, waarmee de gezondheid van werknemers beschermd kan worden.

In dit advies bespreekt de commissie de gevolgen van blootstelling aan benzeen en stelt ze een gezondheidskundige advieswaarde vast. De conclusies van de commissie berusten op de wetenschappelijke publicaties die vóór oktober 2013 zijn verschenen.

Fysische en chemische eigenschappen

Benzeen (CAS 71-43-2) is een kleurloze vloeistof met een zoete geur, die wordt gewonnen uit koolstof- en petroleumbronnen. Benzeen heeft een moleculair gewicht van 78,11 g/mol, een smeltpunt van 5,5°C en een kookpunt van 80,1°C. Verder is benzeen vluchtig en zeer brandbaar.

Gebruik

Benzeen wordt voornamelijk gebruikt in de chemische en farmaceutische industrie als startmateriaal en tussenproduct voor de synthese van diverse chemische stoffen. Het wordt ook toegevoegd aan benzine, als anti-klopmiddel, voor een betere ontbranding.

Monitoring

De blootstelling aan benzeen kan goed gemeten worden door bepaling van benzeen in urine, bloed en uitgeademde lucht, en door de bepaling van S-fenylmercaptuurzuur (SPMA) in urine.

De analyse van benzeen gebeurt meestal met gaschromatografie (GC). Massaspectrometrie (MS) kan gebruikt worden om benzeen te detecteren en te kwantificeren. De detectielimiet van benzeen in urine, bloed en uitgeademde lucht ligt in het gebied van enkele nanogrammen per liter.

Er zijn verschillende GC-methoden om SPMA in urine te analyseren. In aanvulling op de GC-methoden zijn verschillende *high-performance liquid chromatography* (HPLC) methoden beschikbaar. In het algemeen liggen de detectielimieten hiervan nabij de 1 microgram per liter.

Huidige grenswaarden

Er is voor benzeenblootstelling op de werkplek een Europese grenswaarde van 3.25 mg per m^3 lucht vastgesteld, voor een werkdag van 8 uur. Deze grenswaarde wordt ook in Nederland en enkele andere Europese landen toegepast. In de Verenigde Staten zijn grenswaarden van 3,2 mg/m^3 (voor een 8-urige blootstelling) en 16 mg/m^3 (voor een piekblootstelling van 15 minuten) vastgesteld door de *Occupational Safety & Health Administration* (OSHA).

Kinetiek

Benzeen wordt gemakkelijk opgenomen via alle blootstellingsroutes (de luchtwegen, de huid en de mond), waarvan de luchtwegen de belangrijkste route vormen). In de mens varieert de opname na inademing van ongeveer 50 tot 80 procent, afhankelijk van de blootstellingsomstandigheden. Diergegevens wijzen erop dat opname van benzeen via de luchtwegen niet rechtevenredig afhankelijk

is van de concentratie in de lucht. De gegevens wijzen uit dat vloeibaar benzeen door de menselijke huid kan worden geabsorbeerd. De geschatte opname via de huid per tijdseenheid varieert van 200-400 µg per cm^2 per uur. Benzeen wordt na toediening via de mond efficiënt opgenomen in dieren; de mate hiervan varieert van ongeveer 80 procent(in konijnen) tot meer dan 97 procent (in ratten en muizen).

Na opname verdeelt benzeen zich over het lichaam. Benzeen is bij de mens gemeten in verschillende lichaamsvloeistoffen en weefsels, waarbij de hoogste gehaltes zijn gemeten in weefsels met een hoog vetgehalte. Ook in dieren verdeelt benzeen zich na absorptie in vetrijke weefsels, vooral weefsels met een hoge doorbloeding zoals de nier. In de rat werd in het bloed binnen 4 uur een concentratie-evenwicht van benzeen bereikt, in vetweefsel binnen 6 uur en in beenmerg in minder dan 2 uur, na blootstelling aan 1.600 mg/m^3 (500 ppm) benzeen.

Hoewel nog niet alle stappen die leiden tot de toxiciteit van benzeen bekend zijn, is het duidelijk dat het metabolisme in grote mate de toxiciteit van benzeen bepaalt. De beschikbare gegevens wijzen erop dat de afbraakproducten van benzeen voornamelijk in de lever gevormd worden. De eerste stap in de omzetting van benzeen is de oxidatie van benzeen door het enzymsysteem cytochroom P-450, voornamelijk het enzym CYP 2E1, en de vorming van benzeenoxide. Verschillende mechanismen zijn betrokken bij de omzetting van benzeenoxide, waarvan het mechanisme via niet-enzymatische omzetting tot fenol een prominente rol speelt. Na blootstelling aan benzeen via de luchtwegen, is zowel in dieren als mensen uitademing de belangrijkste route waarlangs benzeen het lichaam verlaat. Het merendeel van het benzeen dat geabsorbeerd wordt, wordt echter omgezet en uitgescheiden in de urine na binding met lichaamseigen stoffen.

Effecten

Waarnemingen bij mensen

Benzeendamp irriteert de luchtwegen; benzeen is ook irriterend voor de huid.

Kort na inademing van zeer hoge concentraties benzeen kunnen er bij mensen symptomen optreden die duiden op toxiciteit van het centraal zenuwstelsel, zoals duizeligheid, stuiptrekkingen, trillingen en uiteindelijk een narcotisch effect en overlijden door ademhalingsstilstand.

Beroepsmatige blootstelling wordt sinds lange tijd in verband gebracht met nadelige effecten op het bloed en het beenmerg, waaronder een tekort aan rode én witte bloedcellen. Verschillende onderzoeken met werknemers die zijn blootgesteld aan benzeen tonen deze effecten aan bij hoge maar ook relatief lage blootstellingsconcentraties.

Bij verschillende beroepsgroepen, in verschillende industrietakken, is een verhoogd risico gevonden op leukemie na blootstelling aan benzeen. Het verhoogde risico geldt in het bijzonder voor acute myeloide leukemie, maar recentelijk is gebleken dat blootstelling aan benzeen ook kan leiden tot het myelodysplastisch syndroom (MDS), een beenmergstoornis waarbij de productie van bloedcellen ernstig verstoord is.

Er zijn onvoldoende en tegenstrijdige aanwijzingen om te kunnen concluderen dat benzeen nadelige effecten heeft op de vruchtbaarheid, of de ontwikkeling van het nageslacht van de mens.

Waarnemingen bij dieren

Onderzoek met ratten laat zien dat benzeen op korte termijn niet erg toxisch is, met een geschatte LD_{50}-waarde hoger dan 2.000 mg/kg lichaamsgewicht en een LC_{50} waarde van 44.500 mg/m^3 (13.700 ppm). Afhankelijk van de dosis, zijn de voornaamste acute effecten verdoving en narcose. Onderzoek met konijnen toont bij hen irritatie van de huid.

Onafhankelijk van de blootstellingsroute, zijn de beenmergcellen en bloedcellen het meest gevoelig voor herhaalde blootstellingen aan benzeen. Chronische blootstelling aan benzeen kan leiden tot onderdrukking van het beenmerg, met als gevolg een tekort aan rode en witte bloedcellen.

Studies waarbij dieren blootgesteld zijn via de luchtwegen of de mond laten zien dat benzeen tumoren veroorzaakt in meerdere organen. Doelorganen zijn, onafhankelijk van de blootstellingsroute, het hematopoetische systeem en een spectrum van epitheelweefsels. Bij muizen uit de carcinogeniteit van het hematopoetische systeem zich voornamelijk in het ontstaan van lymfomen. Bij ratten daarentegen, wordt een verhoogd aantal dieren met leukemie gevonden na blootstelling aan benzeen. Daarnaast zijn verschillende typen epitheliale tumoren gevonden in muizen en ratten.

Benzeen en de metabolieten van benzeen veroorzaakten geen mutaties in testen met bacteriën, terwijl de resultaten van testen met zoogdiercellen dubbelzinnig waren. Het merendeel van de testen voor chromosomale afwijkingen in

proefdieren was positief, zowel voor benzeen als voor de metabolieten van benzeen.

In vrouwtjesratten die voor het paren tien weken lang blootgesteld waren aan 975 mg/m^3 benzeen (300 ppm), werden geen nadelige effecten gevonden op de vruchtbaarheid, voortplanting, en lactatie. In muizen daarentegen, zijn er bij deze concentratie aanwijzingen gevonden voor veranderingen in voortplantingsorganen, met name in mannetjes. Deze effecten traden echter op bij concentraties die duidelijk toxisch voor bloedcellen waren, bij zowel mannetjes als vrouwtjes. Er zijn geen ontwikkelingsstoornissen gevonden na blootstelling aan benzeen, zelfs niet bij concentraties die leidden tot toxiciteit bij de moederdieren.

Evaluatie en advies

De Subcommissie Classificatie carcinogene stoffen (een autonome subcommissie van de Commissie GBBS) concludeert, overeenkomstig met de Europese classificatie, dat benzeen kankerverwekkend is voor de mens (classificatie categorie 1A, zie Annex H en I). Ze beschouwt benzeen als een kankerverwekkende stof met een niet-stochastisch genotoxisch werkingsmechanisme. Dit betekent dat de subcommissie ervan uit gaat dat er een veilig blootstellingsniveau, een drempelwaarde, voor benzeen bestaat. De commissie heeft het oordeel van de subcommissie overgenomen en een gezondheidskundige advieswaarde afgeleid.

Onafhankelijk van de blootstellingsroute, zijn het beenmerg en het bloedvormend systeem het meest gevoelig voor de schadelijke effecten van benzeen. De gegevens over het blootstellingsniveau waarbij effecten bij de mens kunnen optreden zijn niet eenduidig. In meerdere studies zijn effecten gevonden bij een benzeenconcentratie die lager was dan 3,3 mg/m^3 (1 ppm), terwijl in andere studies geen effecten zijn beschreven bij deze concentratie. De commissie baseert haar gezondheidskundige advieswaarde op het geheel van de gegevens en hanteert, vanuit een pragmatisch oogpunt, 2 mg/m^3 (0,6 ppm) als een reëel blootstellingsniveau waarbij gezondheidseffecten kunnen optreden. De commissie past voor het afleiden van een gezondheidskundige advieswaarde een onzekerheidsfactor toe, om rekening te houden met het feit dat er bij deze concentratie nog effecten zijn te verwachten. Het toepassen van een standaard onzekerheidsfactor van 3 levert een gezondheidskundige advieswaarde op van 0,7 mg/m^3 (0,2 ppm).

Gezondheidskundige advieswaarde

De Commissie GBBS van de Gezondheidsraad beveelt een gezondheidskundige advieswaarde aan voor beroepsmatige blootstelling aan benzeen van 0,7 mg/m^3 (als een gemiddelde waarde over een achturige werkdag).

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, recommends health-based occupational exposure limits for airborne substances to which people are exposed in the workplace. These recommendations serve as a basis in setting legally binding occupational exposure limits by the Minister. In this report, the Committee considers the implications of exposure to benzene, and recommends a health-based occupational exposure limit for this substance. The Committees' conclusions reflect the content of scientific publications that have appeared in the public literature prior to October 2013.

Physical and chemical properties

Benzene (CAS 71-43-2) is a colourless liquid with a sweet odour, which is commercially produced from coal and petroleum sources. Benzene has a molecular weight of 78.11, a melting point of 5.5°C and a boiling point of 80.1°C. Benzene has a vapour pressure of 99.7 hPa at 20°C and is highly flammable with flammability limits in air of 1.2% (lower limit) and 7.8% (upper limit).

Use

Benzene is used primarily in the chemical and pharmaceutical industries, as a starting material and intermediate in the synthesis of numerous chemicals. It is also used as a gasoline additive, since benzene increases the octane rating and reduces knocking.

Monitoring

The determination of benzene in urine, blood and expired air, and the determination of S-phenylmercapturic acid (SPMA) in urine are suitable approaches for biomonitoring of benzene.

The analysis of benzene generally involves dynamic headspace (purge and trap). Mass spectrometry can be used for the detection and quantification of benzene. The limit of detection for benzene in urine, blood and expired air falls within the low ng/L range.

Commonly used methods for urinary SPMA analysis consist of extracting SPMA from the urine by liquid-liquid extraction, subsequent derivatisation, and detection by gas chromatography/mass spectromethry (GC/MS). In addition to the GC approach, several high-performance liquid chromatography methods are available. Generally, the limit of detection of urinary SPMA analysis are in the range of 1 µg/L.

Exposure limits

At the European level, there is currently a limit value of 3.25 mg/m^3 (1 ppm) for occupational exposure to benzene. The legal time weighted average (TWA) (8h) occupational exposure limit for benzene in the Netherlands is 3.25 mg/m^3 air. Also in Finland, France, and the UK, an occupational exposure limit of 3.25 mg/m^3 is being applied. In Germany, no MAK value (Maximum Concentration at the Workplace) has been derived. In the US, exposure limits of 3.2 mg/m^3 (8h-TWA) and 16 mg/m^3 (15 min-TWA) have been set by OSHA. The American Conference of Governmental Industrial Hygienists (ACGIH) has specified a threshold limit value (TLV) of 1.6 mg/m^3 (8h-TWA value).

Kinetics

Benzene is readily absorbed by all routes (inhalation, dermal and oral), of which inhalation is considered to be the most important route of exposure. In humans, extents of absorption have been reported ranging from approximately 50-80%, depending on exposure conditions. Animal data suggest that the uptake of benzene by the lungs is related to the concentration in a non-linear manner. The amount of benzene absorbed and retained in the tissues and blood during a 6-hour exposure decreased from 33 to 15% in rats, and from 50 to 10% in mice, when exposure was increased from 26 to 2,600 mg/m³ (8-812 ppm). Results from in vivo experiments indicate that liquid benzene can be absorbed through human skin, although not as substantial as the absorption following inhalation or oral exposure.The estimated skin absorption rate ranges from 200-400 µg/cm²*h. Benzene is efficiently absorbed following oral dosing in animals; absorption levels have been reported of > 97% (in rats and mice) and 80% (in rabbits).

Upon absorption, benzene is distributed throughout the body. Benzene has been detected in various biological fluids and tissues of humans, the highest levels amounting in lipid-rich tissues. Also in animals, benzene distributes in tissues rich in lipids, particularly those with high perfusion rates, such as the kidney. In rats, steady state concentrations of benzene were reached within 4 hours in blood, 6 hours in fat and less than 2 hours in bone marrow after exposure to 1,600 mg/m³ (500 ppm).

The metabolism of benzene is an important determinant for benzene-induced toxicity, however the steps leading to benzene toxicity are not yet fully understood. The available data indicate that metabolites are primarily generated in the liver. Similar metabolic pathways exist in animals and human, although remarkable species variability has been observed. The first step in the metabolism of benzene is the oxidation of benzene to benzene oxide by the cytochrome P-450, mainly CYP 2E1. Several pathways are involved in the metabolism of benzene oxide, predominantly the pathway involving non-enzymatic rearrangement to form phenol. In turn, phenol can be oxidised by CYP2E1 to catechol or hydroquinone, which are subsequently oxidised to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively. The phenolic metabolites of benzene can undergo conjugation. Other pathways of benzene oxide metabolism include the reaction with glutathione to form SPMA and iron-catalysed ring-opening to trans, trans-muconic acid.

Following inhalation exposure to benzene, exhalation is the major route of elimination of (unmetabolised) benzene in humans and animals. Most of the

absorbed benzene however, is metabolised and the metabolites are excreted after phase-II-conjugation, predominantly in the urine.

Effects

Observations in humans

Benzene vapour is irritating to the respiratory tract; benzene is also irritating to skin.

Following acute inhalation of high levels of benzene, humans exhibit symptoms of central nervous system toxicity, e.g., dizziness, convulsions, tremors and ultimately narcotic effects and death by respiratory arrest.

Occupational exposure to benzene has long been associated with toxicity to the blood and bone marrow, including lymphocytopenia, pancytopenia, and aplastic anaemia. Several cross-sectional studies with workers who were exposed to benzene have shown haematological effects at a broad range of exposure levels.

Increased risk of either leukaemia in general or acute myeloid leukaemia/ acute non-lymphocytic leukaemia specifically, after exposure to benzene has been observed in several cohorts of workers, in various industries, with long-term exposure to benzene.

There are insufficient, or inconsistent data on adverse effects of benzene exposure on fertility, or the development of offspring, in humans.

Observations in animals

Benzene has been shown to be irritating to the skin of rabbits, inducing moderate erythema, edema, and moderate necrosis following application.

The acute toxicity of benzene is low, and suggest an oral LD_{50}-value exceeding 2,000 mg/kg bw and a LC_{50} value of 44,500 mg/m³ (13,700 ppm) in rats. Depending on the dose, the main clinical signs are sedation and narcosis.

Irrespective of the exposure route, the main and most sensitive targets of toxicity in animals after repeated dose application of benzene are the cells of the bone marrow and haematopoietic system. Chronic benzene exposure can result in bone marrow depression expressed as leucopenia, anaemia and/or thrombocytopenia, leading to pancytopenia, and aplastic anaemia.

Inhalation and oral exposure studies provide evidence that benzene is a multipotential carcinogen in animals. Target organs of benzene, irrespective of exposure route, included the haematopoietic system and a spectrum of tissues of

epithelial origin. In mice, carcinogenicity of the haematopoietic system predominantly involves the induction of lymphomas. In contrast, increased frequencies of leukaemia were found in rats after exposure to benzene. In addition, several epithelial tumours have been found in mice and rats.

Bacterial mutagenicity assays conducted with benzene or its metabolites are predominantly negative, whereas mixed results have been observed in mammalian cell culture assays. In the majority of in vivo micronucleus tests and in vivo chromosomal abberation assays, positive results have been observed for benzene and its metabolites.

In female rats exposed up to 975 mg/m^3 (300 ppm) benzene for 10 weeks during premating, no adverse effects on fertility, reproduction, and lactation were observed. In mice, however, at this benzene concentration led to indications for changes in reproductive organs. Most distinct for the males. These effects however, were accompanied with clear-cut haematotoxicity (anaemia, leucopenia and thrombocytopenia) in both sexes. None of the developmental studies demonstrated a specific effect, even at levels that induced signs of maternal toxicity.

Evaluation and recommendation

The Committee concludes that benzene, as recommended by the Subcommittee on Clasification of Carcinogenic Substances, and in accordance with EU classification, is known to be carcinogenic in humans (classification category 1A, see Annex H and I). The Subcommittee has further concluded that benzene acts by a non-stochastic genotoxic mechanism. The Committee, therefore, decided to apply a threshold approach.

Irrespective of the exposure route, the main and most sensitive targets of toxicity in animals and humans after repeated exposure to benzene are cells of the bone marrow and haematopoietic system. Several studies address haematological effects in humans at low benzene exposure levels, however, the results are not consistent. Some studies report adverse effects below 3.25 mg/m^3 (1 ppm), whereas other studies do not. The Committee applies a pragmatic approach based on the aggregate of the accumulated evidence. It considers a benzene effect level of 2 mg/m^3 (0.6 ppm) an appropriate point of departure to derive a health-based recommended occupational exposure limit (HBR-OEL). The Committee applies an additional uncertainty factor to take into account the use of an effect level instead of a no-effect level, as point of departure. By applying a default uncertainty factor of 3, the Committee derives a HBR-OEL for benzene of 0.7 mg/m^3 (0.2 ppm).

