ANNEX 1

Background document

in support of the Committee for Risk Assessment (RAC) evaluation of limit values for benzene in the workplace

Prepared by the European Chemicals Agency (ECHA)

ECHA/RAC/A77-0-0000001412-86-187/F

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Preamble

The Commission, in view of the preparation of the third and fourth proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD), and in line with the 2017 Commission Communication '*Safer and Healthier Work for All*' - *Modernisation of the EU Occupational Safety and Health Legislation and Policy*'[1], asked the advice of RAC to assess the scientific relevance of occupational exposure limits for some carcinogenic chemical substances.

Therefore, the Commission made a request (8 March 2017[2]) in accordance with Article 77 (3)(c) of the REACH Regulation, to evaluate, in accordance Directive 2004/37/EC, the following chemical compounds: 4,4'-methylenebis[2-chloroaniline] (MOCA), arsenic acid and its inorganic salts, nickel and its compounds, acrylonitrile and benzene.

In support of the Commission's request, ECHA prepared a proposal concerning occupational limit values for benzene at the workplace. This proposal was made publically available at: 'https://echa.europa.eu/echas-executive-director-requests-to-the-committees-previous-consultations' on **10 October 2017** and interested parties were invited to submit comments by **7 November 2017**.

RAC developed its opinion on the basis of the proposal submitted by ECHA. During the preparation of the opinion on occupational limit values for benzene, the ECHA proposal was further developed as the Background Document to ensure alignment. In addition, stakeholders were able to provide comments on the RAC opinion during the evaluation process.

Following adoption of an opinion on 9 March 2018, recommending an Occupational Exposure Limit for benzene by the Committee for Risk Assessment (RAC), this background document was amended to align it appropriately with the view of RAC. It supports the opinion of the Committee for Risk Assessment (RAC) and gives the detailed grounds for the opinion[3].

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1 http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

2 https://echa.europa.eu/documents/10162/13641/ec_note_to_echa_oels_en.pdf/f72342ef-7361-0d7c-70a1-e77243bdc5c1

3 https://echa.europa.eu/documents/10162/13579/interim_wponevaluation_oel_agreed_rac_42_en.pdf/021bc290-e26c-532f-eb3f-52527700e375

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Literature search

This assessment of benzene is based on recent reviews by other organisations, in particular on the report from the German Scientific Committee AGS (2012) and the report from the Dutch Expert Committee on Occupational Safety (DECOS 2014). However, reviews such as ANSES (2014), ATSDR (2007, 2015), Concawe (1999, 2002, 2006, 2012), EPA (1998), EU RAR (2008), IARC (2012) have also been included.

However, this has been complemented by an extensive review of the primary literature from the last ten years and earlier focussing on data from workers, relevant animal data, and on the mode of action of benzene.

1. Chemical Agent Identification and Physico-Chemical Properties

The identification and physico-chemical properties of benzene are described in the tables below:

Table 1: Identity and physico-chemical properties

Endpoint	Value
IUPAC Name	Benzene
Synonyms	Cyclohexatriene, Benzol
EC No	200-753-7
CAS No	71-43-2
Chemical structure	
Chemical formula	C_6H_6
Appearance	Liquid
Boiling point	80.1 °C at 1.013 hPa
Density	0.88 g/cm^3
Vapour pressure	10 kPa at 20 °C
Partition coefficient (log Pow)	2.13 at 25 °C
Water solubility	ca. 1.88 g/L at 23.5 °C
Viscosity	0.604 mPa at 25 °C
Conversion factor	1 ppm (mL/m^3)=3.25 mg/m^3 1 mg/m^3=0.308 ppm (mL/m^3)

2. EU Harmonised Classification and Labelling -CLP (EC)1271/2008

Table 2: EU classification: CLP (EC) 1271/2008 (EU Commission 2008)

Index No	International chemical ID	Chemical formula	EC No	CAS No	Annex VI of CLP hazard class and category	Hazard statement code
601-020-00-8	Benzene	C_6H_6	200-753-7	71-43-2	Flam. Liq. 2	H225
					Skin Irrit. 2	H315
					Eye Irrit. 2	H319
					Asp. Tox. 1	H304
					Muta. 1B	H340
					Carc. 1A	H350
					STOT RE 1	H372

3. Chemical Agent and Scope of Legislation Regulated uses of Benzene in the EU

3.1 Directive 98/24/EC and Directive 2004/37/EC

Benzene is a hazardous chemical agent in accordance with Article 2 (b) of Directive 98/24/EC and falls within the scope of this legislation.

Benzene is a carcinogen (Carc 1A; H350) and a mutagen (Muta 1B; H340) for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC (EU Parliament and Council Directive 2004) and falls within the scope of this legislation.

Annex III of Directive 2004/37/EC (EU Parliament and Council Directive 2004) specifies a limit value for occupational exposure to benzene of 1 ppm (3.25 mg/m^3) and a 'skin notation' indicating that there is a substantial contribution to the total body burden possible via the dermal exposure.

3.2 REACH Registrations

Benzene registered as a monoconstituent substance under the substance identity "Benzene" (CAS No 71-43-2) has 109 active registrants under REACH in 1 Joint Submission and 1 Individual Submission.

Benzene is also a constituent/impurity in many substances. There are 128 registered substances that have a benzene content in a range of 0.1 to 1.0% w/w, and 97 registered substances that have a benzene content of higher than 1.0% w/w. Mainly the registered substances refer to gasoline, naphtha, distillates from petroleum or coal tar, or other type of hydrocarbon substances. For example,

- there are 159 active registrants for the substance "gasoline" (CAS No 86290-81-5)
- there are 30 active registrants for the subtance "naphtha (petroleum), heavy straight-run" (CAS No 64741-41-9) and
- there are 2 active registrants for "Distillates (petroleum), straight-run light" (CAS No 68410-05-9).

The REACH[4] registrations for benzene and some other registered substances that contain benzene are listed[5] below

Table 3: REACH Registrations for the substance "benzene" and some other registered substances that may contain benzene

Substance identity	Tonnage	Type	Status
Benzene	1 000 000 – 10 000 000	Full	Active
Benzene	Intermediate use only	Intermediate	Active
Examples of other registered substances that may contain benzene			
Gasoline A complex combination of hydrocarbons consisting primarily of paraffins, cycloparaffins, aromatic and olefinic hydrocarbons having carbon numbers predominantly greater than C3 and boiling in the range of 30°C to 260°C (86°F to 500°F).	100 000 000 - 1 000 000 000	Full	Active
Naphtha (petroleum), heavy straight-run A complex combination of hydrocarbons produced by distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C6 through C12 and boiling in the range of approximately 65°C to 230°C (149°F to 446°F).	10 000 000 - 100 000 000	Full	Active
Distillates (petroleum), straight-run light A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C2 through C7 and boiling in the range of approximately -88°C to 99°C (-127°F to 210°F).	100 000 - 1 000 000	Full	Active

[4] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396 of 30 December 2006, p. 1; corrected by OJ L 136, 29.5.2007, p. 3)

[5] ECHA https://echa.europa.eu/information-on-chemicals/registered-substances accessed 24 April 2017

3.3 Authorised uses under Annex XIV of REACH

Benzene is not listed on annex XIV of REACH, thus not subject to authorisation.

3.4 Restricted uses under Annex XVII of REACH

The following uses of benzene are restricted in entry 5 of Annex XVII:

1. Shall not be used in toys or parts of toys where the concentration of benzene in the free state is greater than 5 mg/kg (0,0005%) of the weight of the toy or part of toy.

2. Toys and parts of toys not complying with paragraph 1 shall not be placed on the market.

3. Shall not be placed on the market, or used,

- as a substance,
- as a constituent of other substances, or in mixtures, in concentrations equal to, or greater than 0.1% by weight

4. However, paragraph 3 shall not apply to:

a) motor fuels which are covered by Directive 98/70/EC[6];

b) substances and mixtures for use in industrial processes not allowing for the emission of benzene in quantities in excess of those laid down in existing legislation.

c) natural gas placed on the market for use by consumers, provided that the concentration of benzene remains below 0.1% volume/volume'.

3.5 Biocidal Products Regulation (EU)528/2012

No applications for biocidal use.

3.6 Directive 2008/50/EC on ambient air quality and cleaner air for Europe

Directive 2008/50/EC of the European Parliament and of the Council on ambient air quality and cleaner air for Europe entered into force on 11 June 2008. The Directive merges four directives and one Council decision into a single directive on air quality. It sets standards and target dates for reducing concentrations of several of the most dangerous pollutants for human health, including benzene. The Directive gives the possibility for time extensions of three years (PM10) or up to five years (NO_2, benzene) for complying with limit values, based on conditions and the assessment by the European Commission.

The margin of tolerance set for benzene is "*5 µg/m^3 (100%) on 13 December 2000, decreasing on 1 January 2006 and every 12 months thereafter by 1 µg/m^3 to reach 0% by 1 January 2010*". 5 µg/m^3 equals 0.0015 ppm.

[6] Directive 98/70/EC determines that the maximum limit value for benzene in petrol (gasoline) is 1% v/v

3.7 EU Directive 98/70/EC relating to the quality of petrol and diesel fuels in the later text.

Directive 98/70/EC, as amended by Directive 2003/17/EC contains the environmental fuel quality specifications for petrol (gasoline) and diesel fuels in the Community with the main focus on sulphur and for gasoline on lead and aromatics. Directive 2009/30/EC was adopted which revises the Fuel Quality Directive. It amends a number of elements of the gasoline and diesel specifications. In all those directives, the maximum limit value for benzene in petrol (gasoline) is 1.0% v/v limit.

3.8 Directive 2010/75/EU on industrial emissions (integrated pollution prevention and control)

Directive 2010/75/EU of the European Parliament and the Council on industrial emissions (the Industrial Emissions Directive or IED; EU Parliament and Council Directive 2010) regulates pollutant emissions from industrial installations. The IED achieves a high level of protection of human health and the environment by reducing harmful industrial emissions across the EU, in particular through application of Best Available Techniques (BAT). Industrial activities listed in Annex I of the IED (including Refining of mineral oil and gas) are required to operate in accordance with a permit (granted by the authorities in the Member States). The permits must take into account the whole environmental performance of the plant, covering e.g. emissions to air, water and land, generation of waste, use of raw materials, energy efficiency, noise, prevention of accidents, and restoration of the site upon closure.

To define Best Available Technologies (BAT) at EU level, the European IPPC Bureau of the Institute for Prospective Technology Studies at the EU Joint Research Centre in Seville co-ordinates the production of BAT Reference Documents (BREFs). The IED requires that these documents are the reference for setting permit conditions. Best Available Techniques (BAT) Reference Document for the Refining of Mineral Oil and Gas[7] is relevant for permit setting for benzene emmissions from refineries.

4. Existing Occupational Exposure Limits

Annex III of Directive 2004/37/EC (EU Parliament and Council Directive 2004) specifies a limit value for occupational exposure to benzene of 1 ppm (3.25 mg/m^3) and a 'skin notation' indicating that there is a substantial contribution to the total body burden possible via the dermal exposure.

In some EU Member States, lower OEL values, additional short-term exposure limits (STEL) or biological limit values (BLV) are applied. Those are presented in Table 4 below but the list should not be considered as exhaustive.

[7] Available from http://eippcb.jrc.ec.europa.eu/reference/BREF/REF_BREF_2015.pdf

Table 4: Existing Occupational Exposure Limits, indicated as 8-h Time-Weighted Average (TWA), and Biological Limit Values (BLV) for benzene

Country	TWA (8 hrs)		STEL (15 min)		Remarks	BLV	Reference
	ppm	mg/m³	ppm	mg/m³			
EU	1	3.25					Directive 2004/37/EC (EU Parliament and Council Directive 2004)
						28 µg BZ/L blood; 46 µg SPMA/ g creat	SCOEL 1991, 2006
Austria	1	3.2	4	12.8		1.6 mg ttMA/L urine	EU OSHA 2009
Czech Republic		3		10		0.024 µmol SPMA/mmol creat	EU OSHA 2009
Denmark	0.5	1.6					EU OSHA 2009
Estonia	0.5	1.5	3	9			EU OSHA 2009
Finland	1	3.25			Binding value	14 µmol ttMA /L urine; (2 µg ttMA/L for pregnant women)	Finland Ministry of Social Affairs and Health 2016
France	1	3.25					INRS 2016
Germany	0.6	1.9			Tolerable risk 4:1,000	5 µg BZ/ L urine 25 µg SPMA/ g creat 500 µg ttMA/ g creat	AGS 2012, BMAS 2017
	0.06	0.2			Acceptable risk 4:10,000	0.8 µg BZ/ L urine 2.5 µg SPMA/g creat	
	0.006	0.02			Acceptable risk 4:100,000		
Latvia	1	3.25				25 µg phenol/ g creat	EU OSHA 2009
Lithuania	1	3.25	6	19			EU OSHA 2009
Netherlands	0.2	0.7					NL 2017
Poland		1.6				25 µg SPMA/ g creat; 0.5 mg ttMA/ g creat	EU OSHA 2009

Country	TWA (8 hrs)		STEL (15 min)		Remarks	BLV	Reference
	ppm	mg/m³	ppm	mg/m³			
Romania	1	3.25				25 µg SPMA/g creat; 50 mg phenol/L	Romanian Government Decision No. 1218/2006 completed and modified in 2015
Slovakia	1	3.25				5 µg BZ/ L blood; 0.045 mg SPMA/g creat; 2 mg ttMA/L urine	EU OSHA 2009
Slovenia	1	3.25	4				EU OSHA 2009
Spain	1	3.25				2 mg SPMA/ L urine; 4.5 mg ttMA/g creat	EU OSHA 2009; INSHT 2017
Sweden	0.5	1.5	3	9			EU OSHA 2009
NON-EU							
US (ACGIH)	0.5	1.6	2.5	8		25µg SPMA /g creatinine; t,t MA 500 µg/g creatinine	Biotox database
US (OSHA)	1	3.2	5	15			US OSHA 2017
US (NIOSH)	0.1	0.3	1	3.2	10 h TWA		US NIOSH 2017

Abbreviations: BZ: benzene; creat: creatinine; ttMA: t,t-muconic acid; SPMA: S-phenylmercapturic acid

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

Benzene occurs naturally as a component of petroleum and to a lesser extent, as a component of condensate from natural gas production. Other natural sources include gas emissions from volcanoes and forest fires.

The major non-occupational exposure sources for benzene are tobacco smoke, refuelling of combustion engines and emissions from combustion engines (Arnold *et al* 2013).

5.2 Production and Use Information

Benzene is produced in petroleum refinery and chemical plant processes, primarily by catalytic reforming, steam cracking and dealkylation. Benzene can also be recovered

during production of coal-derived chemicals, primarily from coke oven by-products. It is extracted from these sources and purified for industrial use.

Benzene identified as a monoconstituent substance following the nomenclature principles for substance identification (i.e. chemicals that fulfil the definition of substance and have benzene present as a main constituent at > 80 % of their compositions) is manufactured and/or imported in the European Economic Area in a quantity of 1 000 000 to 10 000 000 tonnes per year. Under REACH, the registered substance reporting "benzene" as its substance identity has been registered mainly as transported isolated intermediate or onsite isolated intermediate (ECHA 2017a).

Benzene is used as an intermediate in the production of a wide range of chemical substances such as styrene, cumene, and cyclohexane, which are further used for manufacturing of plastics, various resins, nylon and synthetic fibres. Benzene is also used in the manufacturing of some types of rubbers, lubricants, dyes, detergents, drugs, and pesticides (ATSDR 2007).

The identified uses for benzene as described within the REACH registration dossiers include formulation or re-packing, distribution and professional uses, uses at industrial sites and use in articles, health services, scientific research and development. The following products may contain benzene: laboratory chemicals, coating products, fillers, putties, plasters, modelling clay, non-metal surface treatment products, pH regulators, water treatment products and polymers (ECHA 2017b).

Because benzene occurs naturally as a component of petroleum and also as a component of condensate from natural gas production, there are many petroleum products that contain benzene and are used in diverse industrial processes, fuels, heating, solvents, cleaning agents etc. For example, benzene in gasoline (petrol) has a role as an anti knocking agent. The maximum content of benzene in gasoline was limited in 1998 to 1% v/v (EU Directive 98/70/EC relating to the quality of petrol and diesel fuels). According the restriction (REACH Regulation[8] Annex XVII entry 5), benzene should not be placed on the market above 0.1% per weight as a substance, as a constituent of other substances, or in mixtures. For natural gases for consumer use the limit is 0.1% v/v. For the use in industrial processes, the benzene emissions must be in line with other existing legislation.

5.3 Routes of exposure and uptake

Benzene is readily absorbed by all routes (inhalation, dermal and oral), of which inhalation is the most important route of occupational exposure. Mean inhalation absorption has been reported in humans ranging from approximately 50 to 80% (DECOS 2014).

Dermal absorption of **benzene vapour** is possible; however, the uptake is small compared to the uptake via inhalation (Rauma *et al* 2013).

Liquid benzene can be absorbed through human skin, although this is not as substantial as absorption following inhalation or oral exposure. Under normal condition the contribution of dermal uptake to total uptake might be low as evaporation from the skin surface will decrease the dermally absorbed amount. However, the dermal route can be an important contributor to total benzene exposure in certain situations, such as immersion

[8] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396 of 30 December 2006, p. 1; corrected by OJ L 136, 29.5.2007, p. 3)

of the skin in solution or when the airborne concentration of benzene is very low (Williams *et al* 2011).

Jakasa *et al* (2015) calculated the dermal uptake with 5.85% at an OEL of 1 ppm (3.2 mg/m^3). Williams *et al* (2011) analysed the experimental skin absorption data of benzene (both human and animal; *in vitro* and *in vivo*), and concluded that the steady state absorption rate of benzene ranges from 200 to 400 µg/cm^2*h (DECOS 2014). Considering an OEL of 0.05 ppm (0.16 mg/m^3) this value exceeds by far the critical absorption value (CAV) calculated according to the ECETOC methodology (1998) of 0.08 µg/cm^2*h (with (10 [m^3] x OEL [mg/m^3] x f x 0.1)/2,000 [cm^2], in which 10 m^3 is the human inhalation volume per 8-hour 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).

Kalnas and Teitelbaum (2000) found that for **solvents used for cleaning that contained benzene at concentrations of less than 0.1%**, the amount of benzene absorbed through the skin over a long period was significant, depending on exposure time and exposed skin surface areas.

5.4 Occupational exposure

The studies reviewed in this section report benzene concentrations most often in mg/m^3. Hence, in the tables of this section, benzene exposures are summarized using the unit mg/m^3. In the concluding paragraphs, concentrations may also be provided in ppm to compare with the effect on humans, for which most often benzene concentrations are reported in the unit ppm.

Exposure to benzene occurs in the petroleum and chemical industries and also as a result of exposure to gasoline engine emissions and combustion products.