Health-based recommended occupational exposure limit

The Committee recommends a health-based occupational exposure limit for benzene of 0.7 mg/m^3 (as an eight-hour weighted average concentration).

Chapter 1

Scope

1.1 Background

At request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances that are used in the workplace. The purpose of the evaluation is to recommend a health-based occupational exposure limit, expressed as a concentration in the air, provided the database allows the derivation of such a value.

This advisory report contains an evaluation of the health hazard and recommendation for a health-based occupational exposure limit for benzene.

1.2 Committee and procedure

The present document contains the evaluation of the DECOS, hereafter called the Committee. The members of the Committee are mentioned in Annex B. The submission letter to the Minister can be found in Annex C.

In 2013, the president of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in

deciding on the final version of the advisory report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

1.3 Data

The Committee's recommendation on the health-based occupational exposure limit of benzene has been based on scientific data, which are publicly available. Data were obtained from the online databases Toxline, Medline and Chemical Abstracts, In addition, in preparing this report several review documents were consulted.[1-6] The last search was performed in October 2013.

Finally, a list of abbreviations can be found at the end of this report in Annex J.

Chapter 2

Identity, properties and monitoring

2.1 Identity and physico-chemical properties

Benzene is a colourless liquid with a sweet odour. It is a ubiquitous environmental contaminant that is found in air, water and soil, and comes from both industrial and natural sources. It is commercially recovered from both coal and petroleum sources to be used primarily in the manufacture of organic chemicals.[1,2,6] In Europe, benzene is mainly used to make styrene, phenol, cyclohexane, aniline, maleic anhydride, alkylbenzenes and chlorobenzenes. It is an intermediate in the production of anthraquinone, hydroquinone, benzene hexachloride, benzene sulfonic acid and other products used in drugs, dyes, insecticides and plastics. It is also added to gasoline for its octane-enhancing and anti-knock properties.[6]

A summary of the identity and physical and chemical properties is given in Table 1.

Table 1 Identity, physical and chemical properties of Benzene.[1,2]

IUPAC Name	:	Benzene
Synonyms	:	Cyclohexatriene; Benzol
CAS number	:	71-43-2
EINECS number	:	200-753-7
Chemical formula	:	C_6H_6
Chemical structure	:	
Molecular weight	:	78.11 g/mol
Colour	:	Clear, colourless liquid
Physical state	:	Colourless to light yellow liquid
Melting point	:	5.5°C
Boiling point	:	80.1°C at 1,013 hPa
Density at 20°C	:	0.879 g/mL
Odour	:	Aromatic
Solubility:		
• Water at 25°C		1,800 mg/L
• Organic solvents		Miscible with acetone, chloroform, diethyl ether and ethanol; soluble in carbon tetrachloride
Log K_{ow} [a]	:	2.13 (determined by HPLC)
Vapour pressure	:	99.7 hPa at 20°C
Autoignition temperature	:	498°C
Flashpoint	:	-11°C (closed cup)
Flammability limits in air	:	1.2% (lower limit); 7.8% (upper limit)
Explosive limits	:	1.4% (lower limit); 8% (upper limit)
Conversion factors	:	1 ppm = 3.26 mg/m^3

[a] = octanol-water partitioning coefficient

2.2 EU Classification and labeling

In the European Union, benzene is classified for carcinogenicity, category 1A (H350; May cause cancer) and for germ cell mutagenicity, category 1B (H340; May cause genetic defects)*.

* According to the Regulation on the classification, labelling and packaging of substances and mixtures (1272/2008/EC).

2.3 Validated analytical methods

In this section, the analytical methods which are available for detecting and/or measuring and monitoring benzene in air and in biological samples are described. The intention is not to provide an exhaustive list of analytical methods that could be used to detect and quantify benzene. Rather, the Committee aims to identify well-established methods that are used as the standard methods of analysis. For more details on analytical methods for benzene, the Committee refers to the review documents and subsequent references used.[7,8]

2.3.1 Biological monitoring

The determination of benzene in urine, blood and expired air, and S-phenyl-mercapturic acid (SPMA) in urine are suitable approaches for biomonitoring of benzene (see also Sections 5.5 and 5.6).[7] Despite the sensitive analytical methods available, no "standard" analytical method exists for benzene. For the determination of SPMA in urine, a standardised gas chromatography/mass spectrometry (GC/MS) approach has been published by the Deutsche Forshungs Gemeinschaft.[9]

The analysis of blood and urine for benzene consists of an extraction step which is typically followed by GC/MS, hereby separating benzene from other volatile constituents. Extraction procedures include purge and trap, head space, solilid phase extraction (SPE; i.e., using Tenax or charcoal) and solid phase microextraction (SPME). After extraction, benzene is transferred to a capillary column for GC separation. MS, or flame ionization or can subsequently be used for the detection and quantification of benzene. The limit of detection (LOD) for benzene in urine, blood and expired air falls within the low ng/L range.

Several analytical methods for the determination of SPMA in urine exists. Commonly used methods consist of extracting SPMA from the urine by liquid-liquid extraction with ethyl acetate or by SPE. After subsequent derivatisation, SPMA can be detected by GC/MS with a LOD generally in the range of 1-5 µg/L. Another approach is the detection of SPMA with high-performance liquid chromatography (HPLC) in combination with ultraviolet absorption detection, diode array detection, fluorescence detection, and MS. Generally, the LODs are below 1 µg/L.

2.3.2 *Environmental monitoring*

To determine benzene air levels, samples are collected from the air on either an adsorbent or by trapping whole air in a container.[8] Passive vapour monitors or badges which collect volatile organic compounds based on diffusion are commonly used in occupational settings to measure ppm concentration levels present within the personal or breathing zone air. Environmental measurements, where the concentration is typically several orders of magnitude lower then occupational levels, are generally obtained by active sampling. Active sampling involves an air sampling pump and adsorbent held in an inert trap, and is the most sensitive sampling method. Recently, also passive badges have been used for environmental measurements.

Benzene in air samples is then analysed by GC. The most common detector currently used with a GC is a MS detector. The LOD for personal air monitoring is typically around 0.1 mg/m^3 (0.03 ppm) (for an 8-hour sampling period).

Soil and water can be analysed in a similar fashion to air samples by purging the benzene from the water or soil and trapping the benzene on an adsorbent to facilitate its transference to GC. Alternate analytical methods for introducing a sample for GC analysis include headspace and SPE or SPME systems to concentrate the benzene from the sample followed by liquid concentration and injection.

Chapter 3

Sources

3.1 Natural occurrence

The natural sources of benzene include gas emissions from volcanoes and forest fires.[1,2] Other sources of benzene are crude oil and, to a lesser extent, condensate from natural gas production.[2]

3.2 Man-made sources

Benzene is produced by different petroleum conversion processes in petroleum refinery and chemical plant processes, primarily by catalytic reforming, steam cracking and dealkylation. Benzene is recovered during production of coal-derived chemicals, primarily from coke oven by-products. Benzene is extracted from these sources and purified for industrial use.[2]

Benzene is released from a number of man-made sources. Approximately 60% of the benzene emissions in The Netherlands is caused by road traffic. Other sources are combustion in wood-burning stoves and fire places, accounting for approximately 20% of the benzene emission, and storage and transshipment and chemical industry in the Rijnmond area.[10]

Chapter 4

Exposure

4.1 General population

Exposure to low levels of environmental benzene is unavoidable due to the ubiquitous presence of benzene in the environment from a variety of natural and anthropogenic sources. Major sources of exposure to the general population include combustible fuel emissions and exhaust from motor vehicles, evaporation of gasoline and solvents (especially in attached garages), and industry or hazardous waste sites.[2,7]

The highest air levels of benzene in The Netherlands are present in urban areas with a high industrial activity, such as locations of storage and transshipment, and surrounding highways (Figure 1a).[10] Mean benzene levels have shown a decreasing trend, in particular since the 90's, due to the introduction of the three-way catalytic converter, other technical improvements of motorised transport, and the reduction of the amount of benzene in petrol from 5% to 1% (Figure 1b). The mean benzene concentration for the Netherlands in 2011 was 0.50 µg/m³. Urban concentration levels were up to 4-fold higher than levels measured in rural areas.

An additional source of exposure (for both workers and general population) is tobacco smoking (including second-hand smoking). It has been estimated that it accounts for 90% of the total benzene exposure of smokers.[7]

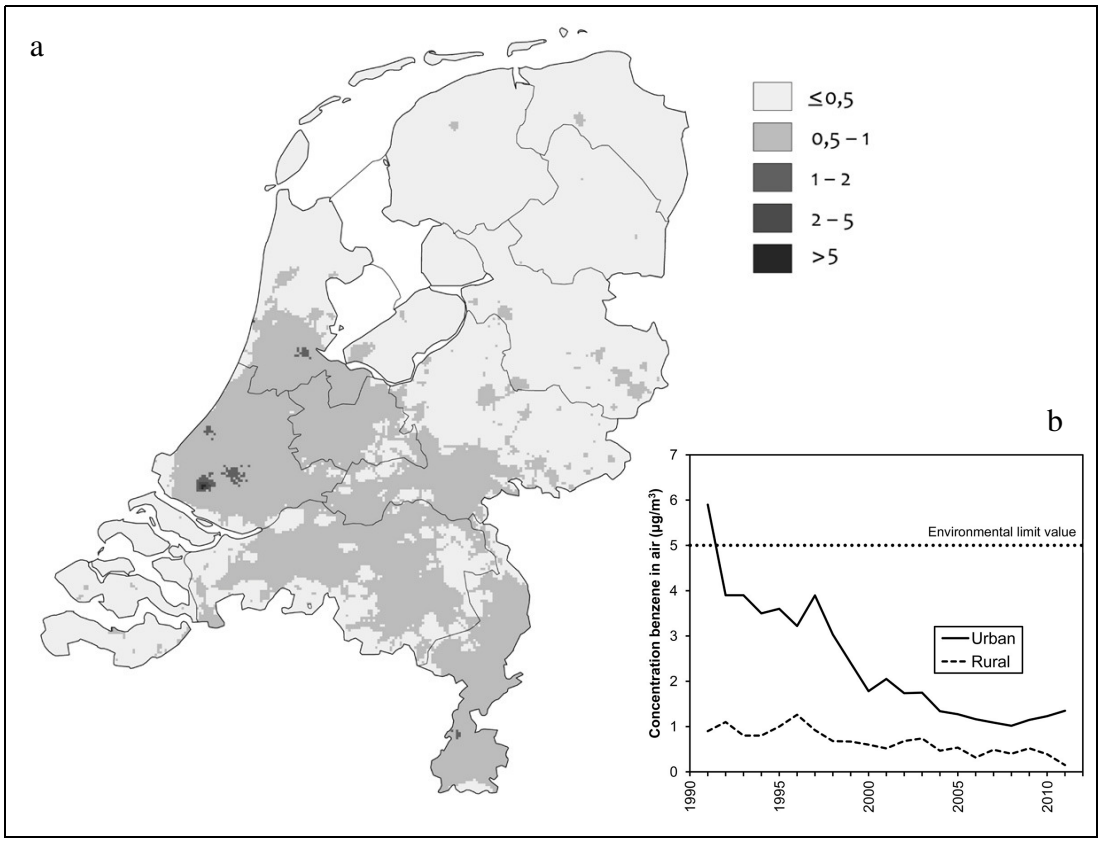

Figure 1 (a) Estimation of annual average benzene concentrations in air (µg/m^3) in the Netherlands (2011), (b) Trend of the benzene concentration between 1990-2011. (Source: RIVM/DCMR, 2012; modified from[10])

4.2 Working population

Primary occupational exposure in Western countries is associated with employment in industries that use or make benzene or products containing benzene.[1,7] In a number of other occupations, exposure to benzene can occur indirectly through the use of petroleum products (e.g., aviation workers, service station workers, bus drivers, cargo tank workers) or solvents. Long term air concentrations for these various exposures have been reported in the range of 0.01 to 2 mg/m^3 (0.003 to 0.6 ppm) (arithmetic mean), measured during 1991-2003.[6]

Chapter 5

Kinetics

The absorption, distribution and metabolism, and the possibilities of biological monitoring of benzene have been studied extensively. In this Chapter, the Committee provides a summary based on review documents.[1,2,7,11]

5.1 Absorption

Benzene is readily absorbed by all routes (inhalation, dermal and oral), of which inhalation is considered to be the most important route of exposure.

Inhalation

Numerous studies on the absorption of benzene after inhalation exposure have been conducted. Mean absorption rates have been described, from approximately 80% (during the first minutes of exposure to 150-350 mg/m^3 (47-110 ppm) to approximately 50% after 4 hours of exposure in the 3.3-33 mg/m^3 (1-10 ppm) range.[12,13] Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median level of 547 ng/L) than in a control group of 13 nonsmokers (median level of 190 ng/L).[14]

Animal data suggest that the uptake of benzene by the lungs is related to the concentration in a non-linear manner.[15] Mean percentage of inhaled ^{14}C-benzene absorbed and retained in the tissues and blood during a 6h exposure decreased

from 33-15% in rats, and from 50-10% in mice, as the exposure concentration was increased from approximately 26-2,600 mg/m³ (8-812 ppm). At similar vapour concentration exposures, mice take up 1.5 to 2.0-fold the dose per kilogram body weight compared to rats.

Dermal

Results from in vivo experiments indicate that liquid benzene can be absorbed through human skin, although not as substantial as absorption following inhalation or oral exposure. In vitro experiments with human skin indicate that benzene can be absorbed dermally.[16]

In studies conducted in rhesus monkeys, miniature pigs, and hairless mice, dermal absorption was < 1% following a single direct (unoccluded) application of liquid benzene.[16-19] In hairless mice, absorption was rapid and absorption rates increased linearly with dose and exposure time.[20] Williams et al. analysed the experimental (both human and animal; in vitro and in vivo) skin absorption data of benzene, and concluded that the steady state absorption rate of benzene ranges from 200-400 µg/cm^2*h.[16]

Oral

Data on the rate of absorption of benzene following oral ingestion in humans are not available. Case reports of accidental or intentional ingestion suggest that benzene is also readily absorbed after oral ingestion.[2]

Benzene appears to be efficiently absorbed following oral dosing in animals. Approximately 80% of the administered radioactivity was eliminated in exhaled air and urine within 2-3 days after oral administration of ^{14}C-labeled benzene to rabbits (340-500 mg/kg bw).[21] Gastrointestinal absorption exceeded 97% in rats and in mice at oral doses between 0.5 and 150 mg benzene/kg bw.[15]

5.2 Distribution

Information on the distribution of benzene in humans is primarily derived from case studies, and relates to exposure by inhalation.[2] The available data suggest that benzene, upon absorption, is distributed throughout the body. Benzene has been detected in various biological fluids and tissues of humans. Benzene is lipophilic and the highest levels have been found in lipid-rich tissues. Benzene also has been shown to be able to cross the human placenta, and has been found in the cord blood in amounts equal to or greater than those in maternal blood.[2]

Animal data also show that benzene distributes in tissues rich in lipids and/or with high perfusion rates, such as the kidney, lung, liver, brain and spleen. Benzene can cross the placenta and distribute to developing offspring.[2] The relative uptake in tissues appears to be dependent on the perfusion rate of tissues. Following inhalation exposure to 1,600 mg/m³ (500 ppm) benzene, steady state concentrations of benzene were reached in male F344 rats within 4 hours in blood (11.5 mg/mL), in 6 hours in fat (164.4 mg/g) and in less than 2 hours in bone marrow (37.0 mg/g).[22] Levels in bone marrow exceeded the respective levels in blood. Benzene metabolites (phenol, catechol, and hydroquinone) were detected in blood and bone marrow of rats following 6 hours of inhalation exposure to benzene. For the more water soluble benzene metabolites, the distribution differs from that of benzene.[23]

5.3 Metabolism

Although the metabolism of benzene has been studied extensively, the steps leading to benzene toxicity are not yet fully understood. The metabolism of benzene is an important determinant for benzene-induced toxicity; it is generally understood that both cancer and noncancer effects are caused by one or more (reactive) metabolites of benzene. Available data indicate that metabolites produced in the liver are carried to the bone marrow where benzene toxicity is expressed. Benzene metabolism may also occur, at least in part, in the bone marrow.

Benzene metabolism appears to follow similar pathways in both animals and humans[24]; however remarkable species variability has been demonstrated.[2] A general scheme of the metabolism of benzene is illustrated in Figure 2.

The first step in the metabolism of benzene is the oxidation of benzene to benzene oxide by the cytochrome P-450 dependent mixed-function oxidase system. The P-450 enzyme CYP 2E1 appears to exhibit the greatest affinity for benzene and is the most active in benzene metabolism. CYP 2B1 is also capable of hydroxylating benzene, but contributes to the metabolism only at higher benzene concentrations.[2] At low benzene exposures, involvement of an enzyme other than CYP 2E1 has been suggested.[25]

Several pathways are involved in the metabolism of benzene oxide[26], which exists in equilibrium with its oxepin.[27] The predominant pathway involves non-enzymatic rearrangement to form phenol.[28] In turn, phenol is oxidised in the presence of CYP2E1 to catechol or hydroquinone, which are subsequently oxidised via myeloperoxidase (MPO) to the reactive metabolites 1,2- and

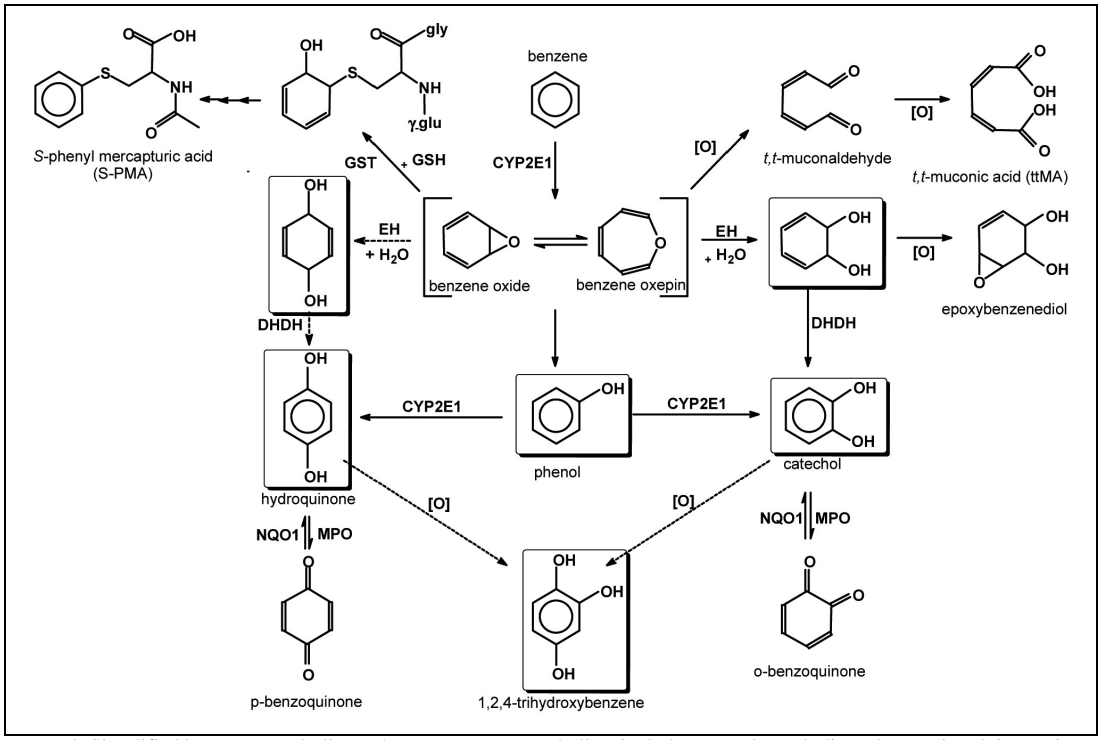

Figure 2 Simplified benzene metabolism scheme. Benzene metabolism includes several metabolic pathways, involving various enzymatic and non-enzymatic steps. The framed compounds can be excreted as glucuronide or sulphate.