Occupational exposures to benzene occur mainly via inhalation, although dermal exposure is also possible. Dermal exposures associated with service station refuelling activities were reported by Concawe (2014). In the preliminary study, patch, surface and hand wipe samples were examined for petroleum hydrocarbons, including also benzene. Hydrocarbon evaporation test confirmed that all benzene (100%) evaporated from the petrol matrix in four hours. Benzene was not detected on the hands of the service workers because of its volatility. Patch samples placed inside and outside the clothing at the level of chest and forearms showed variable level of benzene and other hydrocarbons less than C_{12}. The level of benzene was from 0 to 0.4 µg/cm^2 on patches placed under a cotton t-shirt and from 0 to 2.3 µg/cm^2 on the patches placed over the clothing or on the forearm skin not covered by clothing.

In the years between 1950 and 1960, occupational exposure to benzene was high with estimated benzene concentrations between 10 and 100 ppm or even higher than 100 ppm (see Figure 1).

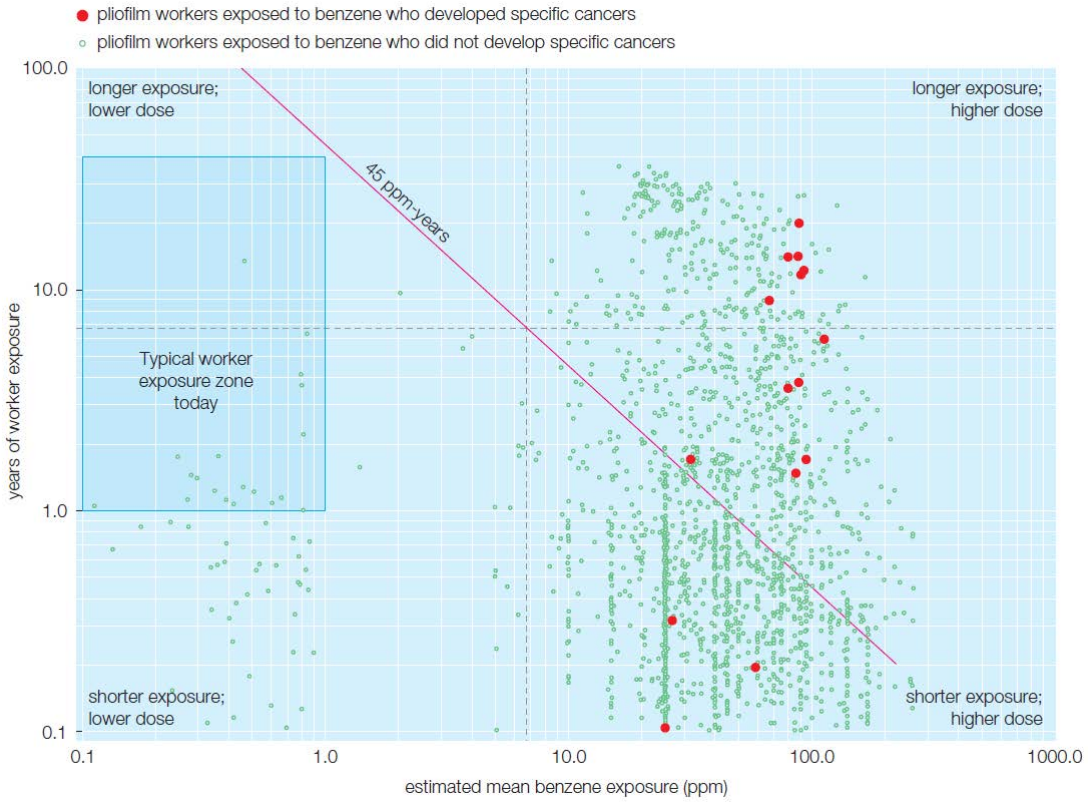

Figure 1: Average benzene exposures for workers evaluated in the 1950-60s 'pliofilm study' compared to typical worker exposure today (Concawe 2012)

Recent publications confirm that occupational exposures to benzene in the EU are usually below 1 ppm (Capleton and Levy 2005; Concawe 2002). However, occasional higher exposures may have occurred in some occupational groups such as road tanker drivers, when loading has been performed without vapour recovery (Capleton and Levy 2005).

Concawe (2002 and 2009) has performed a survey of **European gasoline exposures** for the period 1999-2001 (Table 5 below) and additional exposure measurements during 2002-2007 (Table 6below). The average full-shift exposure concentrations among different job groups were mainly below 1 ppm (3.25 mg/m^3). Concentrations slightly above the current EU Occupational Exposure Limit (OEL) of 1 ppm were reported for laboratory technician blending test gasoline (3.7 mg/m^3, **1.1 ppm**) and for railcar top loading without vapour recovery (4.0 mg/m^3, **1.2 ppm**) in the report from 2002. The exposure to benzene during railcar top loading with vapour recovery is 8-times lower than without vapour recovery. While the vapour recovery became later mandatory in bulk gasoline distribution operations, the exposure to benzene during loading should be clearly lower (around 0.5 mg/m^3) than the current EU OEL. According additional exposure measurements reported in 2009, the most elevated exposures appeared in service station pump repairs inside a workshop, maximum value being 2.9 mg/m^3 (0.89 ppm; Concawe 2002, 2009).

Table 5: Occupational exposure concentration to benzene 1999-2001 (Concawe 2002)

Job group	Full-shift exposure (mg/m³)			Peak exposure (mg/m³)		
	n	average	90-percentile	n	average	90-percentile
On-site refinery operator				6	1.0	1.4
Off-site refinery operator	6	0.3	0.5	7	0.7	0.8
Laboratory technician blending test gasoline for research	7	3.7	8.3	2	0.8	-
Laboratory technician octane rating for research	3	0.3	0.5 (max)	4	0.8	1.0 (max)
Road tanker driver	33	0.6	1.2			
loading				15	1.8	3.8
delivery				7	0.7	1.6
Gantry man	3	0.4	0.5 (max)			
Drum filler	2	0.2	-	10	0.9	1.5
Railcar top loading without VR	16	4.0	10			
Railcar top loading with VR	21	0.5	0.7	3	0.5	0.5 (max)
Other railcar loading workers	5	0.2	0.3 (max)			
Jetty staff	4	0.1	0.1 (max)	6	0.3	
Service station attendants – no VR	26	0.3	0.5			
Service station attendants – with VR	7	0.1	0.1			
Service station shop personnel	13	0.2	0.2			
Miscellaneous service station personnel	6	0.2	0.2	1	0.2	
Marine deck crew				6	0.3	0.6
Miscellaneous ship personnel				4	0.4	0.8 (max)

Abbreviations: VR: vapour recovery

Table 6: Occupational exposure concentration to benzene 2002-2007 (Concawe 2009)

Job group	N	Full-shift exposure, (mg/m³)		Peak exposure, (mg/m³)		Location, date
		Average	Range	average	range	
Refinery maintenance workers	9			0.058	0.003-0.122	Finland, 2006
Refinery maintenance workers	5	0.034	0.006-0.099			Norway, Finland, The Netherlands, 2006
Refinery production laboratory	5	0.022	0.006-0.052			Norway,2006; France, 2007
Research and Development laboratory	5			0.262	0.061-0.560	UK, Germany, 2006
Research and Development laboratory	9	0.155	0.029-0.726			UK, Germany, 2006
Road tanker operations	2			0.045		France, 2007
Rail car operations	8	0.045	0.011-0.152			France, 2007
Gasoline pump repair and maintenance	5	0.828*	0.11-2.9			Finland, 2004
Gasoline pump calibration	5	0.41	0.06-0.92			Finland, 2004
Operation of gasoline-powered garden maintenance equipment	12	0.009**	<0.002-0.02			Belgium, 2005
Aviation gasoline operations	8	0.021	0.02-0.04			UK, 2004; France, 2005

*duration of sampling 1.5 hours to 4 hours; **half-shift sampling

Table 7 provides an overview on occupational exposures to benzene mainly in Europe. The table summarises the ranges of exposures for different types of activities that involve exposure to benzene. For this overview table the data selected only include relatively recent literature and working sites located in Europe. Some older data have been included if recent data from Europe are not available. More detailed information including older literature and studies carried out outside the EU can be found in Table 34 in Appendix 1 that provides more granularity of data regarding the publications including exposure data per activity, and mean and median values.

Table 7: Overview of occupational exposures to benzene in Europe (and USA)

Work area / Occupation	N	Average (mg/m³)	Range (mg/m³)	Location	Comments	References
Upstream petroleum industry-offshore	**380**		**0.003-54**			
Modes of operation and tasks on a production vessel in the Norwegian sector of the North Sea	139	1.40	0.003 (LOD)-54	Norway/ the North Sea	Tasks: cleaning tank, maintenance of a cleaned cargo tank, work near an open hydrocarbon transport system and other tasks. The highest potential exposure is measured during the cleaning of tank; workers used PPE.	Kirkeleit *et al* 2006
Offshore oil and gas production operations-North Sea	241	91% of the samples <0.16	0.065 (LOD)-1.6 (99th percentile)	United Kingdom/ the North Sea		HSE 2000
Refineries	**540**	**0.005-3.7**	**0.002-8.3**			
Refinery, during routine operations; all	373	0.005-0.075	0.005 (LOD)-0.16 (CI 95%) max 3.7	Sweden	Mean concentrations for each exposure group defined reported separately. See Table 34 in Appendix 1 for detailed results per exposure group	Almerud *et al* 2017
Refinery 2, during turnaround	26	0.96	0.007-4.5 (CI 95%)	Sweden	Complete turnaround. Benzene content in the stream around 20% Average for contractors higher. (See Table 34 in Appendix 1)	Akerstrom *et al* 2016
Refinery 2, during turnaround	22	0.15	0.007-1.2 (CI 95%)	Sweden	Partial turnaround. Benzene content in the stream around 1.5% Average for contractors higher. (See Table 34 in Appendix 1)	Akerstrom *et al* 2016

Work area/ Occupation	N	Average (mg/m³)	Range (mg/m³)	Location	Comments	References
Refinery 1 during turnaround	43	0.61	0.23-1.6 (CI 95%)	Sweden	Complete turnaround. Benzene content in the stream around 8% Average for contractors higher. (See Table 34 in Appendix 1)	Akerstrom *et al* 2016
Oil harbour	34	0.31	0.080-1.2 (CI 95%)	Sweden		Akerstrom *et al* 2016
Sewage tank drivers	16	0.36	0.068-1.9 (CI 95%)	Sweden		Akerstrom *et al* 2016
Refinery, offsite refinery operators and laboratory technicians	7	0.3-3.7	0.5-8.3 (90th percentile)	Europe, 1999-2001	Mean concentrations for each exposure group defined reported separately. Higher exposure corresponds to R&D laboratory technicians (see Table 5 for details on other tasks)	Concawe 2002
Refinery, maintenance and laboratory workers	19		0.006-0.73	Europe, 2002-2007	See Table 6	Concawe 2009
Chemical industry	**351**	**0.003-0.035**	**<0.001-0.9**			
Chemical plants (different activities)	19		<0.002-0.83	Germany	Measurements taken to test a new analytical method not to perform a workplace assessment	Breuer *et al* 2013
Petrochemical industry operators	145	0.014	<0.001–0.28	Italy		Carrieri *et al* 2010
Petrochemical industry Outdoor operators	173	0.035	0.002-0.9	Italy		Carrieri *et al* 2012
Petrochemical industry workers	33	0.003 (median)	0.002-0.59	Italy		Fracasso *et al* 2010
Coke oven industry	**57**	**0.12-1.2**	**Max 24**			
Coke plant	36	0.13-1.8	24 max	1994-1995, Belgium		IARC 2012; Hotz *et al* 1997
By-product plant	21	1.2	5.3 max	1994-1995, Belgium		IARC 2012; Hotz *et al* 1997
Tank filling/ tank drivers	**109**	**0.2-0.6**	**0.002-1.2**			

Work area / Occupation	N	Average (mg/m³)	Range (mg/m³)	Location	Comments	References
Train and truck tank filling	8		0.002-0.027	Germany	Measurements taken to test a new analytical method not to perform a workplace assessment	Breuer *et al* 2013
Tank filling (big quantities)	16		<0.006-0.4	Germany		Breuer *et al* 2015
Fuel tank drivers	18 17	0.31 0.28	0.007-1.0	Italy		Lovreglio *et al* 2014, 2016
Road tanker driver	33	0.6	0.2 -1.2 (10th -90th percentile)	Europe, 1999-2001	Conditions of use bottom loading. Most also had vapour recovery systems but no significant difference in exposure was found	Concawe 2002
Other railcar loading operations	5	0.2	Max 0.30	Europe, 1999-2001		Concawe 2002
Railcar top loading with VR	21	0.5	0.2-0.7 (10th -90th percentile)	Europe, 1999-2001		Concawe 2002
Railcar operations	8		0.011-0.15	Europe, 2002-2007	France	Concawe 2009
Service stations / Repairing workshops	**258**	**0.02-0.24**	**0.001-2.9**			
Service station attendants	10		0.001-0.006	Germany	Measurements taken to test a new analytical method not to perform a workplace assessment	Breuer *et al* 2013
Service station attendants and shop personnel	22	0.036-0.053 (Geometric mean)	0.002-0.088	Germany		Breuer *et al* 2015
Service station attendants and shop personnel	13 24	0.020 0.023	0.005-0.066	Italy		Lovreglio *et al* 2014, 2016
Gasoline station workers	89	0.059	0.005-0.28 (5-95 percentile)	Italy		Campo *et al* 2016
Service station workers- 1995	*21*	*0.74*	*0.27-1.6*	*Spain*	*Not included into the merged values for the sector while the measurements are before 2000*	*Periago and Prado 2005*
Service station workers-2000	28	0.24	0.11-0.45	Spain		Periago and Prado 2005
Service station workers-2003	19	0.16	0.035-0.56	Spain		Periago and Prado 2005

Work area / Occupation	N	Average (mg / m³)	Range (mg / m³)	Location	Comments	References
Service station attendants	28	0.040 (median)	(0.008–0.26	Italy		Fracasso *et al* 2010
Repairing workshop	8		0.005-1.5	Germany	Workshops at petrol stations, car and motorbikes and gardening tools dealers. Higher exposures corresponds to the gardening tool workshop	Breuer *et al* 2013
Repairing workshop	12		0.052-0.33	Germany		Breuer *et al* 2015
Gasoline pump repair, maintenance and calibration	10		0.060-2.9	Finland, 2002-2007		Concawe 2009
Aviation gasoline operations	8		<0.030-0.040	UK, France; 2004-2007		Concawe 2009
Traffic / Use of gasoline-engined equipment	**204**		**<0.002-0.2**			
Traffic policeman	70	0.019	0.023–0.059	Italy		Angelini *et al* 2011
Landscaping work	120		<0.002-0.2	Germany		Breuer *et al* 2015
Garden maintenance	14		<0.002-0.020	Belgium, 2005		Concawe 2009
Use of gasoline-derived products	**465**	**0.00003 -3.2**	**Max 9.1**			
Surface cleaning with petroleum solvents	9	1.1	0.55-1.9	USA	Spiked benzene content was 0.07% in paint thinner or engine degreaser. 18-23 minutes time-weighted concentration.	Hollins *et al* 2013
Painting with lacquer	2	-	1.27-2.97	Germany	Benzene concentration during 30 min task. Simulation experiment in 21 m^2 room, 0.05% spiked benzene in lacquer.	HVBG 2001
Painting with lacquer	2		2.77-4.96	Germany	Benzene concentration during 30 min task. Simulation experiment in 21 m^2 room, 0.10% spiked benzene in lacquer.	HVBG 2001

Work area / Occupation	N	Average (mg/m³)	Range (mg/m³)	Location	Comments	References
Various printing operations	281	0.00003-3.2	Max 7.8	USA, 1981-2006	Products containing trace levels of benzene. The highest level measured in sheet fed printing, the content of benzene unknown (personal sample).	IARC 2012
Paint/paint solvents	161	0.026-1.1	Max 9.1	USA, 1981-2006	Products containing trace levels of benzene. The highest level measured in automotive assembly plant (personal sample).	IARC 2012
Mineral spirits (spray cleaning and parts degreasing)	23	<0.020-0.94	Max 1.8	USA, 1981-2006	Products containing <0.01% of benzene. Both area and personal samples, 60 minutes sampling.	IARC 2012

Upstream petroleum industry – offshore production

Kirkeleit *et al* (2006) measured personal benzene exposure during various tasks on offshore production vessels in the **Norwegian sector of the North Sea**. A full-shift sampling (12 hours) was performed by passive dosimeter badges attached to the worker's collar. The mean exposure levels during tank cleaning, maintenance of a cleaned cargo tank, work near an open hydrocarbon transport system and other tasks were 6.2 mg/m³, 0.24 mg/m³, 0.03 mg/m³ and 0.005 mg/m³, respectively.The average exposure concentration of all measurements was 1.4 mg/m³ (**0.43 ppm**; n= 138). The highest exposure was during tank cleaning. The workers used half-mask respirators with combination filter and chemical protective clothing during tank cleaning (Kirkeleit *et al* 2006). HSE has gathered some older measured data from offshore production from **the British sector in the North Sea**. Measured data has been collected during years of 1998 and 1999 from normal operations in eleven installations. The sampling was performed with diffusive sampling tubes placed in worker's breathing zone for duration of 12-hour work-shift. The exposure levels to benzene were less than 0.16 mg/m³ **(<0.05 ppm**) in 91% of the measurements. The 95th percentile benzene concentration was 0.36 mg/m³ (**0.11 ppm**) (HSE 2000).

Refineries

Almerud *et al* (2017) looked at worker exposure to benzene in petroleum refineries. The study concluded that workers have a low average personal exposure to benzene during normal operations in **Sweden**. The average personal benzene exposure among process technicians was 0.015 mg/m³ at refinery 1 and 0.014 mg/m³ at refinery 2. The highest mean exposure to benzene, 0.075 mg/m³, was measured among outdoor process technicians in harbour and tank area.

Campagna *et al* (2012) measured exposure to benzene by personal sampling for oil refinery workers and the general population in **Italy** during 2006 to 2007. The median concentration of airborne benzene was 0.025 mg/m³ (**0.008 ppm**) in oil refinery workers (n=32), and 0.008 mg/m³ in the general population subgroup (n=65).

Akerstrom *et al* (2016) examined the personal exposure to benzene at refinery turnarounds and during work in an oil harbour in **Sweden**. Planned showdown of the refinery unit for maintenance and repair work occurs every 2 to 4 years. The mean benzene exposure levels for refinery workers during three measured turnarounds were 0.15, 0.61 and 0.96 mg/m^3 (**0.05, 0.19 and 0.30 ppm**). The benzene content was different in each of the streams ranging from 1.5 to 20% of benzene. Higher exposures were associated with handling benzene-rich products. Mean exposures for oil harbour workers and sewage drivers were 0.31 and 0.36 mg/m^3 (**0.10 and 0.11 ppm**), respectively. The range in all benzene measurements was from 0.007 to 4.5 mg/m^3 (0.002-1.4 ppm). During these turnarounds, the contractors had about 3- and 12-fold higher benzene exposure compared to the refinery employees. For example , the mean exposure concentration to benzene was 0.43 mg/m^3 for refinery workers and 1.2 mg/m^3 for contractors in the refinery 1, and 0.87 mg/m^3 and 1.1 mg/m^3 in refinery 2 during complete turnaround. The work within the petroleum refinery industry with potential exposure to open product streams containing higher fractions of benzene, pose a risk of personal benzene exposures exceeding 1 ppm. Refinery workers performing these work tasks frequently are contractors, sewage tanker drivers and oil harbour workers.