Abbreviations:CYP2E1 = cytochrome P-450 2E1; DHDH = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; GST = glutathione-S-transferase; MPO = myeloperoxidase; NQ01 = NAD(P)H: quinone oxidoreductase 1.

1,4-benzoquinone, respectively.[29] Benzoquinone formation via myeloperoxidase in the bone marrow is suggested as being a key step in the carcinogenicity of benzene.[30] The sensitivity of bone marrow cells to benzene has been attributed to a relatively high level of peroxidases present in this tissue.[2,31] The reverse reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalysed by NAD(P)H: quinone oxidoreductase 1 (NQ01). Both catechol and hydroquinone may be converted to 1,2,4-benzenetriol via CYP2E1 catalysis.

Alternatively, benzene oxide may undergo epoxide hydrolase-catalysed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalysed conversion to catechol.[29,32,33] Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo

conjugation[29,34] with sulfate or glucuronic acid; the conjugates of phenol and hydroquinone are the major urinary metabolites of benzene.[35,36]

Other pathways of benzene oxide metabolism include: (1) reaction with glutathione to form SPMA[29,37-41], and (2) iron-catalysed ring-opening to trans, trans-muconic acid (ttMA), presumably via the reactive trans, transmuconaldehyde intermediate.[29,42-46]

5.4 Elimination

Available human data indicate that following inhalation exposure to benzene, exhalation is the major route of elimination of unmetabolised benzene in humans and animals. Most of the absorbed benzene however, is metabolised and the metabolites are excreted after phase-II-conjugation predominantly in the urine (sulfates and glucuronides).[24] Small amounts of the glucuronides may enter the bile and are found in the faeces. There is evidence that the elimination via some metabolic routes is saturable. No studies were available regarding excretion in humans after oral exposure to benzene.

Experimental data in laboratory animals have shown an essentially similar pattern of benzene elimination and excretion as in humans. Also in animals, unmetabolised benzene is excreted mainly by exhalation and metabolised benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in faeces. At higher concentrations, relatively high amounts of benzene are excreted by exhalation.

5.5 Possibilities for biological monitoring

Biomarkers of exposure

Several biomarkers of benzene exposure have been studied. These include benzene levels in blood, urine or expired air. In addition, benzene metabolites in urine and biological adducts of benzene have been used as biomarkers of exposure.[8] The following approaches have been evaluated[7]:

- benzene in blood, urine and expired air
- SPMA in urine
- ttMA in urine
- phenol in urine
- catechol and hydroquinone in urine, and
- DNA and protein adducts in blood.

Of the abovementioned parameters, only benzene in blood and urine, and SPMA in urine are considered reliable biomarkers. The use of benzene in expired air is hampered by practical issues during analysis (e.g., variability in breath sampling, transportation and storage; presence of contamination and losses, and absence of standardised methods), whereas the benzene metabolites ttMA, phenol, catechol and hydroquinone lack specificity. For the determination of DNA adducts, sensitive and specific analytical methods are not available.[7]

Urinary SPMA, a benzene metabolite with a mean half life ranging from 9-13 hours, has been shown to be a reliable biomarker for recent benzene exposure.[7,41,47,48] For SPMA, there is no known endogenous or exogenous source, other than benzene exposure and SPMA levels can be detected below 1 µg/L.

5.6 Possibilities for biological effect monitoring

Biomarkers of effect (e.g., complete blood cell counts, red and white blood cell counts, chromosomal aberrations, sister chromatid exchanges, and examination of bone marrow) have been suggested for benzene. The Committee notes however, that these biomarkers can be informative on a population level but cannot easily be used for individual health surveillance, since they are not specific for exposure to benzene.

5.7 Summary

Benzene is readily absorbed by all routes (inhalation, dermal and oral), of which inhalation is considered to be the most important route of occupational exposure. In humans, mean absorption has been reported ranging from approximately 50-80%. In animals, the percentage of benzene absorbed and retained in the tissues and blood during a 6h exposure decreased from 33-15% of the dose in rats, and from 50-10% in mice, when exposure was increased from 26 to 2,600 mg/m³ (8-812 ppm). The estimated skin absorption rate ranges from 200-400 µg/cm²*h. Benzene is efficiently absorbed following oral dosing in animals; absorption levels have been reported of > 97% (in rats and mice) and 80% (in rabbits).

Upon absorption, benzene is distributed throughout the body. Benzene has been detected in various biological fluids and tissues of humans, highest levels amounting in lipid-rich tissues. Also in animals, benzene distributes in tissues rich in lipids, particularly those with high perfusion rates, such as the kidney.

The metabolism of benzene is not yet fully understood. The available data indicate that metabolites are primarily generated in the liver. Similar metabolic pathways exist in animals and human, the first step is the oxidation of benzene to benzene oxide by cytochrome P-450, mainly CYP 2E1. Several pathways are involved in the metabolism of benzene oxide, predominantly the pathway involving non-enzymatic rearrangement to form phenol. In turn, phenol is oxidised in the presence of CYP2E1 to catechol or hydroquinone, which are subsequently oxidised to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively. The phenolic metabolites of benzene undergo conjugation. Other pathways of benzene oxide metabolism include the reaction with glutathione to form SPMA and iron-catalysed ring-opening to ttMA.

Following inhalation exposure to benzene, exhalation is the major route of elimination of unmetabolized benzene in humans and animals. Most of the absorbed benzene however, is metabolised and the metabolites are excreted after phase-II-conjugation predominantly in the urine.

The determination of benzene in urine, blood and expired air, and the determination of SPMA in urine are suitable approaches for biological biomonitoring of benzene.

Chapter 6

Mechanism of action

Benzene-induced carcinogenicity is caused by a complex mechanism involving the metabolism of benzene, subsequent toxicity to blood cells and blood-forming organs, genotoxicity and formation of initiated, mutated bone marrow target cells, altered oncogenic signalling and clonal proliferation.[2,49] In this Chapter, the Committee will focus on haematotoxic and genotoxic effects.

Haematotoxicity

Benzene must be metabolised to induce haemototoxic and carcinogenic effects.[43] Several metabolising enzymes have been shown to be involved, in particular CYP2E1. Other enzymes associated with benzene toxicity include epoxide hydrolase and detoxifying NAD(P)H: quinone oxidoreductase 1 (NQ01).[1] Intermediates associated with hematotoxicity include hydroquinone, p-benzoquinone, catechol and muconaldehyde (see Figure 2).[1,2] The contribution to benzene-induced toxicity of each intermediate is currently not known.

Toxicity is thought to be a result of multiple reactive intermediates that interact with multiple targets within the bone marrow. The bone marrow is particularly sensitive to benzene, among others due to the presence of benzene-metabolising enzymes leading to the production of reactive oxygen species. In turn, reactive oxygen species can lead to a spectrum of cellular effects, including damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins and DNA itself (including structural and numerical aberrations).[1]

Benzene-induced haematotoxicity manifests as pancytopenia (a decrease in various cellular elements of the circulating blood resulting in anaemia, leukopenia, or thrombocytopenia), and aplastic anaemia (along with myelofibrosis), i.e., when all cellular elements in the peripheral blood and bone marrow are reduced.

Haematoxicity is considered to be an early indicator of developing acute myeloid leukaemia (AML)/myelodysplastic syndrome (MDS) after benzene exposure.[50] Persistent cytopenias and other blood disorders frequently precede the onset of leukaemia in patients developing AML secondary to exposure to benzene or alkylating agents.[3] Also, workers suffering from benzene poisoning are at increased risk of developing leukaemia.[51] Currently however, it is not proven that benzene-induced haematotoxicity forms an initial (required) step to neoplastic disease, or simply represents bone marrow damage.[52]

Genotoxicity

Leukaemia develops from genotoxic effects in the CD34 progenitor cells in the bone marrow, a primary target in benzene-toxicity.[53,54] Overwhelming evidence exists that benzene causes chromosomal aberrations in haematopoetic cells in humans and experimental animals.[1,2,55] The Committee considers this induction of chromosomal aberrations the most plausible explanation for benzene carcinogenicity.

Multiple pathways leading to MDS/AML have been identified. These involve different oncogenes and tumour suppressor genes and can be distinguished by their specific chromosomal aberration. Several typical cytogenetic or mutagenic profiles are commonly observed in AML:[56,57]

- unbalanced aberrations (primarily 5q-/-5 or 7q-/-7 and +8)
- balanced rearrangements (e.g., t(11q23), t(8;21) and t(15;17)) or inversions (e.g., inv(16))
- karyotypically normal but with mutations (e.g., mutations of NPM1 or C/EBPα, duplications of FLT3).

These profiles are quite similar for therapy-related MDS/AML (i.e., MDS/AML caused by treatment with alkylating agents, radiation, or topoisomerase II inhibitors) and spontaneous MDS/AML, although the frequencies at which these typical chromosomal aberrations occur may differ.[57] MDS/AML associated with benzene exposure has been reported to share a similar genetic profile with therapy-related MDS/AML, i.e., a high frequency of loss of all or part of chromosomes 5/7.[30,58,59] AML/MDS related to therapy and AML/MDS related to

benzene exposure have therefore been considered biologically similar diseases.[50,57,60] Recent data however, suggest that the pattern of clonal cytogenetic abnormalities in benzene-exposed cases more closely resemble that of spontaneous AML than therapy-related AML.[61]

Several underlying mechanisms of benzene-induced AML/MDS have been suggested in literature (reviewed by McHale et al.[62]), i.e.,:

1 Inhibition of topoisomerase II. The inhibition of topoisomerase II is the most well established explanation for the genotoxic mode of action of benzene, and the most likely mechanism through which benzene induces chromosomal translocations.[62] Several studies have shown that benzene and its metabolites hydroquinone and 1,4-benzoquinone act as inhibitors of topoisomerase II ("topoisomerase II poisons"), potentially leading to DNA strand breaks, aberrant mitotic recombination and subsequent chromosomal aberrations.[30,55,63-66] Topoisomerase II inhibitors are indeed also known to produce leukaemia in humans and some share structural and biological similarities with benzene.[64,67] Furthermore, several genetic pathways that have been implicated in benzene-induced MDS/AML are associated with the inhibition of topoisomerase II.[56] Whysner et al. compared the genotoxic profiles of benzene and its metabolites with those of other genotoxic agents, and concluded that it was most similar to genotoxicity induced by topoisomerase II inhibitors.[55]
2 Adduct formation of reactive metabolites. Adduct formation has been observed for benzene metabolites in multiple organs in animals, and in blood of benzene exposed workers.[68] This mainly involved binding to proteins, for which benzene oxide and *p*-benzochinon have been considered as the most important metabolites involved. Based on the very low level of DNA adducts found, in particular in target tissues, it has been suggested that covalent binding does not play a significant role in benzene-induced carcinogenicity.[55]
3 Oxidative DNA damage. Several benzene metabolites have been associated with the generation of reactive oxygen species (ROS). Subsequently, reactive oxygen species and oxidative damage after exposure to benzene have been linked with the induction of DNA strand breaks and point mutations.
4 Error prone DNA repair. It has been suggested that induction and activation of DNA-PKcs may contribute to benzene carcinogenesis by increasing the error-prone, non-homologous end joining (NHEJ) DNA repair pathway.. This has also been suggested to explain the high susceptibility of haematopoetic stem cells to benzene, as these cells preferentially initiate DNA repair instead of undergoing apoptosis.

5 Epigenetic alterations. Benzene has been shown to alter the expression of many genes in the peripheral blood of exposed workers. Epigenetic changes are major mechanisms by which gene expression is regulated, and epigenetic marks including histone modification, DNA methylation and microRNA expression, activate or repress expression of individual genes (e.g., oncogenes and tumor suppressor genes).

Consequently, these events in the stem or progenitor cell most likely result in genomic instability, and subsequent activation of key protooncogenes, loss of heterozygosity, and inactivation of tumour suppressor genes. Dysregulation of the p53 pathway resulting in alterations in cell cycle checkpoints, apoptosis, or the DNA repair system may be an event leading to haematopoietic malignancies.[52]

Conclusion

Benzene-induced haematotoxicity and genotoxicity result from a complex cascade of events. Multiple mechanisms have been suggested to be involved (cytogenetic alterations, aberrant mitotic recombination, gene mutations and/or epigenetic alterations). Most evidence has been provided for a role of inhibition of topoisomerase II in the induction of chromosomal damage; however, other modes of action, such as oxidative stress and inhibition of DNA repair, may add to the effect.

Based on the weight of evidence. the Subcommittee on Classification of Carcinogen Substances considers that the above mentioned mechanisms, which ultimately may lead to genotoxicity and altered gene expression, are most likely to be explained by mechanisms for which a threshold exists. Therefore, the Subcommittee concludes that benzene acts by an indirect (non-stochastic) genotoxic mode of action (see Annex H).

Chapter 7

Effects

Numerous human studies and animal studies regarding the carcinogenic and non-carcinogenic effects of benzene have been published. As the Committee prefers the use of human data, and considers the available human data on benzene adequate, it will focus its current evaluation of benzene to human studies. Furthermore, the Committee limits its evaluation to the human studies most relevant for the quantitative risk assessment of benzene.

7.1 Observations in humans

7.1.1 Carcinogenicity

Classification of haematological malignancies

There is extensive literature available on benzene carcinogenicity in humans. These carcinogenic effects mainly relate to the haematopoietic and lymphoid system. The classification of tumours of the haematopoietic and lymphoid tissues has been revised by the World Health Organisation in 2008, due to the availability of new scientific and clinical information.[69] This revised classification is based on the integration of clinical, morphologic, immunopheno-typic, genetic and other biological features.[70] In the revised classification, haematological disorders are no longer classified according to their localisation, but according to their cells of origin. The traditional classification and the 2008

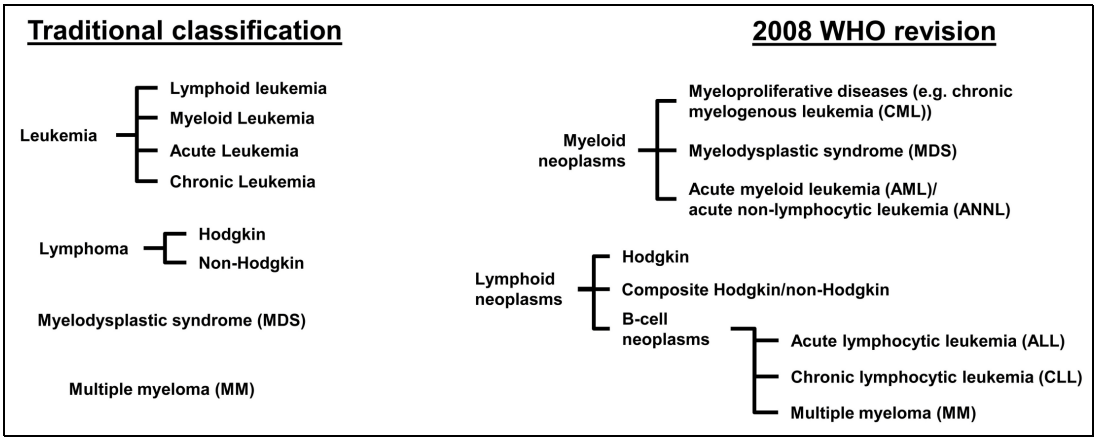

Figure 3 Former and current classification of the main haematopoietic and lymphoid malignancies.

revision of the main haematopoietic and lymphoid tumour types are illustrated in Figure 3.

Due to the revision, the classification of several haematological disorders that have been associated with exposure to benzene have changed in time, hereby complicating an historical analysis of these disorders.[71] For instance, acute lymphatic leukaemia (ALL) has been reported as a type of leukaemia in past studies, whereas in more recent studies, ALL is classified as a lymphoid neoplasm. In addition, in the new classification additional haematological disorders are distinguished. Particularly relevant for benzene is the current discrimination between myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML; also referred to as acute non-lymphatic leukaemia (ANLL)). These malignancies are both associated with benzene exposure and have historically been difficult to discern.

Critical carcinogenic endpoints

Despite the use of different classifications in time, the Committee notes that clear evidence has been published for a relationship of benzene exposure and development of leukaemia and AML/MDS. For other haematological malignancies, this relationship is less clear. Several positive associations have been reported, including two recent meta-analyses in which an association was suggested between benzene exposure and multiple myeloma (MM), chronic lymphocytic leukaemia (CLL), acute lymphocytic leukaemia (ALL), and chronic myeloid leukaemia (CML).[72,73]

Overall, the Committee finds the data available on subtypes of haematological malignancy other than leukaemia AML/MDS, most notably non-Hodgkin lymphoma (NHL), currently inconsistent (see e.g., references[72,74-79]). Therefore, the Committee will focus on the key cohorts for exposure-response analysis – including recent updates, and meta-analyses – on leukaemia in general and AML.[80] These studies are summarised below and represented in Annex E. The malignancies that are less consistently linked to benzene exposure are not specifically discussed by the Committee, as these do not influence the carcinogenic classification of benzene nor do these provide a basis for a quantitative risk analysis.

Cohort studies

Pliofilm cohort

The Pliofilm cohort is an extensively studied cohort consisting of workers exposed to benzene in three rubber hydrochloride manufacturing plants at two locations in Ohio.[81] This cohort has historically been considered to be a carefully performed study, with a low probability of co-exposures which served as a basis for earlier quantitative risk analyses of several authorities or organisations.[4] This cohort has been updated several times.

The Pliofilm cohort initially consisted of a total of 748 workers who were exposed to benzene for at least 1 day between 1940 and 1950.[81] Mean exposure duration was short but high exposures (> 325 mg/m^3 (> 100 ppm)) occurred frequently; 58% of the workers were exposed to benzene less than 1 year. Death rates for age-matched U.S. white males during the same calendar period were used for comparison with death rates observed in the cohort. An increased risk of dying from leukaemia (all myelocytic or monocytic cell types) was found (standardised mortality ratio (SMR) of 5.6, $p < 0.001$). Workers exposed for more than 5 years had a SMR of 21.00.