A total of 16700 personal workplace air samples samples were analysed for benzene at four **USA** refineries from 1976 to 2007. A statistically significant decrease in benzene air concentration was reported after 1990. For all job titles during 1976 to 1989 and 1990 to 2007, the mean benzene concentrations were 0.88 mg/m^3 (**0.27 ppm**) and 0.46 mg/m^3 (**0.14 ppm**) and the 95-percentile values were 1.8 and 0.68 mg/m^3, respectively. When the benzene exposures were examined according different tasks during 1976 to 1989 and 1990 to 2007, the mean benzene concentrations for all tasks were 6.2 mg/m^3(**1.9 ppm**) and 1.3 mg/m^3 (**0.40 ppm**) and the 95th percentile values were 27 mg/m^3 and 4.5 mg/m^3, respectively. Key factors for the decrease in exposure were benzene content of the process stream and the performance of specific tasks. Different fuels contained benzene in the range of <0.1 to 3.0%. The “blinding and breaking” tasks in area of the reformer and tank farm had the highest exposures to benzene. “Non-task” benzene air concentrations (workers not limited to one task) were divided into exposures during routine, startup and turnaround work. The mean concentrations were 0.68, 0.13 and 0.81 mg/m^3, respectively. In general, the “non-task” personal air samples indicated that exposures to benzene were below 1.4 mg/m^3 (95th percentile value) (Burns *et al* 2017).

The exposure to benzene in refineries has decreased after 1990. Key factors for the decrease in exposure have been decreased benzene content of the process stream and the performance of specific tasks.

In the recent studies in Sweden and Italy, the exposure to benzene has been below 0.2 mg/m^3 (**<0.06 ppm**) during routine operations in the refineries. The reports by Concawe 2002 and 2009 show that the mean exposure to benzene is around 0.5 mg/m^3 (**0.15 ppm**) in Europe. In the US refineries, the mean exposure to benzene has also been 0.5 mg/m^3 for all job titles during the years of 1990 to 2007. However, during certain tasks the exposure to benzene may be increased in refineries. The tasks with potentially high benzene exposure are fuel blending and dispensing in the research and development laboratory, gasoline pump calibration and gasoline pump repair and maintenance work, especially when the work is done indoors (maximum exposure was 2.9 mg/m^3 coresponding to **0.89 ppm**) and maintenance and repair work in the refinery (1.1 mg/m^3, range 0.007-3.4 mg/m^3 according to a Swedish study).

Service stations, repair workshop and tank drivers

Breuer *et al* (2013) measured airborne benzene using 22 stationary measurement points in nine chemical plants, at gasoline stations, in repair workshops and in a tank farm in **Germany**. The benzene concentration was well below 0.03 mg/m^3 for most samples. The level of 0.03 mg/m^3 was exceeded in a few samples in a repair workshop for gardening

tools (1.5 mg/m^3). The benzene exposure was further studied during filling of tanker vehicles, in indoor and outdoor areas of filling stations, during maintenance work on motor vehicles, and in landscaping work involving gasoline-engined equipment in Germany. The exposure level was normally below 0.2 mg/m^3 at gasoline station and in landscaping work. The geometric means varied from 0.004 to 0.024 mg/m^3 at gasoline station and from 0.004 to 0.02 mg/m^3 in landscaping work.The highest geometric mean value for benzene was 0.33 mg/m^3 measured at a workshop (Breuer *et al* 2015).

Lovreglio *et al* (2016) measured mean benzene levels of 0.020 mg/m^3 (range 0.005-0.053 mg/m^3, n=13) for fuel filling station attendants, 0.280 mg/m^3 (range 0.007-1.0 mg/m^3, n=17) for fuel tank drivers, and 0.005 mg/m^3 (range <0.003-0.012 mg/m^3, n=20) for controls in **Italy**. The similar exposure levels were achieved also in another similar study performed in Italy (Lovreglio *et al* 2014).

Campo *et al* (2016) investigated the exposure to benzene for fuel filling station attendants using personal air sampling in the area of Milan **Italy**. Petrol station workers had median airborne exposures to benzene of 0.059 mg/m^3 (n=89). Exposure varied between 0.005 and 0.28 mg/m^3 (5–95 percentile). However, the maximum benzene concentration of 3.2 mg/m^3 was in a fuel loading operation. The authors acknowledge significant differences (around two fold) in the average exposure measured compared with other studies carried out in Italy and attributed the difference to the size of the cities studied and the higher volume of fuel dispensed around bigger cities.

Periago and Prado (2005) measured exposure to benzene in 2000 and 2003 from personal breathing zone of occupationally exposed workers in service station in **Spain**. The results were compared to the concentrations measured in 1995. Summer weather conditions were similar for all measurements. A decrease of benzene concentrations was observed. The time-weighted average values were 0.74 mg/m^3, 0.24 mg/m^3 and 0.16 mg/m^3, respectively for the years 1995, 2000 and 2003.

The exposure level to benzene is generally below 0.3 mg/m^3 for fuel filling station attendants in Europe. However, the exposure can be higher during fuel loading operations at petrol station (3.2 mg/m^3) and in repair workshops (1.5 mg/m^3).

Petrochemical industry

Breuer *et al* (2015) analysed airborne benzene levels from stationary samples in chemical plants in **Germany**. The benzene concentration ranged from <0.002 to 0.83 mg/m^3. Carrieri *et al* (2010, 2012) reported that exposure to benzene is low in the petrochemical industry in **Italy**. The mean value for benzene was 0.046 mg/m^3 (0.014 ppm), the median 0.010 mg/m^3 and the range <0.003 to 0.91 mg/m^3 (n=145) for petrochemical industry operators. For outdoor operators, the mean was 0.034 mg/m^3 (0.011 ppm), the median 0.009 mg/m^3 and the range <0.002 to 0.895 mg/m^3)(n=173). Fracasso *et al* (2010) measured median value of 0.029 mg/m^3 for petrochemical industry workers (n=33) in **Italy**.

The exposure levels to benzene ranges from <0.002 to 0.9 mg/m^3 in chemical plants in Europe.

Petroleum based solvent products containing trace levels of benzene

Williams *et al* (2008) reviewed the historical benzene content of various petroleum-derived products and characterized the airborne concentrations of benzene associated with the typical handling or use of these products in the **United States**, based on indoor exposure modeling and industrial hygiene air monitoring data collected since the late 1970s. Analysis showed that products that normally contained less than 0.1% v/v benzene, such as paints and paint solvents, printing solvents and inks, cutting and honing oils, adhesives, mineral spirits and degreasers, and jet fuel typically have yielded time-weighted average (TWA) airborne concentrations of benzene in the breathing zone and surrounding air ranging on average from <0.03 to 0.98 mg/m^3 (<0.01 to 0.3 ppm).

In HVBG (2001) the authors reported on personal exposure measurements performed in an experimental set-up of a 20.8 m³ exposure chamber in **Germany**. Door leaves were manually painted under controlled directed air exchange rates (5.3 h^{-1} or 23.7 h^{-1}) using lacquers which were specifically doped with a certain benzene content ranging from 0.05 to 0.55%. Even for the lowest content of benzene (0.05%) employed under an air exchange rate of 5.3 h^{-1}, which is still higher than normally encountered indoors, the personal exposures during the painting tasks (n=2, 30 minutes) were 1.27 mg/m³ and 2.97 mg/m³. For the painting and drying task (n=2, 90 minutes), the benzene concentrations from stationary samplers were 1.46 and 0.77 mg/m³. For the 0.1% benzene concent in lacquer, the personal exposure during painting (30 minutes) was 4.96 and 2.77 mg/m³ and during painting and drying (90 minutes), the benzene concentrations from stationary samplers were 1.15 and 1.37 mg/m³. It is to be noted that benzene concentration as a constituent of other substances, or in a mixture placed on the market should be less than 0.1% by weight.

Airborne benzene exposures from cleaning metal surfaces with small volumes of petroleum solvents were studied in a simulation study in **USA** (Hollins *et al* 2013). Average breathing zone concentrations of benzene were 0.01, 0.05, and 0.33 ppm (time weighted average), when solvents (paint thinner and engine degreaser) contained approximately 0.003, 0.008 and 0.07% spiked benzene. According to this study and a previous study of Richter *et al* (2013), the higher aromatic content and higher liquid benzene content suppress benzene vapor concentrations due to benzene's greater affinity for similar aromatic molecules in solution, hence results in disproportionately lower vapour release from liquid solvent at higher solvent benzene concentrations.

Petroleum based solvents which may contain benzene are used in different tasks (surface cleaning, painting, degreasing etc.). Depending on the task, its duration, aromatic content and benzene content, solvent airborne benzene concentrations may result in rather high levels. For example painting with lacquer that contained 0.1% benzene resulted in high short-term exposures of 5 mg/m^3 (30 minutes exposure). Also the time-weighted concentrations exceed the 0.3 ppm level (1 mg/m^3) when the petroleum based solvents contain less than 0.1% benzene.

Other occupations

IARC collected typical benzene exposure levels in different occupational groups in **Europe and North America** during the years 1981 to 2003.

IARC (2012) summarizes the airborne benzene concentrations from different occupational groups and petroleum based solvent products containing trace levels of benzene showing that the mean airborne benzene concentrations are below 2 mg/m^3 and the current EU OEL of 3.25 mg/m^3 (**1 ppm**). When comparing different occupational groups, the coke oven industry has the highest exposure to benzene. The median concentration is 1.79 mg/m^3 (**0.55 ppm**). The data is from the period 1994 to 1995. When the exposure from petroleum based solvent products containing trace levels of benzene were compared, the highest benzene exposure occurs in sheet fed printing process for operators, where the mean concentration is 3.2 mg/m^3 (**0.99 ppm**) and the range is 1.0 to 5.9 mg/m^3 (**0.31-1.81 ppm**). The job categories and usages where the mean benzene concentration is above 0.32 mg/m^3 are the following: use of paints or paint solvents in automotive assembly plant (1.1 mg/m^3; **0.34 ppm**), use of printing solvents or silk screening inks in various printing (2.7 mg/m^3; **0.84 ppm**), offset duplicating (0.39 mg/m^3; **0.12 ppm**), rotogravure printing (0.42 mg/m^3; **0.13 ppm**), and the use of mineral spirits in parts decreasing – over tank (0.94 mg/m^3; **0.29 ppm**) (Williams *et al* 2008, IARC Volume 100F 2012).

The exposure to benzene is around 1.8 mg/m^3 in coke oven industry. The printing processes may have mean exposures higher than 0.3 mg/m^3. The highest exposure to benzene has been 5.9 mg/m^3 in sheet-fed printing.

Occupational exposure to benzene as recorded in National databases:

The exposure to benzene has reduced from 1985 to 2002 according the **German** MEGA database, which includes exposure measurements in the workplace atmosphere. One reason for the decreasing exposure is that the benzene concentration has decreased in gasoline and also in lacquers and thinners. According to the MEGA database, the highest benzene exposures occur during transfer and filling of gasoline tanks and vessels and their cleaning. During the period from 1998 to 2002, the 90th percentile value for benzene exposure level was 3.4 mg/m^3 during transfer and filling up tasks and 2.2. mg/m^3 during cleaning of/in tanks and vessels. In the foundry the exposure level was below 1.2 mg/m^3, in laboratories 0.8 mg/m^3, and in repair/maintenance/test bench 0.7 mg/m^3. The 50th percentile values for all these tasks were less than the detection limit which was around 0.1 mg/m^3. The measured benzene exposure was less than the detection limit (0.1 mg/m^3) also during cold/hot moulding of plastics, thermal processing methods, bonding/coating/lacquer application and cleaning (excluding cleaning of buildings) (DGUV 2007).

According to data from an occupational measurement database compiled in **Finland**, during the period of 2004 to 2007, the median measured benzene exposure was 0.002 mg/m^3, the arithmetic average was 0.09 mg/m^3 and the 95th percentile was 0.96 mg/m^3. The number of samples was 83. The highest exposures were in a sector of motor vehicles' sale, repair and maintenance and the retail sale of fuel and in a sector of environmental care and maintenance. Exposure to benzene has been monitored also by measuring *t*,*t*-muconic acid (ttMA) in urine. The number of samples was 501. The average value for ttMA has been 3.4 µmol/l (nonsmokers 2.2 µmol/l and smokers 3.9 µmol/l). The reference value for non-occupationally exposed workers is 0.5 µmol/l and the action limit value is 14 µmol/l in Finland. The action limit was exceeded in activities in oil refinery and handling of contaminated soil. There were 1200 workers who are exposed to benzene in Finland. These workers are employed in coking plants, manufacturing benzene and its reaction products, manufacturing fuels, loading and transferring, repairing of motors and laboratory work (FIOH 2010).

Summary of occupational exposure in Europe

Some review articles (Burns *et al* 2017; Capleton 2005; IARC 2012) and also two occupational databases show that the exposure to benzene are typically below 1 ppm in Europe and North America, the OEL valid at that time. The highest occupational exposures occur during filling and transferring (loading) of gasoline, cleaning of tanks and vessels, repair, maintenance and laboratory work. Many of these tasks have a relatively high exposure of short duration. The recent occupational exposure studies for benzene exposed workers are included in the Table 34 in Appendix 4.

Offshore crude oil and gas production

Kirkeleit *et al* (2006) measured personal benzene exposure during various tasks at offshore production vessel in the **Norwegian sector of the North Sea**. The mean benzene exposure levels during tank cleaning, maintenance of a cleaned cargo tank, work near an open hydrocarbontransport system and other tasks have been 6.2 mg/m^3 (**1.9 ppm**), 0.24 mg/m^3 (**0.07 ppm**), 0.03 mg/m^3 (**0.01 ppm**) and 0.005 mg/m^3 (**0.002 ppm**), respectively.The average exposure concentration of all measurements was 1.4 mg/m^3 (**0.43 ppm**; n= 138). The highest exposure was during tank cleaning. The workers used half-mask respirators with combination filter and chemical protective clothing during tank cleaning. HSE (2000) has gathered some older measured data from offshore production from **the British sector in the North Sea**. Measured data has been collected during years of 1998 and 1999 from normal operations in eleven installations. The exposure levels

to benzene were less than 0.16 mg/m^3 (**<0.05 ppm**) in 91% of the measurements. The 95th percentile benzene concentration was 0.36 mg/m^3 (**0.11 ppm**).

Refinery

The exposure to benzene in refineries is below 0.2 mg/m^3 (**<0.06 ppm**) during routine operations in Sweden (Almerud *et al* 2017). However, during turnarounds and maintenance work the exposure can be higher according to a Swedish study (Akerstrom *et al* 2016). The higher mean benzene concentration was 1.1 mg/m^3 (**0.33 ppm**; range 0.007-3.4 mg/m^3) during turnaround work with higher benzene content for contractors. The reports by Concawe 2002 and 2009 show that the mean exposure to benzene is around 0.5 mg/m^3 (**0.15 ppm**) in Europe, but during laboratory work it can be higher especially during fuel blending and dispensing tasks. In the latest report, the average exposure was 0.2 mg/m^3 (**0.06 ppm**) in the research and development laboratory. Another activity that may lead to benzene exposure is gasoline pump repair and maintenance work, especially when the work is done indoors (maximum exposure was 2.9 mg/m^3 (**0.89 ppm**).

Service stations

The mean benzene exposure values among service station attendants are below the level of 0.3 mg/m^3 (**<0.1 ppm**). In Germany and Italy the measured exposures have been in the range of 0.005 to 0.09 mg/m^3 (**0.002-0.03 ppm**) (Campo *et al* 2016; Lovreglio *et al* 2016; Breuer *et al* 2013, 2015). In Spain, the benzene concentrations have been higher among workers in service station, being in range of 0.04-0.56 mg/m^3 (**0.01-0.17 ppm**) (Periago and Prada 2005).

Repair workshop

The benzene exposure range from 0.005 to 1.5 mg/m^3 (**0.002-0.46 ppm**) in repair workshops in Germany, the mean value being 0.3 mg/m^3 (**0.09 ppm**) (Breuer *et al* 2013 and 2015).

Petrochemical industry

The latest exposure data from the literature shows that the benzene exposure in petrochemical industry range from <0.002 to 0.9 mg/m^3 (**<0.001-0.28 ppm**), the mean value being around 0.1 mg/m^3 (**≤0.03 ppm**) in Germany and Italy (Breuer *et al* 2013; Carrieri *et al* 2010, 2012; Fracasso *et al* 2010).

The use of petroleum based solvent products

According to IARC (2012) and Williams *et al* (2008), printing and degreasing operations with substances containing benzene may result in mean benzene exposure around or above 3.2 mg/m^3 (**0.99 ppm**) and 0.9 mg/m^3 (**0.29 ppm**), respectively. The highest exposure to benzene has been 5.9 mg/m^3 (**1.8 ppm**) in sheet fed printing.

According to Williams *et al* (2008) if the benzene content in the product is less than 0.1% v/v, the airborne benzene concentration range on average from <0.3 to 1.0 mg/m^3 (**<0.1-0.3 ppm**). However, task based concentrations may result high short-term exposure. For example, painting with lacquer that contain 0.1% benzene, resulted nearly 5 mg/m^3 (**1.5 ppm**) exposure (task duration was 30 minutes).

Use of gasoline-engined equipment

The use of gasoline-engined equipment in gardening, landscaping or forest work may lead to the exposure to benzene. The benzene exposure range has been from <0.002 to 0.2 mg/m^3 (**<0.001-0.06 ppm**), and the mean values have been around 0.01 to 0.07 mg/m^3 (**0.003-0.02 ppm**) (Breuer *et al* 2015; Concawe 2009; Neri *et al* 2016).

5.5 Human exposures via the environment

The general population is usually exposed to benzene via inhalation. The main sources are traffic exhausts and cigarette smoke. Benzene exposures to the general population have been reduced significantly from these sources by lowering the benzene content of gasoline and prohibiting smoking in many public places (Capleton 2005; Weisel 2010). Natural sources of benzene include volcanoes and forest fires (Arnold *et al* 2013).

Wide ranges of benzene concentrations are reported which may be due to factors such as sample location (e.g., rural versus urban; outdoor versus indoor), season and time of measurement (e.g. winter; afternoon), number of observations, average sampling time and other factors (e.g. mean versus maximum concentrations) (Arnold *et al* (2013).