An update of this cohort, providing more detailed exposure estimates, included 1,165 white males employed between 1940 and 1965 who were followed through 1981.[82] Cumulative exposure for each cohort member was estimated from historical air-sampling data, or alternatively, from interpolation based on existing data. A statistically significant increased mortality from all leukaemias (9 observed versus 2.7 expected; SMR = 3.37; 95% CI 1.54-6.41) and multiple myeloma was noted (4 observed versus 1 expected; SMR = 4.09; 95% CI 1.10-10.47). Stratification of exposure identified a relationship between cumulative exposure and an increasing risk of leukaemia.

Assessment of the Pliofilm cohort after an additional follow-up period up to 1996 (i.e., 15 years after the original report from Rinsky (1981)) also showed an increased leukaemia mortality risk at cumulative exposures exceeding 650 mg/m^3-years (200 ppm-years).[83] For MM, a non-significant increased relative risk was found whereas for NHL, no association was found. Although in this update 5 new leukaemia cases were observed, the relative risk had declined compared to the previous analysis, suggesting that the excess risks diminished with prolonged time since exposure. Exposures in the most recent 10 years were most strongly associated with leukaemia risk; there was no significant relationship observed between leukaemia deaths and benzene exposures of more than 20 years ago.

Although several exposure estimates have been derived in time[84-86], the temporal trend observed in this cohort has been undisputed and has been confirmed by others.[87-89]

NCI/CAPM[*]

The largest cohort study conducted to date is the NCI/CAPM study.[90-93] In this study incidence rates (occurrence of disease and cause of death) for lympho-haematopoietic malignancies and other haematological disorders were evaluated in a cohort of 74,828 benzene exposed and 35,805 non-exposed workers employed in 672 factories at 12 cities in China. The workers were employed from 1972 to 1987, and were followed for an average of nearly 12 years. Estimates of benzene exposure were derived from work histories and available historic benzene measurements. Mean exposure was estimated to be 73.4 mg/m^3 (22.5 ppm) (time-weighted average), with a mean exposure duration of 9.3 years. A total of 82 exposed cases were diagnosed with leukaemia.

For all haematological neoplasms (leukaemia, ANLL, ANLL/MDS and NHL), increased RRs and statistically significant trends were found when benzene exposure was expressed as either average or cumulative exposure level. Analysis by duration of exposure (< 5, 5-9, or ≥ 10 years) did not show increased risk with increasing exposure duration.

UK Petrol cohort

In a case-control study in the United Kingdom, 91 cases of leukaemia were compared with matched controls (four per case) in a cohort of workers in the petroleum distribution industry who were exposed to low levels of benzene.[94,95]

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[*] Study by the U.S. National Cancer Institute (NCI) and the Chinese Acadamy of Preventive Medicine (CAMP).

Exposure to benzene was estimated based on work histories obtained, and specific information on the exposure conditions at the terminals. Analyses were done for all leukaemia and separately for acute lymphoblastic leukaemia, CLL, AML and acute monocytic leukaemia, and CLL. Potential confounding or effect modifying variables considered were smoking, employment status at end date, socioeconomic status based on the job title of longest duration, age, and the starting date of work, ever had a previous job, and ever had a previous job as a driver.

No significantly increased risk was observed in the risk of overall leukaemia or subtypes. The authors note the suggestion of a relationship between myeloid leukaemia (in particular acute myeloid and monocytic leukaemia) and exposure to benzene.

The UK Petrol cohort was included in a pooled analysis, recently published by Schnatter et al.[96] (see below).

Dow cohort

Ott et al. described a retrospective cohort study in 594 individuals exposed to benzene at a chemical plant in Michigan between 1938 and 1970, with follow up from 1940 to 1973.[97] Workers were exposed to an estimated 6.5-29.5 mg/m^3 (2-9 ppm) (TWA), based on data derived from work histories and industrial hygiene records. No significant increases in total leukaemia were found in chemical industry workers. A statistically significant increased number of cases of myelogenous leukaemia was noted (4 observed compared to 0.9 expected; $p < 0.011$). The study, however, suffers from limitations in design (*e.g.,* a small sample size).

In a subsequent update of this cohort, with an additional follow up of 9 years included 956 employees, the mortality risk of myelogenous leukaemia was increased but did not reach statistical significance (SMR = 1.94; 95% CI 0.52-4.88).[98]

In 2004, a follow-up study was published on this cohort study in which cause-specific mortality was determined in a prospective study of 2,266 chemical workers.[99] The risk for leukaemia was slightly above background (SMR = 1.14). No significantly increased risk was found for any of the lymphohaematopoietic cancers. Only a weak trend of increasing SMRs for leukaemia, and possibly ANLL, with increasing cumulative exposure to benzene was noted.

Chemical Manufacturers Association (CMA) cohort

This cohort study consisted of 4,602 male workers in US chemical industry between 1946 and 1975.[100,101] The control group consisted of 3,074 unexposed

males from the same company in the same period. Benzene exposure was estimated based on work histories and benzene measurements. Half of the exposed workers were exposed to average benzene concentrations of less than 3.3 to over 160 mg/m^3 (1-50 ppm) for six months to over 35 years, on average 8 hours per day. The control group consisted of 3,074 unexposed workers from the same chemical industry. Death rates were also compared to the general population.

No increased risk of leukaemia or other types of lympho-haematopoietic cancer were noted among the exposed and unexposed group when compared with the general population. When compared to the unexposed group, the exposed group showed a statistically significant positive trend in the risk of leukaemia and other types of lympho-haematopoietic cancer.

Australian Health Watch study

A large nested case-control study within a cohort of 17,525 employees in the Australian petroleum industry working for more than 5 years found an increased risk of leukaemia.[102-104] Workers were employed between 1935 and 1985, the majority after 1965. Exposure to benzene was retrospectively estimated based on individual occupational history and benzene exposure measurements supplied by Australian petroleum companies.[102] 79 cases of lympho-haematopoietic cancers were identified between 1981 and 1999, including 33 cases of leukaemia – 11 of these cases were diagnosed as ANLL. A strong association was found between leukaemia risk and exposure to benzene. Increasing risk was reported from 2.6-5.2 mg/m^3-years (0.8-1.6 ppm-years) onwards, to significantly increased ORs of 5.9 and 98.2, for exposure estimates of > 26-32 mg/m^3-years (> 8-16 ppm-years) and 52 mg/m^3-years (>16 ppm-years), respectively. Risk for the subtype ANNL was increased at exposures greater than 26 mg/m^3-years (8 ppm-years) (OR = 7.17; 95% CI 1.27-40.4).[103] NHL and MM were not associated with benzene exposure.

In a re-analysis of the exposure-response relationship, the 7 leukaemia cases with the highest cumulative exposure (52 mg/m^3-years (>16 ppm-years)) were compared with a different reference exposure category (i.e., the two lowest exposed categories).[104] This new reference category contained 9 cases of leukaemia, as opposed to 3 cases in the previous analysis. An increase in leukaemia risk with increasing cumulative benzene exposure was observed, hereby confirming the earlier analysis of this cohort by Glass in 2003.

This cohort was included in a pooled analysis, recently published by Schnatter et al.[96] (see pooled analysis below).

Canadian cohort

In 1996, Schnatter conducted a nested case-control study on lymphohaematopoetic cancers in a previously defined cohort[105,106], consisting of a total of 6,672 petrochemical workers (average age 38 years) exposed to benzene for a median 18 years 63 and followed up for an average of approximately 15 years.[107] A total of 31 cases (16 leukaemia cases, 7 cases of MM and 8 cases of NHL) were matched with 4 controls for each case. Benzene exposure was estimated based on work histories and historical industrial hygiene surveys, and ranged from 0.04-20.2 mg/m^3 (0.01-6.2 ppm). Additional potential confounders including smoking, dermal contact, hobbies, previous exposures and occupations, diagnostic radiation exposure and familial cancer were accounted for.

No significant association was found between the cumulative benzene exposure and mortality due to leukaemia, NHL or multiple myeloma. However, the power of the study was limited and the authors report incomplete correction for confounders.

This cohort was included in a pooled analysis, recently published by Schnatter et al.[96] (see pooled analysis below).

Pooled analysis of the AHW, Canadian and UK Petrol cohorts

Recently, Schnatter et al. presented an update of three nested case–control studies among petroleum workers from Australia, Canada, and the United Kingdom.[96] Cases were re-assessed based on the new classification for hematopoetic malignancies and exposure to benzene was standardised across the three studies. Cumulative benzene exposure showed a monotonic dose-response relationship with MDS (highest vs lowest tertile, > 2.93 vs ≤ 0.35 ppm-years, OR = 4.3; 95% CI 1.3 to 14.3). In contrast to previous findings in these cohorts, there was little evidence of a dose–response relationships for AML.

Shoe factory worker cohort

A cohort consisting of 891 men and 796 women employed in the shoe factory industry was followed between 1939 and 1984.[108] Workers were followed from 1950 to 1999. Exposures were estimated based on work histories and limited air sampling data. Estimated benzene concentrations ranged from 0-300 mg/m^3 (0-92 ppm), and mean cumulative exposures of 190.4 ± 304.2 mg/m^3-years (58.4 ± 93.9 ppm-years), respectively. Duration of exposure and duration of employment were not reported. The general population was used as control reference.

Leukaemia risk was significantly increased at >652 mg/m^3-years (> 200 ppm-years) for men only (SMR = 7.0; 95% CI 1.9-18.0) and for men and women combined (SMR = 5.1; 95% CI 1.4-13.0). Leukaemia subtypes were not evaluated.

Offshore worker cohort study
A recent historical cohort study included 27,919 offshore workers and 366,114 controls from the general working population.[109] Workers were employed between 1981 and 2003. Benzene exposure and variability therein, was however not quantified in this study. Based on previous studies assessing benzene exposure for this type of industry, the authors estimated that exposure of the workers ranged from below 0.003 to 2.3 mg/m^3 (0.001 to 0.7 ppm).

An increased risk of AML (RR = 2.89; 95% CI 1.25-6.67) and multiple myeloma (RR = 2.49; 95% CI 1.21-5.13) was observed. An increased risk of AML was only present among workers who had their first registered engagement in this industry in the period 1981-1985 (RR = 3.26). In contrast, for the period 1986-2003 there was no statistically significant increased risk for AML found.

Meta-analyses

A meta-analysis by Raabe and Wong consisted of 19 cohorts with a total of 208,741 workers in the petrochemical industry employed between 1937 and 1989 in the United States and the United Kingdom.[110] Average exposure levels were estimated to be 0.70 mg/m^3 (0.22 ppm) based on mean benzene exposure levels reported for general plant operations in petroleum refineries. No increased risk of AML or other types of leukaemia were found. Analyses that were limited to studies of refinery workers or studies with at least 15 years of follow-up yielded similar results.

In a more recent analysis, Vlaanderen et al. fitted meta-regression models to 30 aggregated risk estimates, extracted from 9 observational studies to assess the benzene-leukaemia exposure-response relationship.[111] In addition, relative risks (RRs) were calculated for several cumulative exposure levels, based on either all studies, or cohort studies only, using four different modelling scenarios (i.e., natural spline or linear model with and without intercept). All scenarios predicted a significantly increased, exposure-dependent RR. The highest RRs were predicted by applying a natural spline on all studies, whereas these RRs dropped considerably after correction for the predicted intercept, or when only cohort studies were used.

Khalade et al. conducted a systematic review and meta-analysis on the relationship between occupational benzene exposure and the risk of leukaemia, including all types combined and the four main subgroups separately (AML, ALL, CLL and CML).[112] This meta-analysis included 15 studies providing 16 risk estimates in total. A statistically significant increase in summary effect size for all leukaemias combined was found. Dose-response analysis for the 9 studies in which exposures were expressed in ppm-years resulted in significant increased risks of leukaemia for the different exposure categories and a significant trend. The risk for AML was also increased, but the trend was not statistically significant. Some evidence of an increased risk was also noted for CLL, no association was found for CML.

A meta-analysis of benzene exposure and leukaemia subtypes, including nine cohorts and 13 case-control studies from several industries, found a high and significant risk of AML.[113] Furthermore, a positive dose-response relationship across study designs was noted. No clear evidence for a dose-response relationship was obtained for other leukaemia subtypes (CLL, CML and ALL). This meta-analysis however, did not attempt to couple exposure concentrations to AML incidence but rather described patterns among industrial sectors and different study designs in relation to relative risk.

Vlaanderen et al. conducted a meta-analysis on a total of 44 publications on 5 different haematological malignancies (HL, NHL, MM, ALL and CLL).[72] The authors aimed to identify the most informative subgroups of cohort studies by the stratification of three different study quality dimensions. These study quality dimensions involved (a) the year of start of follow-up, (b) the strength of the reported association benzene-AML, and (c) the quality of the exposure assessment. For MM, ALL and CLL the relative risk increased with increasing study quality for all three stratification approaches, thereby suggesting an association with the exposure to benzene.

A similar approach by these authors was applied for CML. The overall meta-relative risk (mRR) was non-significantly elevated (1.23; 95% CI 0.93-1.63).[73] An increasing meta-RRs with increasing study quality was reported for all dimensions. For studies with start of follow-up after 1970 this increase was statistically significant (1.67; 95% CI 1.02-2.74. For AML, the highest study quality stratum significance and exposure quality showed an elevated but non-significant increased mRR.

Conclusion

The Committee concludes that benzene is carcinogenic in humans by causing leukaemia and more specifically, AML/ANNL.

7.1.2 Genotoxicity

The Committee limits its evaluation of the genotoxicity data (mainly on chromosomal aberrations in peripheral white blood cells) to studies with low exposure levels (i.e., below 33 mg/m^3 (10 ppm) of benzene (Table 2). For a complete overview of the genotoxicity data, the Committee refers to extensive review documents available (e.g., references[1,2,6,55,59]).

Chromosomal aberrations

A large biomonitoring study (n=171) in Chinese factory workers exposed to benzene also included the assessment of chromosomal aberrations.[114] A broad range of benzene exposures was studied, on the day of biological sample collection, exposures ranged from 0.20-400 mg/m^3 (0.06-123 ppm) with a median exposure of 10.4 mg/m^3 (3.2 ppm). The median of the 4-week mean benzene exposures was 12.4 mg/m^3 (3.8 ppm), and the median lifetime cumulative exposure was 166.1 mg/m^3 (51.1 ppm-years). The results analysed using 4-week mean exposures showed a significant trend in the increase of chromatid breaks, total chromatid-type aberrations, total chromosomal-type aberrations, and total aberrations. When the low portion of the benzene exposure spectrum (< 1.6 mg/m^3; mean 0.5 mg/m^3 (< 0.5 ppm; mean 0.14 ppm); n=16) was examined, there were positive associations for total chromatid aberrations, total chromosomal aberrations, total aberrations, chromatid breaks, and acentric fragments.

The specificity of benzene-induced aneuploidy and the influence of genetic polymorphisms on chromosomal aberrations were studied in workers at a coke oven plant by Kim et al.[115] The benzene concentration ranged from 0.03-2.4 mg/m^3 (0.01-0.7 ppm) (geometric mean 1.8 mg/m^3 (0.6 ppm)). Multiple regression analysis indicated that the frequencies of chromosome aberrations were significantly associated with benzene exposure and polymorphisms in the metabolic enzyme genes.

In a cytogenetic monitoring study by De Jong et al., 32 workers exposed for periods between 1 and 13 years to low levels of benzene (0.1-2.5 mg/m^3; < 0.03-0.8 ppm)) in a petrochemical complex in the Netherlands were monitored

for the induction of structural chromosomal aberrations.[116] Control samples were obtained from 42 employees working in an remote administrative office, and matched for sex, age and smoking habits. No increase in frequencies of chromosome aberrations was observed.

Table 2 Selection of genotoxicity studies with human peripheral lymphocytes at low benzene concentrations.

Industry	Number of workers exposed	Mean exposure (range)		Result (result at < 3.3 mg/m³ (1 ppm))	Reference
		mg/m³	ppm		
Chromosomal aberration tests in pheripheral lymphocytes					
Shoe and glue manufacturing	130 16 (subgroup)	10.4 (0.2-400) 0.5 (0-1.6)	3.2 (0.1-122) 0.14 (0-0.5)	+ (+) + (+)	Qu et al. (2003)[114]
Coke oven plant	82	1.8 (0.0-2.4)	0.6 (0.0-0.7)	+ (+)	Kim et al. (2004)[115]
Petrochemicals	32	< 0.3 (< 0.1-2.5)	< 0.1 (< 0.0-0.8)	- (-)	De Jong et al. (1988)[116]
Oil-refinery	42	7.2 (2.9-20.5)	2.2 (0.9-6.3)	+ (ND)	Major et al. (1994)[117]
Oil-refinery	49 subgroup	(3.0-68.7) (1.0-18.4)	(0.9-21.5) (0.3-5.6)	+ (ND) + (ND)	Tompa et al. (1994)[118]
Benzene production	22	(0.7-40.3)	(0.2-12.4)	+ (ND)	Sarto et al. (1984)[119]
Shoe manufacturing	49	< 16.2	< 5	+ (ND)	Bogadi Sare et al. (1997)[120]
Shoe manufacturing	38 45	25.5 (5.2-48.6) 15.9 (6.2-40.8)	7.8 (1.6-15.0) 4.9 (1.9-12.6)	+ (ND)	Karacic et al. (1995)[121]
Single strand breaks					
Shoe manufacturing	20	4.2 (0.8-16.1)	1.3 (0.2-4.8)	+ (ND)	Popp et al. (1992)[123]
Several, with exposure to gasoline	33	0.4 (0.0-2.0)	0.13 (0.0-0.6)	+ (+)	Nilsson et al. (1996)[124]
Micronucleus assay					
Shoe manufacturing	35	2.6	0.8	+ (+)	Liu et al. (1996)[125]
Sister chromatid exchange					
Shoe manufacturing	38 45	25.5 (5.2-48.6) 15.9 (6.2-40.8)	7.8 (1.6-15.0) 4.9 (1.9-12.6)	+ (ND) - (ND)	Karacic et al. (1995)[121]
Oil-refinery	42	7.2 (2.9-20.5)	2.2 (0.9-6.3)	+ (ND)	Major et al. (1994)[117]
Oil-refinery	49 subgroup	(3.0-68.7) (1.0-18.4)	(0.9-21.5), (0.3-5.6)	+ (ND) + (ND)	Tompa et al. (1994)[118]
Benzene production	22	(0.7-40.3)	(0.2-12.4)	- (ND)	Sarto et al. (1984)[119]
Other: chromosomal aberrations in sperm					
Various, including shoe and glue manufacturing	33 9	9.4 (< 78) < 3.3	2.9 (< 24) < 1	+ (+) + (+)	Xing et al. (2010)[126] Ji et al. (2012)[127]

ND: Not determined; positive results were observed but exposure below 3.3 mg/m³ (1 ppm) was not specifically addressed.

Chromosomal aberrations were measured in peripheral blood lymphocytes of 42 oil-refinery workers exposed to benzene, and 42 controls.[117] The benzene concentrations in the ambient air samples varied from 3-20 mg/m³ (mean: 7 mg/m³) (0.9-6.3 ppm; mean 2.2 ppm). The continuous low-dose benzene exposure statistically significantly increased the numbers of chromosomal aberrations.

Tompa et al. assessed the induction of chromosomal aberrations, SCEs and UV-induced DNA synthesis as indicators of genotoxic effects in peripheral blood lymphocytes of 49 workers occupationally exposed to benzene (3-68.7 mg/m³;

0.9-21.1 ppm).[118] Most of the workers were followed up in a period in which the benzene concentrations were reduced to 1-18.4 mg/m^3 (0.3-5.6 ppm). Overall, the frequencies of chromosomal aberrations were significantly higher in the exposed groups than in controls.

A cytogenetic study was performed with 22 healthy workers engaged in benzene production and exposed to low concentrations of benzene and 22 matched controls. Exposure concentrations ranged from 0.7-40.4 mg/m^3 (0.2-12.4 ppm).[119] Benzene exposure was confirmed by biological monitoring. A statistically significant increase of structural chromosomal aberrations was observed in the exposed workers.

The incidence of structural chromosome aberrations and SCE was studied by Bogadi Sare et al. in the peripheral blood lymphocytes cell genome of 49 female shoe-makers.[120] Workers were exposed to concentrations of benzene up to 49 mg/m^3 (15 ppm). Chromosomal aberration analysis revealed a significant increase in dicentric incidence in the exposed group compared to the controls, however the authors noted a presence of potential confounders.

Structural chromosome aberrations in peripheral blood lymphocytes were studied in female workers employed in the shoe-making industry, the first group in 1987 and the second group in 1992.[121] Mean benzene exposures were 25.5 and 15.9 mg/m^3 (7.8 and 4.9 ppm) for the first and the second group, respectively. The results were compared with those obtained from 35 controls. This cytogenetic study showed a significant increase in dicentric chromosomes in exposed groups I and II when compared to the control group.

Single strand breaks

The induction of single strand breaks was studied in workers exposed to benzene in one of five occupational work places, including six industrial process types, namely, printing, shoe-making, methylene di-aniline (MDA), nitrobenzene, carbomer, and benzene production.[122] Benzene concentration in breath was less than 9.8 mg/m^3 (3 ppm). The mean value of DNA damage was 1.73 ± 0.81. Dose-dependent DNA damage occurred at higher levels of exposure, and DNA damage exhibited a strong correlation with benzene breath levels.

Peripheral lymphocyte DNA damage was investigated in a group of 20 female workers of a shoemaking plant who were exposed to benzene (mean concentration of 4.16 mg/m^3; range 0.80-16.10 mg/m^3 (1.3; 0.2-5.0 ppm)) and toluene.[123] The relative DNA elution rate was statistically significantly higher in workers compared to controls.

Nilsson et al. determined the induction of single-strand breaks in DNA of leukocytes, and urinary levels of the oxidative DNA adduct 8-hydroxydeoxyguanosine (8OHdG), in 33 men occupationally exposed to benzene from gasoline and in 33 controls.[124] The average exposure to benzene over a shift was determined by personal air sampling. The 8-hr TWA exposure to benzene was 0.4 mg/m^3 (range: 0.01-2.0 mg/m^3) (0.13; 0.003-0.6 ppm). Exposed workers had a significant increase of single strand breaks ($p = 0.04$) over the shift compared with controls. Urinary 8OHdG increased over the shift in exposed workers but not in controls.

Induction of micronuclei

The induction of MN in blood lymphocytes was measured in 87 shoe makers exposed to different concentrations of benzene, and 30 controls.[125] In the 35 individuals in the lowest exposure category, exposed to a mean concentration of 2.6 mg/m^3 (0.8 ppm), a statistically significant increase in micronucleated lymphocytes was reported.

Sister chromatid exchange (SCE)

In several studies with peripheral lymphocytes, the induction SCE was also assessed, mostly with positive results.[117-119,121,123]

Other

Xing et al. used multicolour fluorescence in situ hybridization to measure the incidence of sperm with numerical abnormalities of chromosomes X, Y, and 21 among 33 benzene-exposed men and 33 unexposed men from Chinese factories.[126] Exposure levels ranged from below the detection limit to 78 mg/m^3 (24 ppm) (median, 9.4 mg/m^3 (2.9 ppm)). From the exposed men, 27% (n = 9) were exposed to concentrations of $\leq$ 3.3 mg/m^3 (1 ppm). Increased sperm aneuploidy was observed within low- and high-exposed groups, including the $\leq$ 3.3 mg/m^3 (1 ppm) exposure group.

These authors subsequently compared aneuploidies in blood lymphocytes and sperm within the same individuals.[127] The results showed that benzene exposure was positively associated with the gain of chromosome 21 but not sex chromosomes in blood lymphocytes. This was in contrast to analysis of sperm, where the gain of sex chromosomes, but not chromosome 21, was significantly increased in the exposed workers. Furthermore, a significant correlation in the

gain of sex chromosomes between blood lymphocytes and sperm was observed among the unexposed subjects, but not among the exposed workers.

Conclusion on genotoxicity

The Committee notes that multiple studies are available that indicate that benzene is genotoxic in humans by inducing cytogenic effects. Nearly all studies were performed with isolated peripheral lymphocytes. Several studies however, suffer from poor exposure information and methodological insufficiencies, in particular lack of a proper control groups and insufficient information on benzene exposure (i.e., the occurrence of peak concentrations co-exposures to other chemicals).[2]

7.1.3 Haemototoxicity

Occupational exposure to benzene has long been associated with toxicity to the blood and bone marrow, including lymphocytopenia, pancytopenia, and aplastic anaemia. Several cross-sectional studies with workers who were exposed to benzene have shown haematological effects at a broad range of exposure levels. The Committee limits its assessment to haematological effects observed at low concentrations, i.e., at or below 1 ppm: these are relevant for setting a health-based recommended occupational exposure level (HBR-OEL).

Lan et al. assessed the white blood cell and platelet count in 250 shoe workers exposed to benzene and 140 controls.[128] For each subject, individual benzene and toluene exposure was monitored repeatedly up to 16 months before phlebotomy, and postshift urine samples were collected from each subject. Subjects were categorised into four groups (control; < 3.3 mg/m^3 (< 1 ppm); 3.3-32.5 mg/m^3 (1-10 ppm); and > 32.5 mg/m^3 (> 10 ppm)) by mean benzene levels measured during the month before phlebotomy. All types of white blood cells measured, but also platelets were significantly decreased in workers from the lowest exposure group (mean exposure of 1.9 mg/m^3 (0.6 ppm)) to the highest exposure group (93.3 mg/m^3 (28.7 ppm)). Also in a subpopulation that included workers exposed to a mean exposure level of 0.9 mg/m^3 (0.3 ppm) benzene, the decrease in the number of peripheral blood cells was statistically significant. In addition, highly significant dose-dependent decreases in colony forming capacity of progenitor cells were observed. The effect on colony formation appeared greater than the effect on differentiated white blood cells and

granulocytes, suggesting a relatively higher sensitivity towards benzene of progenitor cells as compared to mature cells.

In a study by Qu et al., personal benzene exposure was monitored and peripheral blood cells were counted in a total of 181 Chinese factory workers.[114,129,130] The population studied had a broad range of benzene exposures: on the day of biological sample collection, exposures ranged from 0.2-396 mg/m^3 (0.1-122 ppm) with a median exposure of 10.4 mg/m^3 (3.2 ppm). The median of the 4-week mean benzene exposures was 12.4 mg/m^3 (3.8 ppm), and the median lifetime cumulative exposure was 166.1 mg/m^3-years (51.1 ppm-years). In this study, a decrease in neutrophil number and red blood cells was observed at benzene exposures of 1.6 mg/m^3 (0.5 ppm) or lower (4-week mean of 0.5 mg/m^3 (0.1 ppm)). The Committee notes that 24/51 control subjects were males, whereas no males were included in the exposure group.

In a study on the haematotoxic effects of benzene, 928 workers in 5 factories in China were monitored weekly and 12 peripheral blood indices were examed.[131] According to the authors, the most sensitive parameters to benzene appeared to be neutrophils and the mean platelet volume, where effects were seen, with a linear exposure-response relationship, for benzene air concentrations of 25.3-26.7 mg/m^3 (7.8-8.2 ppm). Logistic regression analysis revealed statistically significant occurrence of anemia in the < 3.3 mg/m^3 (< 1 ppm) category.

In studies conducted in the Western chemical industry, in general, no haematological effects have been observed at benzene exposure levels at or below 3.3 mg/m^3 (1 ppm).

Collins et al. found no adverse effects on routinely collected haematological parameters in chemical workers exposed to either 0.0-4.6 mg/m^3 (0.0-1.4 ppm) or 1.8 mg/m^3 (0.6 ppm) (8h TWA mean).[132,133]

Swaen et al. compared 8,532 blood samples collected during routine health surveillance of workers exposed to low levels of benzene at a chemical plant in The Netherlands, with 12,173 samples of employees with no occupational benzene exposure.[134] A Job Exposure Matrix was constructed to estimate benzene exposure. Depending on the job and operational status, the mean 8h TWA benzene air concentration ranged from 0.5-3.0 mg/m^3 (0.1-0.9 ppm). No adverse effect on any of the haematological parameters was observed.

Table 3 Overview of studies of adverse effects on haematological parameters at low benzene concentrations.

Industry	Number exposed	Mean exposure (range)		Effect observed < 3.3 mg/m³ (1 ppm)	Reference
		mg/m³	ppm		
Shoe production	250	0.9	0.3	+	Lan et al. (2004)[128]
Glu and shoe production	131	0.5 (0-1.6)	0.14 (0.0-0.5)	+	Qu et al. (2002)[129]
Rubber/shoe / pharmaceutical	928	7.4 (0.1-872)	2.3 (0.0-268)	+	Schnatter et al. (2010)[131]
Chemical	200	0.0-4.6	0.0-1.4	-	Collins et al. (1991)[132]
Chemical	387	1.8	0.6	-	Collins et al. (1997)[133]
Chemical	701	(0.5-3.0)	(0.1-0.9)	-	Swaen et al. (2010)[134]
Petroleum production	1,200	< 2.0	< 0.6	-	Tsai et al. (2004)[135]

A large study included 1,200 petrochemical workers with routinely collected haematological parameters, with mean benzene exposure (TWA-8h) of 2.0 mg/m³ (0.6 ppm) from 1977 to 1988 and 0.5 mg/m³ (0.1 ppm) since 1988 who were compared to 3,227 unexposed controls. No increased abnormality in any of the included haematological parameters was found.[135]

An overview of findings in blood cell counts in the low exposure range is provided in Table 3.

7.2 Other effects

Several other effects have been associated with exposure to benzene. As these effects are generally observed at exposure levels far exceeding exposure levels at which haematological effects occur, the Committee considers them as non-critical for deriving a health-based recommend occupational exposure level. Therefore, only a short summary for these other effects is provided here, based on review documents.[1,2,11]

Irritation and sensitisation

High concentrations of benzene vapours are irritating to the mucous membranes of the eyes, nose, and respiratory tract. In humans, benzene is a skin irritant.[136] By defattening the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis.[137] The ingestion of liquid benzene causes local irritation of the mucous membranes of the mouth, throat, esophagus and stomach.[2]

Acute and short-term toxicity

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects at levels ranging from 975-9,750 mg/m^3.[1] Very high concentrations of benzene vapours produce narcotic effects and can lead to death by respiratory arrest. Case reports have been described that report an acceleration (of the respiratory rate) followed by drowsiness, fatigue, dizziness, headache and nausea after inhalation of a high concentration of benzene vapour. At high exposure levels, pulse rate increases, there may be a sensation of tightness in the chest accompanied by breathlessness, and ultimately people exposed may lose consciousness. Convulsions and tremors have occurred , from which it can be concluded that death may follow in a few minutes or several hours following severe exposure. Cyanosis, hemolysis, and congestion or hemorrhage of organs were reported in the cases for which there were autopsy reports.[138-141]

Long-term toxicity

With the exception of haematological toxicity, genotoxicity and carcinogenicity (described in Section 7.1.1 to 7.1.3), limited data are available regarding toxicity in humans following long-term inhalation exposure to benzene.[1] Most findings are inconclusive due to uncertainties in exposure assessment and limitations in reporting. Both humoral and cellular immunological effects have been described in humans exposed to benzene. In 2008, the Dutch Health council evaluated the effects of organic solvents, including benzene, on reproduction.[142] The evaluated data did not indicate that effects on development or fertility could be attributed to exposure to benzene.

7.3 Observations in animals

An enormous amount of animal data on benzene toxicity is available, and involves relatively high benzene exposure levels. In view of the available human data, the Committee considers the animal data not relevant for establishing a HBR-OEL. Only a short summary is provide here, for more details the Committee refers to extensive reviews available.[1,2,11,55]

Irritation and sensitisation

Benzene has been shown to be irritating to the skin of rabbits, inducing moderate erythema, edema, and moderate necrosis following application.[143] Benzene can also cause irritation of the mucous membranes (eye, respiratory tract and mouth, esophagus and stomach).[2]

Acute and short-term toxicity

Acute inhalation toxicity is low with a LC_{50} value of 44,500 mg/m³ (13,700 ppm) after a 4-hour exposure for rats.[144] Depression of the central nervous system appeared to be related to death. The main pathological findings were congestion of the lungs and liver. A dermal LD_{50} value of > 8,260 mg/kg bw for rabbits and guinea pigs has been reported.[145] Acute oral toxicity data for rats suggest that the oral LD_{50} is above 2,000 mg/kg bw, ranging from 810 mg/kg bw to 10,000 mg/kg bw. [146-148] Depending on the dose, the main clinical signs are sedation and narcosis. Pathological findings include among others hyperemic and haemorragic lungs, adrenals and spine.

Long-term toxicity

Irrespective of the exposure route, the main and most sensitive targets of toxicity in animals after repeated dose application of benzene are the cells of the bone marrow and haematopoietic system.[1,2,11] The rapidly proliferating stem cells, myeloid progenitor cells and stromal cells are sensitive targets. Chronic benzene exposure has been reported to result in bone marrow depression expressed as leucopenia, anaemia and/or thrombocytopenia, leading to pancytopenia, and aplastic anaemia at concentrations > 33 mg/m³ (10 ppm).

Carcinogenicity

Several studies with inhalation and oral exposure provide evidence that benzene is carcinogenic in animals.[149-162] Target organs of benzene, irrespective of exposure route, included the haematopoietic system and a spectrum of tissues of epithelial origin.

In mice, carcinogenicity of the haematopoietic system predominantly involves the induction of lymphomas. In contrast, increased frequencies of leukaemia in comparison to controls were found in rats after exposure to benzene.

In addition, several epithelial tumours have been found in mice (e.g., Zymbal gland, lung, Harderian gland, preputial gland, forestomach, mammary gland and liver) and rats (e.g., Zymbal gland, oral cavity, forestomach, nasal cavity, and skin).

An overview of carcinogenic effects observed in animals is provided in Annex F.

Genotoxicity

Bacterial mutagenicity assays conducted with benzene or its metabolites are predominantly negative, whereas mixed results have been observed in mammalian cell culture assays.[1,2,55]

In the vast majority of in vivo micronucleus tests and in vivo chromosomal abberation assays, positive results have been observed for benzene and its metabolites (i.e., phenol, hydroquinone, catechol, and benzenetriol). Only one micronucleus test was conducted with a target organ, the Zymbal gland in the rat, which was also positive.[163] Benzene and some metabolites tested caused structural chromosomal aberrations and sister chromatid exchange (SCE), although some of the SCE results were considered only weakly positive. Furthermore, benzene has been tested positive in a majority of DNA damage assays.[55] Two in vivo mutagenicity assays in transgenic mice have been described in which marginal responses were noted.[164,165] In contrast to the conclusion of the authors, the Subcommittee on Classification of Carcinogenic Substances considered these as negative results (for details on the view of the Subcommittee, see Annex H).

Finally, most of the genotoxicity studies conducted in Drosophila melanogaster (heritable translocations, sex-linked recessive lethal mutations, somatic mutation and recombinations) gave negative results.

An overview of the outcome of the in vivo genotoxicity assays is provided in Annex G.

Reproduction toxicity

Fertility

Aspects related to male and female fertility have been investigated in laboratory animals in studies of different quality and validity and with the inhalatory route of administration only. In a fertility study with female rats exposed up to 300

ppm benzene for 10 weeks during premating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation.[166]

Available data from subchronic toxicity studies indicate that mice are more sensitive to benzene exposure than rats. With respect to possible effects on the organs of the reproductive system, no effects for either sex have been observed in rats with concentration levels of up to and including 300 ppm (960 mg/m^3) benzene. In mice, however, this benzene concentration level led to some indications for changes in reproductive organs. These appeared to be more distinct for the males (testes weight and histopathology affected) than for the females (occasional ovarian cysts), but were accompanied with clear-cut haematotoxicity (anaemia, leucopenia and thrombocytopenia) in both sexes.[2]

Developmental effects

There are numerous inhalation studies available in which rats or mice have been exposed to benzene during pregnancy.[166-172] None of these studies demonstrated a specific embryotoxic or teratogenic potential even at levels that induced signs of maternal toxicity. However, impairment of fetal development as evidenced by decreased body weights of the offspring and increased skeletal variants as well as delayed ossification were observed at levels > 162.5 mg/m^3 (> 50 ppm) often associated with maternal toxicity.

7.4 Summary and evaluation

Epidemiologic studies and case studies provide clear evidence of a causal association between exposure to benzene and leukaemia, especially AML/ ANLL. More recently, an increased risk of MDS is being linked with the exposure to benzene. Also for MM, CLL, CML and ALL, although to a lesser extent, associations with benzene exposure have been reported. The association with other B-cell lymphomas such as follicular lymphoma and diffuse large B-cell lymphoma remains unclear.

Benzene induces tumours in several target organs, including the haematopoietic system and several organs of epithelial origin, in rats as well as in mice.

Convincing evidence exists that exposure to benzene causes the induction of micronuclei, chromosomal aberrations, sister chromatid exchanges and DNA strand breaks, both in humans and in animals. Gene mutation assays are overall negative, whereas no DNA adducts have been measured in target tissues in vivo after exposure to either benzene or its metabolites.

Impairment of the haemopoietic system is the primary (non-carcinogenic) adverse health effect after long-term exposure to benzene. This includes the manifestation of bone marrow depression, leading to aplastic anaemia, leukopenia, agranulocytosis, and pancytopenia.

In humans, high concentrations of benzene vapour are irritant to mucous membranes of the eyes, nose and respiratory tract. Liquid benzene on direct contact may cause erythema dryness and cracking of the skin. In animals, benzene is irritant to the skin and may cause serious damage to eyes.