Benzene exposures from gasoline combustion processes

To improve air quality in the EU, Directive 2008/50/EC (EU Parliament and Council Directive 2008) set a limit value for the protection of human health of 5 µg/m^3 for benzene. One major source of benzene in urban air is from gasoline. The maximum content of benzene in gasoline was limited in 1998 to 1% v/v according to the EU Directive 98/70/EC relating to the quality of petrol and diesel fuels (EU Parliament and Council Directive 1998).

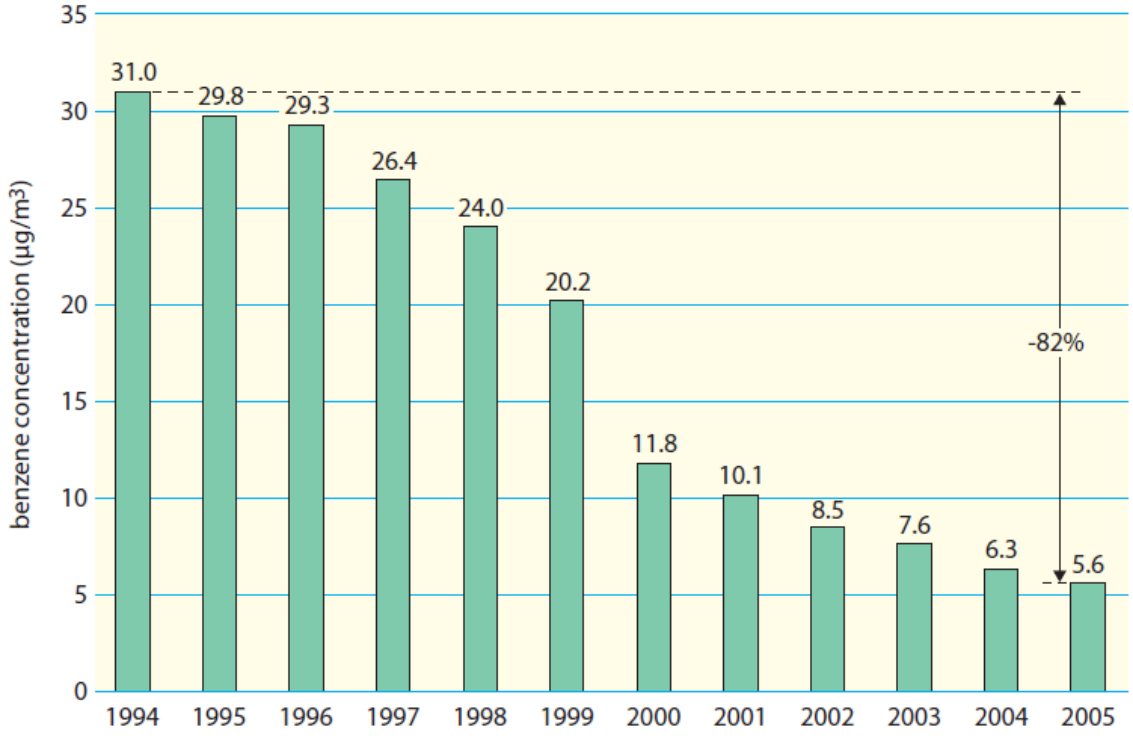

Figure 2: Evolution of the average annual concentration of benzene at the traffic impacted monitoring station of Place Victor Basch, Paris (Concawe 2006)

Subsequently, benzene concentrations in urban areas decreased. For example, the benzene concentrations at a traffic impacted monitoring station in Paris reduced from 31 µg/m^3 (0.0095 ppm) in 1994 to 5.6 µg/m^3 (0.0017 ppm) in 2005 (Concawe 2006, see Figure 2).

Monitoring stations in Rome, Italy, showed average benzene concentrations of 13.1±3.9 µg/m^3 (maximum 24.8 µg/m^3) in 1998 to 1999 (Crebelli *et al* 2001).

In 2003, the population of some European cities in Italy (Milan and Catania), Greece (Athens and Thessaloniki) and Cyprus (Nicosia) was still exposed to benzene concentrations higher than 5 µg/m^3. The personal exposure to benzene has ranged from 2.0 µg/m^3 (Helsinki) to 9.4 µg/m^3 (Thessaloniki; Bruinen de Bruin *et al* 2008).

The annual report from the department of Environment, Food and Rural affairs in the UK shows the modelled annual mean background concentrations of benzene in 2015 in the UK. Modelled background concentrations were below 0.5 µg/m^3 over most of the UK, with marginally higher concentrations for most urban areas. A few small areas had concentrations in excess of 1 µg/m^3. However, background concentrations everywhere are well below the limit value of 5 µg/m^3 for benzene (DEFRA 2015).

Benzene exposures from tobacco smoking

An overarching consideration for both occupational and general population sources of exposure to benzene exposure is tobacco smoking. Benzene concentrations can be 10 to 20 times higher in exhaled breath of cigarette smokers than in non-smokers. For cigarette smokers, smoking accounts for about 90% of this group's exposure to benzene. For non-smokers, environmental tobacco smoke, depending upon lifestyle and local restrictions on smoking, can be a significant source of benzene exposure (see Arnold *et al* 2013).

Following the EU Council Recommendation on smoke-free environment in 2009, indoor exposure to benzene from cigarette smoke should have been reduced.

Benzene concentrations in the air (personal sampling) in a general urban and sub-urban population were measured with 6 µg/m^3 for non-smokers (range 6-11 µg/m^3) and 10 µg/m^3 (range 9-15 µg/m^3) for smokers (Campagna *et al* 2012).

In 2007, when smoking in public was still allowed in Germany, median benzene concentrations were measured in restaurants or cafes with 8.9 µg/m^3 (maximum 22.5 µg/m^3), in bars with 8.1 µg/m^3 (maximum 64 µg/m^3) and in discotheques with 19.7 µg/m^3 (maximum 49.5 µg/m^3; Bolte *et al* 2008).

Summary and conclusion

The air quality limit value of 5 µg/m^3 for benzene has not been reached in all the urban air areas in Europe. Tobacco smoking is a major source of benzene. The median airborne benzene concentrations ranged from 8 to 20 µg/m^3 in restaurants, bars and discotheques when smoking in public was allowed in Germany.

6. Monitoring Exposure

6.1 Biomonitoring Exposure

The metabolism of benzene is described in section 7.1.1. In summary, in the first step benzene is oxidized to benzene oxide mainly by cytochrome P450 2E1 (CYP2E1). Several pathways are involved in the metabolism of benzene oxide:

- Benzene oxide can undergo conjugation with glutathione (GSH), resulting in the formation and urinary excretion of S-phenylmercapturic acid (SPMA).
- Benzene oxide may be further metabolized to benzene dihydrodiol and catechol.
- Benzene oxide spontaneously rearranges to phenol, which subsequently undergoes either conjugation (glucuronic acid or sulfate) or oxidation. The oxidation reaction gives rise to 1,4-hydroquinone, 1,2-hydroquinone (catechol) and 1,2,4-benzene triol.

- Benzene oxide equilibrates spontaneously with the corresponding oxepine valence tautomer, which can lead to ring opening to yield a reactive alpha,beta-unsaturated aldehyde, *trans,trans*-muconaldehyde, further aldehyde metabolites and finally *trans,trans*-muconic acid (ttMA) which is eliminated in the urine.

Several biomarkers of benzene exposure have been investigated. These include benzene levels in blood, urine and expired air. In addition, benzene metabolites in urine and biological adducts of benzene have been used as biomarkers of exposure. The following approaches have been evaluated (DECOS 2014):

- benzene in blood, urine and expired air;
- S-phenylmercapturic acid (SPMA) in urine;
- *t*,*t*-muconic acid (ttMA) in urine;
- phenol in urine;
- catechol and hydroquinone in urine; and
- DNA and protein adducts in blood.

For all approaches analytical methods are available which are sufficiently sensitive (Arnold *et al* 2013).

However, for low benzene concentrations (<1 ppm), benzene and SPMA in urine seems to be the most reliable biomarkers.

6.1.1 Biomonitoring

Benzene in biological matrices

Benzene in blood, urine or expired breath unequivocally indicates the uptake of benzene. However, because of the short half-life of benzene, its concentrations in these biological matrices reflect only recent exposure. Under identical conditions of exposure and because of the lipophilic properties of benzene, its concentration in blood is higher than in urine or expired breath. Therefore, benzene blood levels are diagnostically the most sensitive of these three measures and enable an assessment of background exposure among different population groups. Benzene levels in urine are an alternative to benzene blood levels. Measuring benzene in urine, however, is hampered by possible contamination (Arnold *et al* 2013).

Benzene in expired breath has not proven to be a reliable biomarker for assessing benzene exposure (Arnold *et al* 2013).

Benzene in blood

The half-life of benzene in blood was determined to be 8 hours (Brugnone *et al* 1992). Due to this short half-life, blood sampling has to be performed at the end of exposure and sampling and storage require specific techniques (SCOEL 1991, addendum 2006). In addition, blood sampling requires invasive collection methods. Furthermore, the amount of blood that can be obtained and the frequency of sampling is limited (Arnold *et al* 2013). As a result, benzene in blood is infrequently used as a biomarker.

Background exposure

Benzene in blood of the general population reflects mainly environmental benzene exposures from combustion processes and from tobacco smoke. Brugnone *et al* (1999) measured benzene concentrations in the blood of 171 non-smoking persons with 0.123±0.074 µg/L (range 0.015-0.462 µg/L) and in 72 smoking persons with 0.264±0.178 µg/L (range 0.028-0.940 µg/L). Brugnone *et al* (1992) reported differencess in blood benzene concentrations between rural population (0.200±0.176 µg/L; range 0.007-1.003 µg/L) and urban population (0.296±0.270 µg/L; range 0.007-2.241 µg/L).