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects at high exposure levels. Convulsions and tremors occur frequently, and death may follow in a few minutes or several hours following severe exposure.

The acute toxicity of benzene in animals is low. The oral LD_{50} is estimated to be > 2,000 mg/kg bw and depending on the dose, the main clinical signs are sedation and narcosis. An LC_{50} value of 44,500 mg/m³ (13,700 ppm) is reported in rats; depression of the central nervous system appeared to be related to death.

With the exemption of carcinogenic, haematotoxic and genotoxic effects, no clear evidence for critical effects after long-term exposure to benzene is available. Information on the reproductive toxicity of benzene in humans is limited. There are no indications that benzene is teratogenic in humans. In animals, some effects associated with reproduction were observed, however only at haematotoxic exposure levels of benzene.

Based on the available carcinogenicity data, the Subcommittee on Classification of Carcinogenic Substances confirms the classification of benzene in category 1A (the compound is known to be carcinogenic to humans). Based on the data available on the mode of action, the Subcommittee furthermore concluded that benzene acts by a non-stochastic genotoxic mode of action (see Annex H for further details on the Subcommittee's opinion).

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

As of 2010, the European Union has an environmental exposure limit value for benzene of 5 µg/m^3 (year-average).

8.2 Working population

The currently applicable occupational exposure limits are summarised in Table 4.

In addition, biological limit values* have been set several international authorities. For example, ACGIH established biological exposure limits (biological exposure index (BEI)) of 25 µg/g creatinine for SPMA, and 500 µg/g creatinine for ttMA.[173] DFG (Deutsche Forschungsgemeinschaft) applies biological exposure limits (*Expositions equivalents für krebserzeugende Arbeitsstoffe (EKA)***; exposure-equivalents for carcinogenic substances) of 5 µg benzene/L in blood, and 45 µg SPMA/g creatinine and 2 mg/L ttMA in urine.[174]

* Time of sampling: end of shift.
** EKA value corresponding to external benzene exposure of 3.3 mg/m^3.

Table 4 Existing Occupational Exposure Limits (OELs) for benzene.

Country - Organisation	OEL (mg/m^3)	Time-weighted average	Type of OEL[a]
The Netherlands[b] - Ministry of Social Affairs and Employment	3.25	8h	ns
European Union[c]	3.25	8h	BLV
Denmark[b]	1.6	8h	ns
Finland[b]	3.25	8h	BLV
France[b]	3.25	8h	BEL
Germany			
- AGS[d]	1.9 (1)/ 0.2 (2)		
- DFG[175]	none	-	-
Norway[b]	3	8h	ns
Spain[b]	3.25	8h	ns
Sweden	1.5	8h	ns
	9	15 min	ns
United Kingdom[b]	3.25	8h	WEL
USA[1]			
- ACGIH	1.6		TLV
	8.0		STEL
- OSHA	3.2	8h	PEL
	16.0	15 min	STEL
- NIOSH	0.3	10h	REL
	3.2	15 min	STEL

[a] Abbreviations: ns = not specified; TLV = threshold limit value; STEL = short-term exposure limit; PEL = permissible exposure limit; REL = recommended exposure limit; WEL = workplace exposure limit; BLV = binding limit value; BEL = binding exposure limit

[b] Source: Social Economic Council (http://www.ser.nl/en/grenswaarden/benzeen.aspx)

[c] Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work, Annex III (https://osha.europa.eu/nl/legislation/directives/exposure-to-chemical-agents-and-chemical-safety/osh-directives/directive-2004-37-ec-indicative-occupational-exposure-limit-values).

[d] IFA Gestis database (http://limitvalue.ifa.dguv.de/Webform_gw.aspx) ((1) Workplace exposure concentration corresponding to the proposed tolerable cancer risk. (2) Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk.)

8.3 Classification

In the European Union, benzene is classified for carcinogenicity (category 1A/ H350; may cause cancer); and for mutagenicity (category 1B/H340, may cause genetic defects).

In 1982, the International Agency for Research on Cancer (IARC) concluded that there is *sufficient evidence* that benzene is carcinogenic to man and that there is

limited evidence that benzene is carcinogenic in experimental animals.[5] Therefore, IARC classified benzene as a Group 1 carcinogen (carcinogenic to humans).

In the subsequent updates of the monograph, the body of evidence for carcinogenicity in experimental animals was considered to be *sufficient*. The conclusion to classify benzene as a Group 1 carcinogen was confirmed in an update of the monograph in 1987.[176]

In the latest update, IARC concluded that there is *sufficient* evidence in humans for the carcinogenicity of benzene (Group 1).[6] IARC further concluded that benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia, whereas a positive association has been observed between exposure to benzene and acute lymphocytic leukaemia, chronic lymphocytic leukaemia, multiple myeloma, and non-Hodgkin lymphoma. There is *sufficient* evidence for the carcinogenicity of benzene in experimental animals.

Chapter 9

Hazard assessment

9.1 Hazard identification

Irrespective of the exposure route, the main and most sensitive targets of toxicity in animals and humans after repeated exposure to benzene are the rapidly proliferating stem cells, myeloid progenitor cells and stromal cells of the bone marrow and haematopoietic system.[1-6] As has been summarised in Chapter 7, the available data on the critical toxic effects of benzene in humans mainly involve the development of leukaemia (in particular leukaemia from myeloid lineage), the induction of chromosomal aberrations, and the reduction of the number of peripheral blood cells.

9.2 Quantitative assessment of the health risk

The Subcommittee on Classification of Carcinogenic Substances of DECOS has concluded that benzene acts by a non-stochastic genotoxic mechanism (Annex H). DECOS has decided to adopt this conclusion of the Subcommittee, and therefore applies a threshold approach by deriving a HBR-OEL for benzene.

Several exposure-response analyses of the benzene-leukaemia association have been reported. However, as the power at low levels of benzene exposure is low, these studies do not allow the determination of a reliable point of departure for derivation of a HBR-OEL. Studies on the induction of chromosomal aberrations

show limitations in design and reporting and as a consequence, also cannot serve as a basis to derive a reliable point of departure. Data on haematological effects after benzene exposure include quantitative data on low benzene exposures, from properly studies in several occupationally exposed populations. The Committee therefore considers the data on haematotoxicity most suitable for derivation of a HBR-OEL for benzene.

The Committee notes that a large amount of data is available concerning the haemototoxicity of benzene at low exposure levels. At benzene concentrations below 3.25 mg/m^3 (1 ppm), several haematological studies have shown adverse effects whereas several others have not. For the purpose of deriving a HBR-OEL, all of these studies have their strengths and weaknesses. The Committee has therefore decided to apply a weight of evidence approach to derive a HBR-OEL, using the aggregate of evidence of the available studies.

Assessment of relevant studies

The Committee considers the cross-sectional study published by Lan et al.[128] important for its weight of evidence approach, as it provides information on the exposure-response relationship in the exposure range below 3.25 mg/m^3 (1 ppm).

This study on shoe factory workers in China (two-third females) was designed particularly for studying effects of low benzene exposures. Exposure was assessed using personal monitoring for 16 months prior to biological sample collection. Exposure in the last month before blood collection was used in the analyses due to the relatively short half-life of most peripheral blood cells. Lan et al. reported significantly reduced white blood cells (of all types) in workers exposed to 1.9 mg/m^3 (0.57 ppm) benzene. In a subgroup of workers (gender not specified), exposed to low benzene levels, effects were observed at a mean benzene concentration of 0.9 mg/m^3 (0.29 ppm). Details on the exposure assessment in general (Vermeulen et al.[177]) and the individual measurements in the low exposure subgroup (online supplemental to the publication*) were published separately.

The Committee considers the exposure assessment reported by Lan et al. a realistic estimate of the exposure of the population that was studied. Although subjects were categorised by mean benzene exposure levels measured the month before blood sampling, long-term benzene exposure of these workers is anticipated to have been similar, as it was reported that little or no task rotation

* www.sciencemag.org/cgi/content/full/306/5702/1774/DC1.

occurred. Also, the benzene levels in the factories during the 16 months preceeding blood sampling showed only seasonal fluctuations in exposure, mainly caused by differences in ventilation pattern.[177] Further, the Committee considers the likelihood of bias due to the involvement of peak exposures low. First, the low exposure subgroup was not involved in gluing and exposed only to background levels of benzene. The excursions above 3.3 mg/m^3 (1ppm), ranging from 3.3-5.7 mg/m^3 (1.01-1.74 ppm), were reported to occur infrequently. Also a contribution of dermal exposure in these shoe workers is considered unlikely. The overall probability of dermal exposure has been reported to be low, and importantly, the low exposure group had no direct contact with benzene-containing glue.[178]

The Committee notes that some comments can be made on the effects that were observed by Lan et al. at low exposure concentrations. Noteworthy, a more pronounced reduction in various blood cell types was found in the < 3.3 mg/m^3 (< 1 ppm) category than in the 3.3 to < 33 mg/m^3 (1 to < 10 ppm) category. These cut off effects have been discussed by the authors in a response to a letter to the editor.[179,180] In their response, Lan et al. presented spline regression analyses for white blood cells based on all individual measurements, showing a monotonic decrease along the whole exposure range (0.7 to < 49 mg/m^3 (0.2-15 ppm)). With respect to the differences in blood cells counts between the low exposed and the controls, it can be argued that these are due to demographic and lifestyle differences between the unexposed and exposed population. However, the Committee considers the selection of controls from workers involved in clothes-manufacturing from the same geographical region, in this case, appropriate.

A second study that provides information on haematological effects at exposure levels below 3.3 mg/m^3 (1 ppm) is that of Qu et al., who conducted a biomonitoring study in workers from three different factories in China.[129] An analysis based on all 130 workers exposed to a broad range of exposures and 51 controls revealed a concentration-dependent decrease in red blood cells, white blood cells, and neutrophils (with and without adjustment for sex, age, smoking, and toluene exposure). Furthermore, a statistically significant reduction of red blood cells, white blood cells, and neutrophils was observed in a subpopulation workers exposed to low levels of benzene (4-wk mean exposure of 0.46 mg/m^3 (0.14 ppm)). Qu et al. also measured chromosomal aberrations and observed statistically significant effects in the low exposure group.

The Committee notes several limitations of this study, in particular in relation to the effects reported in the low exposure group. First, a possible contribution of dermal exposure to the effects observed has not been addressed. Also, the control

group and exposure groups are not properly matched. The low exposure group consisted only of 16 individuals, all women (all non-smoking), whereas about half of the control subjects were female (of which 31% smoked). Since there has not been corrected for both gender (since women have relatively lower levels of haemoglobine, haematocrite, and red blood cells) and smoking (as smoking is a known cause of increased neutrophils), the effect levels reported need to be interpreted with caution as these can lead to an overestimation of the true benzene hazard. The Committee considers the study of Qu et al. supporting, but not critical, to its weight of evidence approach.

The findings of Lan et al. and Qu et al. at benzene concentrations below 1 ppm are supported by several other studies. In a study involving 5 factories in China, with workers exposed to a broad range of exposures, a statistically significant increase in risk was found for a clinically relevant reduction in red blood cells.[131] Several other studies addressed endpoints other than blood cell counts.[91,96] In two studies on leukaemia, a statistically significantly increased risk was observed at cumulative exposures of benzene that correspond to mean exposure levels of < 3.3 mg/m^3 (< 1 ppm)*. Hayes et al. observed an increased risk of haematologic neoplasms < 130 mg/m^3-years (< 40 ppm-years) (equivalent to a time-weighed average exposure of 3.3 mg/m^3 (1 ppm)), while in a pooled analysis, Schnatter et al. reported an increased risk of MDS at 9.5 mg/m^3-years (> 2.9 ppm-year) (equivalent to a time-weighed average exposure of 0.1 ppm).[96] However, as the exposure period in the study by Schnatter et al. was far less than 40 years, the corresponding mean exposure level is anticipated to have been higher. As no clear indications of an adverse effect were seen below an average exposure of 0.7 mg/m^3 (0.2 ppm) in the same study, the Committee considers the risk of MDS at this mean exposure level in practice to be negligible.

In addition to the studies mentioned above, the Committee points to several studies that have been conducted on workers in the (petro)chemical industry (mainly males), which have not shown effects of benzene below an exposure concentration of 3.3 mg/m^3 (1 ppm).[132-135] These routine health surveillance studies involved large numbers of workers and blood samples, and have been conducted using standard clinical methods. Importantly, the working conditions in these studies represent the occupational exposure conditions in The Netherlands and Europe. The estimation of benzene exposures is based on sampling strategies targeted at representative jobs and workplace combinations.

* When assuming 40 years of occupational exposure.

Therefore, the subsequent air measurements do not provide information on the workers exposed but rather on the job or task for which it was taken. The Committee notes that such sampling strategy is the method applied in routine monitoring industry, rather than using individual monitoring which is more suitable for the detection of subtle changes in blood cell numbers.

Recommendation of the health-based occupational exposure limit

The Committee has decided to apply a weight of evidence approach to derive a HBR-OEL for benzene, taking into account the aggregate of the accumulated evidence, including the apparent discrepancies between some of the reported results. The Committee therefore does not derive a point of departure by rounding off one (or more) of the reported effect levels, but pragmatically sets a point of departure. Based on the studies discussed above and summarised in Table 5, in which both NOAELs and LOAELs in the range of 0.5 to 3.3 mg/m^3 are reported, the Committee considers a benzene effect level of 2 mg/m^3 (0.6 ppm) a realistic starting point for deriving a HBR-OEL. The Committee applies a default uncertainty factor of 3, because of the use of a LOAEL instead of a NOAEL. In view of the use of an aggregate of evidence based on multiple studies, the Committee does not apply any additional uncertainty factors (e.g., for intra-individual differences or the size of the study population), and sets a HBR-OEL for benzene at 0.7 mg/m^3 (0.2 ppm), 8h time-weighted average (8h-TWA).

Skin notation

To decide whether a skin notation should be recommended to the substance, the Committee uses the ECETOC criteria for assigning a skin notation.[181] According to the ECETOC methodology, a skin notation is needed for a substance, in absence of relevant circumstantial evidence on human skin exposure, when the Critical Absorption Value (CAV; the rate of absorption above which dermal exposure is considered to be an important contributor to the total exposure) exceeds:

$$(10\ [m^3] \times OEL\ [mg/m^3] \times f \times 0.1)/2{,}000\ [cm^2]$$

in which 10 m^3 is the human inhalation volume per 8h working day, f is the absorption factor for inhalation (here assumed to be 1), 0.1 denotes the 10% criterion, 2,000 cm^2 is the surface area of the hands and forearms, and OEL is the

Occupational Exposure Limit, in this case the HBR-OEL. Thus the CAV will be:

*(10 [m^3] x 0.7 [mg/m^3] x 1 x 0.1)/2,000 [cm^2] = 0.35 µg/cm^2*h*

Williams et al. analysed the experimental skin absorption data of benzene, and concluded that the steady state absorption rate ranges from 200-400 µg/cm^2*h.[16] This rate exceeds the CAV of 0.35 µg/cm^2*h by far. The Committee therefore recommended to apply a skin notation.[182]

9.3 Groups at extra risk

A high variation of the level of toxicity has been observed among workers exposed to comparable levels of benzene, but no specific group at risk has yet been identified. This variation may be partly explained by biological factors such as gender, age, and extrinsic factors such as physical activity, coexposures smoking and dietary habits.[1,2]

In addition, genetic factors play a role such as single-nucleotide polymorphisms (SNPs) in genes related to metabolism of benzene and genes otherwise involved in benzene-induced toxicity (e.g., cytokine and chemokine coding genes).[1,2,183]

9.4 Health-based recommended occupational exposure limit

DECOS recommends a health-based occupational exposure limit for benzene of 0.7 mg/m^3 (0.2 ppm), as an eight-hour weighed average concentration.

Table 5 Epidemiological studies addressing effects at low (<3.3 mg/m³; 1 ppm) benzene concentrations.

Population	Experimental design	Effect studied	Mean (No) Effect level	Critical effect	Reference
Haematotoxicity (key studies)					
Workers in shoe manufacturing factories	Individual measurements of 250 exposed workers and 140 controls	Blood cell counts	1.9 mg/m³ (0.6 ppm) (LOAEL) Subgroup: 0.9 mg/m³ (0.3 ppm) (LOAEL)	Reduction in blood cell counts	Lan et al. (2004)[128]
Workers in glue factory or a small shoe factory	Biomarker study in 105 benzene-exposed workers and 26 unexposed workers	Blood cell counts	1.6 mg/m³ (0.5 ppm) (LOAEL)	Reduction in blood cell counts	Qu et al. (2002)[129]
Workers in rubber, shoe and pharmaceutical industry	Individual monitoring of 928 workers	Blood cell counts	3.3 mg/m³ (1 ppm) (LOAEL)	Anaemia (based on logistic regression analysis)	Schnatter et al. (2010)[131]
Workers in chemical industry	Routine biomonitoring study with 387 workers and 553 unexposed workers	Blood cell counts	1.8 mg/m³ (0.6 ppm) (NOAEL)	Not observed	Collins (1991)[132]; Collin et al. (1997)[133]
Workers in chemical industry	Routine biomonitoring study in volving 701 exposed workers (8,532 blood samples) and 1,059 non-exposed workers (12,173 blood samples)	Blood cell counts	3.0 mg/m³ (0.9 ppm) (NOAEL)	Not observed	Swaen et al. (2010)[134]
Workers in petro-chemical industry	Routine haematology surveillance study in 1200 exposed workers and 3,227 controls	Blood cell counts	2.0 mg/m³ (0.6 ppm) (NOAEL)	Not observed	Tsai et al. (2004)[135]
Genotoxicity (supporting study)					
Workers in glue factory or a small shoe factory	Biomarker study in 105 benzene-exposed workers and 26 unexposed workers	Chromosomal aberrations	1.6 mg/m³ (0.5 ppm) (LOAEL)	Chromatid and chromosomal aberrations	Qu et al. (2002)[129]
Carcinogenicity (supporting studies)					
Workers in a variety of industries	Cohort study with 74,828 exposed workers and 35,805 controls	NHL, leukaemia, ANLL, ANLL/ MDS, other hematologic neoplasms; separately and combined	Cumulative exposure: < 130 mg/m³-y (< 40 ppm-y) Equivalent to < 3.3 mg/m³ TWA (< 1 ppm ppm TWA)[a]	Increased risk of haematologic neoplasms combined	Hayes et al. (1997)[85]
Petroleum workers	Pooled analysis of 3 nested case-control studies from Australia, Canada, and the UK	AML, CML, and CLL and two myeloid neoplasms (MDS and MPD)	Cumulative exposure: > 9.5 mg/m³-y (> 2.9 ppm-y) Equivalent to > 0.2 mg/m³ TWA (> 0.1 ppm ppm TWA)[a]	Increased risk of MDS	Schnatter et al. (2012)[96]

[a] Assuming 40 years of occupational exposure.

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187 Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands; 2010: publication no. A10/07E.

A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	Human data
F	Animal data
G	Genotoxicity data
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I	Classification of substances with respect to carcinogenicity
J	List of Abbreviations

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

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Quality of Life, Zeist, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- T.M. Pal
  Occupational Physician, Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam

- A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Antoni van Leeuwenhoek, Amsterdam
- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- G.M.H. Swaen
  Epidemiologist, Dow Benelux NV, Terneuzen *(until April 1, 2013)*; Exponent, Menlo Park, United States *(from August 15, 2013, until February 1, 2014)*
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- P.B. Wulp
  Occupational physician, Labour Inspectorate, Groningen
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- A.S.A.M. Van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- S.R. Vink, *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 chairperson 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 inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *Benzene*
Your Reference: DGV/MBO/U-932342
Our reference : U-8057/SV/fs/459-Q69
Enclosed : 1
Date : February 21, 2014

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to *benzene*.

This advisory report is part of an extensive series in which health-based recommended exposure limits are derived for the concentrations of various substances in the workplace. The advisory report in question was prepared by the Health Council's Dutch Expert Committee on Occupational Safety (DECOS) and assessed by the Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor W.A. van Gool,
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in 2013 for public review. The following organisations and persons have commented on the draft document:

- V. Gálvez Pérez, Instituto Nacional de Seguridad e Higiene en el Trabajo, Madrid, Spain.
- D. Gombert, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, Maisons-Alfort Cedex, France
- T.J. Lentz, National Institute for Occupational Safety and Health, Cincinnati (OH), USA
- A. M. Rohde and G. Wallace, CONCAWE/European Chemical Industry Council (CEFIC), Aromatics Producers Association, Brussels, Belgium
- T. Scheffers, Theo Scheffers Arbo Consultancy, Maastricht, The Netherlands

Annex **E**

Human data

Cohort (Reference)	Cohort description	Exposure assessment	Haemato-logical malignancy	Exposure (ppm) TWA[a]	No of cases/ deaths	Relative risk (95% CI) Numbers in **bold** = statistically significant)
Pliofilm (Rinsky 1981)[81]	748 rubber workers	Estimation based on work histories and air sampling data	Leukaemia mortality	Estimated range 35-100 ppm	 7	SMR 5.6
Pliofilm (update) (Rinsky 1987)[82]	1,165 rubber workers (white males) Control: 10 subjects for each case, matched for age and year of first employment	Estimation based on work histories and air sampling data	Leukaemia mortality	ppm-years 0-40 40-199 200-399 ≥ 400 Total	 2 2 2 3 9	SMR 1.1 (0.1-3.9) 3.2 (0.4-11.75) **11.9** (1.3-42.9) **66.4** (13.3-193.9 **3.4** (1.5-6.4)
Pliofilm (update) (Wong 1995)[184]	1,165 rubber workers (white males)	Estimation based on work histories and air sampling data	AML	ppm-years < 40 40-200 200-400 > 400	 1 0 2 3	SMR 1.2 (0.0-6.6) 0 (0-14.8) **27.2** (3.3-98.2) **98.4** (20.3-287.75)
Pliofilm (update) (Rinsky 2002)[83]	1,845 rubber workers (males and females, all races) (including 1,291 workers exposed to at least 1 ppm-day (control=554 unexposed workers)	Estimation based on work histories and air sampling data	Leukaemia mortality	ppm-years 1 ppm-day-40 40-200 200-400 ≥ 400	 6 4 2 3	SMR 1.5 (0.5-3.3) 3.2 (0.9-8.9) 5.6 (0.1-24.1) **24.0** (4.8-78.5)

Pliofilm (update) (Silver 2002)[89]	See Rinsky (2002) Follow-up until 1996	See Rinsky (2002)	Leukaemia mortality	See Rinsky (2002) Average 174.5 ± 214.4 ppm-years)	1 in 1950 to 15 in 1996	SMR From 32.5 (0.8-180.6) in 1950 to 2.5 (1.4-4.1) in 1996
Pliofilm (update) (Richardson 2008)[88]	1,845 rubber workers (males and females, all races) (control: see Rinsky 1987) Follow-up until 1996	Estimation based on work histories and air sampling data	Leukaemia mortality	ppm-years < 1 1-< 50 50-250 250-500 ≥ 500	 5 3 4 4 1	 1 0.8 (0.2-3.2) 2.5 (0.6-10-2) **10.5** (2.3-46.6) 13.9 (0.7-116.1)
NCI-CAPM (Hayes 1997, Dosemeci 1994, Travis, 1994, Yin, 1987, 1989, 1994)[90-93,185,186]	74,828 workers (males and females) in 672 factories in China (control = 35,805 unexposed workers)	Estimation based on work histories and benzene measurements	All haematologic neoplasms	< 10 10-24 ≥ 25	24 16 18	RR **2.2** (1.1-4.2) **3.1** (1.5-6.5) **2.8** (1.4-5.7)
			Leukaemia	< 10 10-24 ≥ 25	15 13 10	2.0 (0.9-4.5) **3.7** (1.6-8.7) 2.3 (0.9-5.7)
			ANNL	< 10 10-24 ≥ 25	7 9 5	2.0 (0.6-7.0) **5.8** (1.8-18.9) 2.6 (0.7-9.9)
			ANNL/MDS	< 10 10-24 ≥ 25	11 9 8	**3.2** (1.0-10.1) **5.8** (1.8-18.8) **4.1** (1.2-13.2)
Dow Chemical (Ott 1978)[97]	594 workers in chlorobenzol, alkylbenzene and ethylcelulose production	Estimated based on industrial hygiene measurements and work histories.	Leukaemia	2-9	1	1 case of leukaemia (vs. 0.9 expected; no SMR calculated)
Dow Chemical (update) (Bond 1986)[98]	956 workers in chlorobenzol, alkylbenzene and ethylcelulose production	See Ott 1978	Leukaemia	ppm-months 0-500 500-1,000 ≥ 1,000	 2 0 1	SMR (0-year lag) 1.7 (CI not reported) 2.5 ,, 1.6 ,,
Dow Chemical (update) (Bloemen 2004)[99]	2,266 workers in chlorobenzol, alkylbenzene and ethylcelulose production	See Ott 1978	Leukaemia and aleukaemia	< 5 5-14 15+	3 5 4	SMR (0-year lag) 0.8 (0.2-2.2) 1.6 (0.5-3.7) 1.2 (0.3-3.1)
			AML	< 5 5-14 15+	0 3 1	0.0 (0.0-2.6) 2.7 (0.6-7.8) 0.9 (0.02-5.1)

Chemical Manufacturers Association (CMA) (Wong 1987)[100]	4,602 exposed workers (males) in US chemical industry (control=3,074 unexposed males from same company in same period)	Estimation work histories and benzene measurements	Lymphatic and haematopoietic	ppm-months non-exposed < 180 180-719 ≥ 720	 3 5 5 5	**RR** (Mantel-Haenszel; no CI) 1.0 2.1 3.0 3.9 (χ^2=5.4 (p=0.02))
			Leukaemia and aleukaemia	non-exposed < 180 180-719 ≥720	0 2 1 3	undefined χ^2=6.5 (p=0.01)
UK Petrol Nested case control study (Lewis 1997, Rushton, 1997)[94,95]	23,306 distribution workers in petroleum distribution industry (cohort, control = men from same oil company of equivalent age)	Based on work history records	All leukaemia, acute lymphoblastic, chronic lymphocytic, acute myeloid and monocytic, and chronic myeloid leukaemia	Exposure categories up to 45 ppm-y		No statistically sign. increased risk
Australian Health Watch (AHW) (Glass 2000, 2003, 2005)[102-104]	17,525 employees (males and females) in Australian petroleum industry (cohort, control = from same company of equivalent age)	Estimation work histories and benzene measurements	Leukaemia	≤ 0.1 > 0.1-0.2 > 0.2-0.4 > 0.4-0.8 > 0.8-1.6 > 1.6-3.2 > 3.2	OR 5 9 4 4 6 3 2	OR 1.0 **3.8** (1.2-12.7) 2.2 (0.5-9.4) **6.3** (1.5-26.2) 1.5 (0.3-6.7) **5.6** (1.0-31.3) **19.6** (1.4-270.8)
Canadian cohort (Schnatter 1993)[106]	6,672 male petroleum marketing and distribution workers, 226 locations (control = unexposed workers (number not specified))	Estimated based on work histories and historical industrial hygiene surveys	Myeloid/ lymphatic leukaemia	Within the range of 0.01 to 6.2 ppm	NA	No increase risk of leukaemia observed
Canadian cohort (update) (Schnatter 1996) [107]	Nested case-control study involving 31 cases of lymphohaematopoietic cancer (control=124 unexposed subjects from the same cohort matched for age)	Estimated based on work histories and historical industrial hygiene surveys	Leukaemia	Within the range of 0.01 to 6.2 ppm	NA	No increase risk of leukaemia observed
Shoe Factory (Costantini 2003)[108]	1,687 workers (males and females) (control=general population death rates)	Estimation based on work histories and limited air sampling data	Leukaemia mortality	ppm-years < 40 40-99 100-199 ≥ 200	 3 2 2 4	SMR 1.3 (0.3-3.7) 4.1 (0.5-14.7) 2.5 (0.3-9.1) **5.1** (1.4-13.0)

Offshore cohort (Kirkeleit 2008)[109]	27,919 Norway petroleum industry workers (control=366,114 persons from general working population)	Estimation	All blood/bone marrow neoplasms	0.001 to 0.69	20	**1.90** (1.19-3.02)
			AML		6	**2.89** (1.25-6.67)
(Schnatter 2012) (pooled analysis)[96]	UK Petrol, AHW and Canadian cohorts	Estimated using historical monitoring data	AML, CLL, CML, MPD	ppm-years 0 -> 2.93 ppm	6	No increased risk
			MDS	≤ 0.348	8	1.00
				0.348-2.93	15	1.73 (0.55-5.47)
				> 2.93		4.33 (1.31 -14.3)
				≤ 0.016	7	1.00
				0.016-0.081	5	1.99 (0.51-7.76)
				0.081-0.259	7	1.85 (0.51-6.75)
				> 2.59	10	3.12 (0.9-10.8)
Meta analysis						
(Raabe and Wong 1996)[110]	208,741 mainly petroleum refinery workers in the US and UK		AML	Generally less than 1 ppm for most petroleum refinery jobs (0.22 ppm based on an industry-wide survey including 14,824 samples)		Meta-SMR 0.9
(Vlaanderen 2010)[111]	Meta regression analysis of 6 cohort studies and 3 nested case-control studies)	0.32-554.3 ppm-years	Leukaemia	ppm-years 10 20 40		Highest RR (natural spline; all studies) **1.5** (1.1-2.2) **1.7** (1.3-2.3) **2.1** (1.5-3.0)
(Khalade 2010)[112]	Various cohorts derived from a total of 15 studies		Leukaemia	Summary effect size		**1.4** (1.2-1.6)
				< 40 ppm-years		**1.6** (1.1-2.4)
				40-99.9 ppm-years		**1.9** (1.3-2.9)
				> 100 ppm-years		**2.6** (1.6-4.4)
			AML	< 40 ppm-years		1.9 (1.0-4.0)
				40-99.9 ppm-years		2.3 (0.9-5.9)
				> 100 ppm-years		**3.2** (1.1-9.5)
(Schnatter 2005)[113]	9 cohorts and 13 case-control studies	Qualitatively	AML	NS		Increased risk across study designs

[a] In those references where exposure is expressed in both ppm-years and ppm, only exposure in ppm is reported.

Annex **F**

Animal data

Carcinogenicity studies reported in the EU RAR.[2]

Route	Species	Study design	Mortality rate	Hyperplasia related to tumor response	Tumor response	Ref. from EU RAR
Inhalation	Mouse/C 57Bl/6J (males) AKR/J (males)	300 ppm, 6h/d, 5d/w lifetime;	Reduced median survival (41 vs 75 weeks in control	Granulopoietic/ myeloid bone marrow hyperplasia in C57Bl mice (13/32 animals	8/40 animals with hematopoietic lymphoma (6 lymphocytic lymphoma, 1 plasmocytoma, 1 hemocytoblastic leukaemia) 2/40 control animals with lymphocytic lymphoma	Snyder et al. (1980)[161]
		100 ppm, 6hr/d,5d/w, lifetime	Ø	without tumors)	No increase of tumour rate of lymphomas (29/49 in treated animals, 24/50 in controls)	
	Mouse/ C57Bl/6J and CD-1 (males)	Intermitt.: 300 ppm, 6h/d, 5d/w, 1 w interrupted by 2 weeks unexposed, until death	Ø	-	C57Bl/intermittend: tumor bearing animals (TBA): total TBA 25/ 54 vs 8/46 controls; malignant TBA 24/54 vs 2/46 controls; Zymbal gland carcinoma 19/54 vs 0/46 controls CD-1/interrmittend: total TBA 25/54 vs 4/46 controls; malignant TBA 2/54 vs 1/46 controls; lung adenoma 4/54 vs 3/46 controls	Snyder et al. (1988)[162]

	1,200 ppm 6hr/d,5d/w, 10 weeks, untreated until death			C57Bl/10 weeks: no significant tumor response CD-1/10 weeks: total TBA 45/71 vs 36/71controls; malignant TBA 24/71 vs 22/71 controls; benign TBA.35/ 71 vs 21/71 controls; lung adenoma 33/71 vs 17/71 controls; Zymbal gland carcinoma 4/71 vs 0/71 controls	
Mouse/ C57Bl/6 BNL (female)	300 ppm, 6h/d, 5d/w, 16 weeks, observation until death	Increased	-	Lymphomas/ leukaemia all types: 20/89 vs 8/88 controls; thymic lymphoma: 10/89 vs 1/88 controls; nonthymic lymphoma: 6/89 vs 2/88 controls; myelogenous leukaemia 0/89 vs 3/88 controls; leukaemia NOS: 4/89 vs 2/88 controls; Zymbal tumors: 16/89 vs 1/88 controls; ovarian tumors: 8/89 vs 0/88 controls	Cronkite et al. (1984, 1985)[149,150]
Mouse/C BA/Ca BNL	100, 300 ppm, 6h/d, 5d/w, 16 weeks, observation until death	Increased at 100 and 300 ppm	-	300 ppm: Myelogenous neoplasms, males: 19.3% vs 0% control; females: 11% vs 1.7% control 100 ppm: Myelogenous neoplasms, males: 2.4% vs 0% control	Cronkite et al. (1989)[151]
Mouse/ AKR, C57Bl, CD-1 (no data on sex)	6 hr/d, 5 d/ w, Lifetime AKR: 100 and 300 ppm, C57Bl and CD-1: 300 ppm	No data	CD-1 mice: Granulocytic hyperplasia 1/40	CD-1 mice: chronic myelogenous leukaemia 1/40, acute myeloblastic leukaemia 1/40 vs none in control	Goldstein et al. (1982)[154]
Mouse CBA/Ca (males)	300 ppm, 6h/d, 5d/w, 16 weeks, observation until month 22 after start	Increased	Increased granulocytic hyperplasia in bone marrow (42/116 vs 9/117 controls) and in spleen (7/114 vs 0/116 controls)	Malignant lymphoma 14/118 vs 2/119 controls lung adenoma 42/118 vs 17/119 controls preputial gland squamous cell carcinoma 71/ 118 vs 0/118 controls Zymbal gland carcinoma 14/125 vs 1/125 controls forestomach squamous cell carcinoma 9/125 vs 6/125 controls	Farris et al. (1993)[152]
Rat Sprague-Dawley (no data on sex)	100, 300 ppm 6 h/d, 5 d/w, lifetime	No data	-	100 ppm: chronic myelogenous leukaemia 1/ 40 vs none in control	Goldstein et al. (1982)[154]

Rat/ Sprague-Dawley adult females and male and female 12-day embryos	Pregnant females + male/ female offspring: 200 ppm, 4h/d, 5d/w, 7 week, then 200 ppm 7h/d, 5d/w, 12 week, then 300 ppm, 7h/d, 5d/w, 85 week	Increased in offspring after 104 weeks of treatment	At week 118: liver: nodular hyperplasias in 5/48 adult females (parent), 1/74 offspring male, 5/59 offspring females nodular dysplasia 2/59 offspring females versus none of each lesions was seen in controls	Increase of tumor incidences at week 150: No. of animals with tumors were related to the No. of animals at study begin: Zymbal gland carcinoma: adult females (parent) 3/54 vs 1/60 controls; offspring males 6/75 vs 2/158 controls; offspring females 8/65 vs 0/149 controls oral cavity carcinomas: adult females (parent) 2/54 vs 0/60 controls; offspring males 1/75 vs 0/158 controls; offspring females 10/65 vs 0/149 controls nasal cavity carcinomas: adult females (parent) 1/54 vs0/60 controls; offspring males 1/75 vs0/158 controls; offspring females 2/65 vs 0/149 controls skin carcinomas: adult females (parent) 0/54 vs 0/60 controls; offspring males 1/75 vs 0/158 controls; offspring females 1/65 vs 0/149 controls; forestomach carcinomas: adult females (parent) 0/65 vs 0/149 controls; offspring males 0/75 vs 0/158 controls; offspring females 3/65 vs 1/149 controls hepatomas: adult females (parent) 1/54 vs 0/60 controls; offspring males 2/75 vs 1/158 controls; offspring females 7/65 vs 0/149 controls; hemolymphoreticular neoplasia: adult females (parent): 0/54 vs 2/158 controls; offspring males 6/75 vs 12/158 controls; offspring females 0/65 vs 1/149 controls	Maltoni, (1982; 1983; 1985)[156-158]
	Male+ female offspring: 200 ppm, 4h/d, 5d/w, 7week, then 200 ppm, 7h/d, 5h/d, 12 week, observation until death		At week 118: liver: nodular hyperplasia 2/64 offspring males and 7/59 offspring females versus none in the controls	No. of animals with tumors were related to the No. of animals at study begin: Zymbal gland carcinoma: offspring males 4/70 vs 2/158 controls; offspring females 1/59 vs 0/149 controls; oral cavity carcinomas: offspring males 2/70 vs 0/158 controls; offspring females 6/59 vs 0/149 controls nasal cavity carcinomas: offspring males 1/70 vs0/158 controls; offspring females 1/59 vs 0/149 controls hepatomas: offspring males 2/70 vs 1/158 controls; offspring females 5/59 vs 0/149 controls	

Oral	Mouse B6C3F1	0, 25, 50, 100 mg/kg bw/d (gavage)	Decreased with increasing doses	Hematopoietic marrow hyperplasia males: 11/48, 0/50, 25/49 vs 0/49 control; females: 14/45,8/50, 13/49 vs 3/49 control; hyperplasia Zymbal gland males: 4/34, 12/40,10/39 vs 0/42 control; females:1/32, 2/37, 6/31 vs 1/ 43 control; alveolar/bronch. hyperplasia: males: 3/48, 7/50, 10/49 vs 2/49 control; females: 11/48, 12/50, 14/49 vs 1/49 control hyperplasia of preputial gland: males: 18/28, 9/29, 1/35 vs 1/21 control	Malignant lymphomas: males: 9/48,9/50,15/49 vs 4/49 controls; females: 24/45,24/50,20/49 vs 15/49 controls Zymbal gland carcinomas: males: 1/34,4/40,21/39 vs 0/43 controls; females: 0/32,1/37,3/31 vs 0/43 controls squamous cell papilloma or carcinoma (combined) of the forestomach: males: 2/42,3/44,5/38 vs 2/45 controls; females: 3/40,6/45,5/42 vs 1/42 controls alveolar/bronchiolar carcinomas: males: 11/48,12/50,14/49 vs 5/49 controls; females: 3/42,6/50,6/49 vs 0/49 controls alveolar/bronchiolar adenomas: females: 2/42,5/50,9/49 vs 4/49 controls; alveolar/bronchiolar adenomas or carcinomas (combined): males: 16/48,19/50,21/49 vs 10/49 controls; females: 5/42,10/50,13/49 vs 4/49 controls Harderian gland adenomas: males: 9/46,13/49,11/48 vs 0/49 controls Harderian gland adenomas or carcinomas (combined): females: 6/44,10/50,10/47 vs 5/48 controls squamous cell carcinomas of preputial gland males: 3/28,18/29,28/35 vs 0/21 controls mammary gland carcinomas: females: 2/45,5/50,10/49 vs 0/49 controls mammary gland carcinosarcomas females: 0/45,1/50,4/49 vs 0/40 controls ovarian granulosa cell tumors females: 1/44,6/49,7/48 vs 1/47 controls overian benign mixed tumors females: 1/44,12/49,7/48 vs 0/47 controls hepatocellular adenomas: females:8/44,5/50,4/49 vs 1/49 controls hepatocellular adenomas or carcinomas (combined) 12/44,13/50,7/49 vs 4/49 controls	NTP, (1986)[160]; Huff et al. (1989)[155]
	Rat/F-344	Males: 0, 50, 100, 200 mg/kg bw/d females 0, 25, 50, 100 mg/kg bw/d	Increased with dose	-	Zymbal gland carcinomas: males: 6/46,10/42,17/42 vs 2/32 controls females: 5/40,5/44,14/46 vs 0/45 controls squamous cell papilloma of the skin: males: 2/50,1/50,5/50 vs 0/50 controls squamous cell carcinoma of the skin: males: 5/50,3/50,8/50 vs 0/50 controls squamous cell papilloma or carcinomas (combined) of the oral cavity: males: 9/50,16/50,19/50 vs 1/50 controls females: 5/50,12/50,9/50 vs 1/50 controls	NTP, (1986)[160]; Huff et al. (1989)[155]

Rat/ Sprague-Dawley	50, 250 mg/kg bw/d, 4-5 d/w, 52 weeks	No data	SD: liver acanthomas and dysplasias: males: 25%, females: 17.5% control males and control females: 0%	52 weeks: Zymbal gland carcinomas: 50/ males: 0%; 50/females: 6.5%; 250/males: 0%; 250/females: 22.9%;control/males: 0% control/females: 0%; leukaemia: 50/males: 0%; 50/females 6.7%; 250/males: 11.4% 250/females:2.9%; control/ males: 0%; control/females 3.3%;oral cavity carcinomas; 250/males: 0% 250/females: 5.7%; control/males: 0% control/females: 0%	Maltoni et al. (1989)[159]
	500 mg/kg bw/d, 4-5 d/w, 104 weeks in Sprague-Dawley (SD) and Wistar (W) rats			104 week: Zymbal gland carcinomas: SD-males: 45%; SD-females 40%; SD-control males: 2% SD-control females:0%; W-males: 17.5% W-females: 15%; W-control males:0% W-control females 0% leukaemia: SD-males: 2.5%; SD-females: 7.5%;SD-control males: 6%; SD-control females: 2%; W-males: 5%; W-females: 10%; W-control males: 2.5%; W-control females:7.5% oral cavity carcinomas: SD-males 52.5%; SD-females: 50%; SD-control males: 0%; SD-control females:0%; W-males: 5%; W-females: 10%; W-control males: 2.5%; W-control females: 0% forestomach carcinomas in situ: SD-males:0%; SD-females: 15%; SD control males: 0%; SD-control females: 0% forestomach invasive carcinomas: SD-males: 2.5% SD-females: 0%; SD-control males: 0% SD-control females:0% skin carcinomas: SD-males: 22.5% SD-females: 0%; SD-control males:0% SD-control females 2% liver angiosarcomas: SD-males:5% SD-females 7.5%; SD-control males: 0% SD-control females. 0% liver hepatomas: SD-males: 7.5% SD-females: 2.5%; SD-control males: 6% SD-control females: 0% nasal cavity carcinomas: SD-males: 7.5% SD-females: 2.5%; SD-control males: 0% SD-control females:0%; W-males: 5% W-females: 2.5%; W-control males:0% W-control females: 0%	

Ø = no treatment-related effect; NOS = not other specified; TBA = tumor bearing animals.

Annex G

Genotoxicity data

The genotoxicity results are derived from the review by Whysner et al.[55]

Results of benzene genotoxicity studies in humans.

	Results	Number of studies
Micronucleus	+	2
	–	5
Chromosomal aberrations	+	15[a]
	(+)	2
	–	5
Aneuploidy	+	5
	–	2
Sister chromatid exchange	+	5
	-	9
DNA damage/SS. DS breaks	+	3
	–	1

[a] Seperate exposure group within the same study.

Results obtained in rodents for benzene and its metabolites.

		Results[a]	Number of studies[b]
Micronucleus	Benzene	+	59
		(+)	1
		−	5
	Phenol	+	4
		(+)	2
		−	6
	Hydroquinone	+	21
		−	1
	Benzoquinone	+	2
		(+)	1
	Catechol	+	3
		(+)	1
		−	3
	Benzenetriol	+	1
		−	1
	Muconaldehyde	−	1
Chromosomal aberrations	Benzene	+	18
		−	2
	Phenol	+	2
	Hydroqinone	+	5
Aneuploidy	Benzene	+	2
	Hydroquinone	+	3
Sister chromatid exchange	Benzene	+	3
		(+)	4
	Phenol	+	1
	Muconaldehyde	+	1
DNA damage/SS, DS breaks	Benzene	+	5
		−	4
	Phenol	−	1
Transgenic mouse mutation	Benzene	+[c]	2

[a] + indicates positive results; (+) indicates a weakly positive result or positive but in a study of limited quality; and − indicates negative results.

[b] Investigations of different strains of animals and different tissues within one publication are counted as separate studies.

[c] Result reported by the authors. The Subcommittee on Classification Carcinogenic Substances considers the marginal responses reported in the transgenic mouse mutation assay as negative results (see Annex H).

Note: only categories for which at least one result is present are included.

Results of in vitro genotoxicity tests for benzene and its metabolites in cells derived from rodents and humans.

	Results[a]	Number of studies
Micronucleus		
Benzene without activation	-	1
Benzene with activation	-	1
Phenol	+	3
	-	1
Hydroquinone	+	9
	(+)	4
	-	7
Benzoquinone	+	5
Catechol	+	2
	-	1
Benzenetriol	+	3
Muconaldehyde	+	2
Chromosomal aberrations		
Benzene without activation	+	6
	-	7
Benzene with activation	+	3
	-	2
Hydroquinone	+	2
	-	2
Catechol	+	2
Aneuploidy		
Benzene without activation	+	2
	-	2
Benzene with activation	-	1
Phenol	-	1
Hydroquinone	+	4
	-	2
Catechol	+	1
Benzotriol	+	1
Sister chromatid exchange		
Benzene without activation	+	1
	-	11
Benzene with activation	+	1
	-	6
Phenol	+	3
	(+)	1
	-	2
Hydroquinone	+	8
Benzoquinone	+	1
	-	1
Catechol	+	7

Benzenetriol	+	2
Muconaldehyde	-	1
DNA damage/SS breaks		
Benzene without activation	+	2
	-	8
Benzene with activation	+	2
	-	3
Phenol	-	3
Hydroquinone	+	4
	(+)	2
Benzoquinone	+	4
	(+)	1
	-	2
Catechol	+	2
	-	4
Benzenetriol	+	6
	-	2
Muconaldehyde	-	3
Unscheduled DNA synthesis		
Benzene without activation	+	1
	-	6
Benzene with activation	-	2
Phenol	+	1
Hydroquinone	+	1
Catechol	+	1
Muconaldehyde	-	1
Mammalian gene mutation		
Benzene without activation	+	3
	(+)	1
	-	12
Benzene with activation	+	2
	(+)	2
	-	13
Phenol	+	1
	-	2
Hydroquinone	+	3
Benzoquinone	+	2
Catechol	+	3
Benzenetriol	+	1
Muconaldehyde	+	2
Muconic acid	-	3

[a] + indicates positive results; (+) indicates a weakly positive result or positive but in a study of limited quality; and - indicates negative results.

Annex **H**

Advice of the Subcommittee on Classification of Carcinogenic Substances

H.1 Scope

For carcinogens, the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council derives either a health-based recommended occupational exposure limit (HBR-OEL) or a health-based calculated occupational cancer risk value (HBC-OCRV), dependent on their mechanism of action. For non-genotoxic carcinogens and non-stochastic genotoxic carcinogens, it is assumed that the carcinogenic effects only occur when exposure levels exceed a certain threshold. For such substances, the Committee derives a HBR-OEL. For stochastic genotoxic carcinogens, and genotoxic carcinogens for which the mechanism of action is unknown but for which a stochastic mechanism is not unlikely, it is assumed that any level of exposure is associated with a certain risk for developing cancer. For these substances, a HBC-OCRV is derived.

In order to establish the appropriate approach, the Subcommittee on the Classification of carcinogenic substances was requested by DECOS to evaluate the carcinogenic properties of benzene and in particular, its genotoxic mode of action. The members of the Subcommittee are listed at the end of this Annex.

H.2 Carcinogenicity of benzene

Human data

Increased risk of either leukaemia in general or acute myeloid leukaemia/acute non-lymphocytic leukaemia specifically, after exposure to benzene has been observed in several cohorts of workers exposed to benzene in various industries (in particular the Pliofilm cohort of rubber workers in the US, the Chinese Academy of Preventive Medicine (CAMP) cohort of benzene-exposed workers in China and the Australian Healthwatch cohort of petroleum workers)(i.e. Rinsky et al. 1981, 1987, 2002; Wong et al. 1995; Silver et al. 2002; Richardson et al. 2008; Hayes et al. 1997; Travis et al. 1994; Yin et al. 1994; Glass et al. 2003). In addition, several meta-analyses underline the positive relationship between benzene exposure and leukaemia, or acute myeloid leukaemia (AML) specifically (Schnatter et al. 2005; Vlaanderen et al. 2010; Khalade et al. 2010). In one pooled analysis, an increased risk of MDS was observed (Schnatter et al. 2012).

Several positive associations have been reported between benzene exposure and other haematological malignancies, including (based on meta-analyses) multiple myeloma (MM), chronic lymphocytic leukaemia (CLL), acute lymphocytic leukaemia (ALL), and chronic myeloid leukaemia (CML) (Vlaanderen et al. 2011; Vlaanderen et al. 2012).

The Subcommittee concludes that there is sufficient evidence for a causal relationship between benzene exposure and haematological malignancies in humans.

Animal data

In rats as well as in mice, benzene induces tumours in several target organs, involving the haematopoietic system and several organs of epithelial origin.

Tumours that have been reported in rats exposed by inhalation included carcinomas of the Zymbal gland and oral cavity. Tumours found at other sites involved carcinoma of the nasal cavity, mammary gland tumours and hepatomas (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; Snyder et al. 1984). Mice developed a variety of tumours, including lymphomas, myelogenous leukaemias, Zymbal gland tumours, ovarian tumours and lung tumours (Cronkite et al. 1986, 1984, 1985, 1989; Farris et al. 1993; Snyder et al. 1980).

Oral administration induced a similar spectrum of tumours (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986). Exposure of rats to benzene induced Zymbal gland carcinomas, squamous cell papillomas and carcinomas of the oral cavity and skin. In mice, benzene caused tumours that included lymphomas, Zymbal gland carcinomas, lung alveolar/bronchiolar adenomas and carcinomas, Harderian gland adenomas, preputial gland squamous cell carcinomas, and mammary gland carcinomas.

The Subcommittee concludes that benzene is carcinogenic to man, based on epidemiological evidence for AML and total leukaemia, and supporting evidence in experimental animals. The Subcommittee recommends, in accordance with the EU-classification, to classify benzene in category 1A ('*the compound is known to be carcinogenic to humans*').*

H.3 Genotoxicity of benzene

Cytogenicity

An important basis for the Subcommittee's evaluation of the genotoxicity of benzene are the results obtained in standardised, guideline-compliant assays (reviewed in Whysner et al. 2004; JRC-IHCP 2008). These assays are designed to identify the potential to induce (numerical and structural) chromosomal aberrations and gene mutations. From these results, the Subcommittee concludes that a large amount of convincing evidence shows that exposure to benzene causes the induction of micronuclei, chromosomal aberrations, sister chromatid exchanges and DNA strand breaks, both in humans and in animals.

Typical genetic profiles of MDS/AML are found in patients associated with benzene exposure, i.e. higher levels of chromosomal changes commonly observed in AML, including 5q-/-5 or 7q-/-7, +8, and t(8;21) (Smith et al. 2010).

The Subcommittee concludes that benzene is genotoxic by the induction of structural and numerical chromosomal aberrations.

Mutagenicity

Standardised mutagenicity assays have indicated a low mutagenic potential. The majority of the gene mutation assays in bacteria did not reveal any mutagenic

* See Annex I for the classification system of the Health Council.

responses after exposure to benzene and its metabolites. In vitro mammalian gene mutation assays conducted with benzene are predominantly negative (Whysner et al. 2004; JRC-IHCP 2008).

Billet et al. (2010) studied the mutational pattern induced by benzene on the *TP53* gene in human type II-like alveolar epithelial A549 cells by using the Functional Analysis of Separated Alleles in Yeast (FASAY). Seventeen mutations linked to benzene exposure were found, including one – or two – base deletions and single nucleotide substitutions, of which A>G and G>A transitions were the most prevalent.

Two transgenic mouse mutation assays are available that both have shown marginal responses at relatively high benzene exposure levels (Mullin et al. 1995; Provost et al. 1996), in particular an increase in the frequency and length of deletion mutations (Mullin et al. 1998). The Subcommittee notes that one study only applied one dose whereas in the other, a dose-response relationship is only reported in the spleen. Furthermore, a relatively high variation is observed in the controls, within each study as well as between both studies.

Mutations have been found in the glycophorin A (GPA) gene mutation assay (Rothman et al. 1995). The GPA assay measures somatic cell mutation frequency in MN heterozygous peripheral erythrocytes; mutations that are considered to have occurred exclusively in precursor erythroid cells or stem cells in the bone marrow. Both NN variants (which are associated with chromosomal damage and mitotic recombination) and NØ variants (which are associated with gene inactivation through point mutations and deletions) were scored. In 24 workers heavily exposed to benzene (mean exposure was approximately 234 mg/m^3; 72 ppm), an increase in NN but not in NØ mutant variants was observed compared to 23 matched controls.

The Subcommittee concludes that it cannot be excluded that benzene has a low mutagenic potential.

Adducts

Several DNA-adduct formation (with ^{32}P-postlabeling) studies and DNA-binding studies (with ^{14}C-labeled benzene) have been published (reviewed Whysner et al. (2004)). The Subcommittee considers ^{32}P-postlabeling analysis most specific to measure covalent binding. In most in vivo studies with either benzene or its metabolites, DNA adducts were below the limit of detection. Only relatively low levels of DNA binding have been reported, with an apparent lack

of concordance of target organs between DNA-binding studies and the results obtained in bioassays.

In line with the considerations made by Whysner et al. (2004), the Subcommittee concludes that DNA adducts do not play a significant role in the carcinogenicity of benzene.

Mechanisms of action

Several mechanisms of actions have been implicated with the carcinogenic properties of benzene, including:

- Inhibition of topoisomerase II
- Adduct formation of reactive metabolites
- Oxidative stress
- Error-prone DNA repair
- Epigenetic alterations.

For more details on these mechanisms of action and benzene carcinogenicity, the Subcommittee refers to reviews published recently by McHale et al. (2012) and Wang et al. (2012).

The Subcommittee notes that all mechanisms of action that have been proposed, with the exception of the formation of adducts, are currently considered to be thresholded phenomena. The Subcommittee considers covalent binding by benzene, in view of the low electrophilic nature of the prominent metabolites of benzene, the absence of positive findings in standardised gene mutation assays and the lack of substantial adduct formation, of no concern for the risk assessment of benzene.

Whereas there is a lack of evidence for a direct mechanism of genotoxicity, there is a large amount of evidence suggesting that benzene acts by thresholded mechanisms of action. (McHale et al. (2012); Wang et al. (2012); Whysner et al. (2004)) The Subcommittee acknowledges that currently, not all findings can undeniably be attributed to a particular mode of action (either direct or indirect). In particular, the induction of gene mutations and unbalanced chromosomal aberrations have been noted in this context. The Subcommittee concludes however, that also these findings can be the result of indirect genotoxicity and do therefore not provide evidence for a direct genotoxic mode of action per se.

The Subcommittee further acknowledges that the contribution to the toxicity and carcinogenicity of benzene of each of the proposed mechanisms of actions, cannot be quantified. In this context, a systems biology approach has been

proposed for benzene to identify potential biomarkers of exposure, early effect and susceptibility (Zhang et al. 2010), which may lead to more refined risk assessment approaches.

Overall, the weight of evidence points to an indirect genotoxic mode of action (e.g., inhibition of topoisomerase II, generation of oxidative stress, etc.), whereas there is no evidence to substantiate a direct genotoxic mode of action. Therefore, the Subcommittee considers an indirect genotoxic mode of action most likely for benzene.

H.4 Recommendation for classification

Based on the available data, the Subcommittee recommends, in accordance with EU classification, to classify benzene in category 1A (*the compound is known to be carcinogenic to humans*). The Subcommittee concludes that benzene acts by a non-stochastic genotoxic mode of action.

H.5 References

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Annex I

Classification of substances with respect to carcinogenicity

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

Source: Health Council of the Netherlands. A10/07E.[187]

Annex **J**

List of Abbreviations

AML	acute myeloid leukaemia
ALL	acute lymphatic leukaemia
ANNL	acute non-lymphatic leukaemia
CLL	chronic lymphatic leukaemia
CML	chronic myeloid leukaemia
GC	gas chromatography
HBC-OCRV	health-based calculated occupational cancer risk value
HBR-OEL	health-based recommended occupational exposure limit
HPLC	high-performance liquid chromatography
LOD	limit of detection
MDS	myelodysplastic syndrome
MM	multiple myeloma
MPO	myeloperoxidase
MS	mass spectometry
NHL	non-Hodgkin lymphoma
NQ01	NAD(P)H: quinone oxidoreductase 1
SMR	standardised mortality ratio
SPE	solid phase extraction
SPMA	S-phenylmercapturic acid
SPME	solid phase microextraction
ttMA	trans, trans-muconic acid

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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