Occupational exposure

Brugnone *et al* (1992) measured benzene concentrations in the blood of 114 non-smoking rural workers with 0.180±0.155 µg/L (range 0.007-0.840 µg/L) and in 179 non-smoking urban workers with 0.221±0.167 µg/L (range 0.007-0.924 µg/L). Those concentrations did not differ from that of the general population.

Benzene in urine

Benzene levels in urine are measured as an alternative to benzene blood levels (Arnold *et al* 2013). Reliable data on the half-live of benzene in urine have not been found; however, a short half-life can be expected. Furthermore, due to the volatility of benzene, evaporation during urine sampling and handling may occur. In addition, measuring benzene in urine is hampered by possible contamination (Arnold *et al* 2013). Hence, appropriate sampling and storage of urine samples is required.

Background exposure

Arnold *et al* (2013) reported urinary benzene concentrations for the non-smoking general population with 0.10 to 0.25 µg/L and for smokers with 0.20 to 0.80 µg/L.

The 95th percentile for benzene in urine of 86 non-smoking persons from the general population in a metropolitan area (Cagliari, Sardinia, Italy) was determined with 0.312 µg benzene/L urine (Campagna *et al* 2014). For smokers, median benzene concentrations in urine were reported with 0.819 µg/L (maximum 2.024 µg/L) (Campagna *et al* 2012).

DFG (2017a) derived a reference value (BAR; 95 percentile for general non-smoking population) for benzene in urine with 0.3 µg/L urine mainly based on the data from Campagna *et al* (2014).

Occupational exposure

Table 35 in Appendix 1 lists studies in which benzene in urine was measured in Chinese shoe factory workers (Ji *et a*l 2012; Lan *et al* 2004; Marchetti *et al* 2012; Xing *et al* 2010; Zhang *et al* 2012) and in other workers exposed to benzene concentrations below 10 ppm (Campagna *et al* 2012; Campo *et al* 2016; Fustinoni *et al* 2005, 2011; Hopf *et al* 2012; Lagorio *et al* 1998; Lovreglio *et al* 2014; Manini *et al* 2006, 2008; Ong *et al* 1996; Violante *et al* 2003).

Table 8 below shows the correlation between benzene concentrations in the air and benzene concentrations in urine as published by DFG (2017a, b) which is based for the low concentration range (0.03 and 0.06 ppm) on the studies by Campagna *et al* (2012), Fustinoni *et al* (2005), and Manini *et al* (2008). For higher concentrations, DFG calculated the benzene concentrations in urine based on correlations of benzene in urine and ttMA and SPMA in urine.

Table 8: Correlation between benzene concentration in the air and benzene concentration in urine (DFG 2017a, b)

Benzene in air		Benzene in urine
mL/m³ (ppm)	mg/m³	µg/L
		0.3 (95 percentile for general population)*
0.03	0.1	0.5*
0.06	0.2	0.8*
0.15	0.5	1.5
0.3	1.0	2.75
0.6	2.0	5.0
1.0	3.3	7.5
2.0	6.5	12.5

* For non-smokers

Conclusion

Benzene in urine is a suitable biomarker for monitoring benzene exposure as low as 0.03 ppm (0.1 mg/m³) and above. The 95 percentile for the general non-smoking population is 0.3 µg benzene /L urine. 0.1 ppm benzene (0.33 mg/m³) correlates to about 1 µg benzene/L urine and 0.05 ppm benzene (0.16 mg/m³) to about 0.7 µg benzene/L urine. To monitor benzene exposures below 0.1 ppm (0.33 mg/m³) smoking habits need to be taken into consideration.

Applying the correlation between benzene in urine and benzene in air to values of benzene in urine observed in smokers without occupational benzene exposure indicates that smokers attain an internal dose corresponding to air borne concentration of roughly 0.06 ppm with maximum values corresponding to about 0.2 ppm.

S-phenylmercapturic acid (SPMA)

S-Phenylmercapturic acid (SPMA), is formed at amounts of about 0.1% during the metabolism of benzene (SCOEL 1991, addendum 2006). There is significant inter-individual formation of this metabolite ranging from 0.05 to 0.3% (Qu *et al* 2003a). SPMA derives from the condensation of benzene oxide with glutathione. SPMA is generally considered as a specific urinary biomarker of benzene. The mean half-life of SPMA ranges from 9 to 13 hours; a second phase of slow elimination has an estimated half-life of about 45 hours. Since accumulation of SPMA is not likely, SPMA should be considered as a biomarker of recent exposure (ca. 24 hours), but does not reflect mid- and long-term exposure to benzene. The drawback of using SPMA as a biomarker is that it is a metabolic detoxification product and is not involved in benzene toxicity; therefore, its use for anything other than evaluating potential exposure is limited (Arnold *et al* 2013).

Using SPMA as a biomarker at low concentrations has the benefit, compared to benzene, that there are no problems with respect to contamination or loss of material due to volatility. Also some authors found SPMA to be a more sensitive parameter than benzene in urine (Lovreglio *et al* 2017).

It is to be noted that in urine a precursor of SPMA exists that can turn into SPMA by acid treatment of the urine sample. The amount of measured SPMA in urine depends on the degree of hydrolysis and is therefore a function both of the urine pH and of the storage conditions of the sample. The average SPMA in pH 2 samples is 45% to 60% of the total, while free SPMA varies from 1% to 66% (Paci *et al* 2007). Sterz et al (2010) confirmed

that pre-treatment of urine with HCl to adjust the pH to 0.5–1 is essential for complete conversion of pre-SPMA to SPMA.

Furthermore, sufficiently sensitive chromatographic methods to detect SPMA in urine, especially at low concentrations, are required. Fustinoni *et al* (2011) recommend the use of LC/MS/MS. Methods based on immunoassay techniques have been developed which are useful mainly for screening purposes. However, there is the possibility of interference by other chemically related compounds that are present in urine (Maestri *et al* 2005).

The correlation and the reference value derived by DFG (2017a, b), on which the proposed biomonitoring values for SPMA are based, were derived from data using acidification of the urine sample and sensitive chromatographic methods. Therefore, only SPMA measuments that fulfill those criteria can be compared to the proposed biomonitoring values.

Background exposure

In a most recent publication, the mean level of SPMA in the urine of a general population in Italy occupationally not exposed to benzene was about 0.23±0.30 µg SPMA/g creatinine for non-smokers and about 2.07±2.46 µg/g creatinine for smokers. Limit of detection was indicated with 0.026 µg SPMA/L urine (Tranfo *et al* 2017).

DFG (2017a) has derived the reference value (BAR; 95 percentile for the non-smoking general population) for SPMA with 0.5 µg/g creatinine based on data from Schettgen *et al* (2008, 2010) and Scherer *et al* (2007). Schettgen *et al* (2008) found a 95 percentile of 0.29 µg SPMA/g creatinine (n=56 non-smokers), Schettgen *et al* (2010) of 0.31 µg SPMA/g creatinine (n=43 non-smokers), and Scherer *et al* (2007) of 0.5 µg SPMA in urine collected within 24 hours (n=100 non-smokers), which is about 0.3 to 0.5 µg SPMA/g creatinine considering a creatinine excretion of 1.0-1.6 g /24 hours.

Occupational exposure to benzene

Qu *et al* (2003a) mentioned that SPMA formation correlates well with personal benzene exposures across a broad range of exposures from 0.06 ppm to 122 ppm. Based on information from a European biomonitoring study investigating occupationally exposed groups in Italy (78 gasoline filling station attendants, 77 urban policemen, 153 bus drivers) and in Bulgaria (158 petrochemical workers) and controls, SPMA has been shown to correlate with benzene concentrations in the air at benzene levels of 0.1 ppm and higher (Farmer *et al* 2005).

Table 36 in Appendix 1 lists studies in which SPMA in urine was measured in workers exposed to benzene concentrations below 10 ppm (Carrieri *et al* 2010, 2012; Lovreglio *et al* 2014; Lv *et al* 2014; Manini *et al* 2008; Mansi *et al* 2012; Marcon *et al* 1999; Rekhadevi *et al* 2011). It is to be noted that several studies were not considered because either SPMA was measured with methods other than HPLC/MS (Fracasso *et al* 2010; Fustinoni *et al* 2005; Seow *et al* 2012) or it was not explicitly described that an acid treatment of the urine sample was performed before quantification (Angelini *et al* 2011; Campagna *et al* 2012; Campo *et al* 2016; Crebelli *et al* 2001; Fustinoni *et al* 2011; Li *et al* 2017; Maestri *et al* 2005) or because the finding could not be reproduced in a later investigation (Manini *et al* 2006).

Table 9 below shows the correlation between benzene concentrations in the air and SPMA concentrations in urine as published by DFG (2017a, b) which is based for the low concentration range (0.03 and 0.06 ppm) on the studies by Angelini *et al* (2011), Carrieri *et al* (2010), Manini *et al* (2008), and Mansi *et al* (2012). In the higher concentration range of 0.15 ppm and above, the correlation as published by van Sittert *et al* (1993) has been applied.

Table 9: Correlation between benzene in air and SPMA excretion in urine (DFG 2017a, b)

Benzene in air		S-Phenylmercapturic acid (SPMA) in urine
mL/m³ (ppm)	mg/m³	µg/g creatinine
		0.5 (95 percentile for general population)*
0.03	0.1	1.5*
0.06	0.2	2.5*
0.15	0.5	5
0.3	1.0	12
0.6	2.0	25
1.0	3.3	45
2.0	6.5	90

* For non-smokers

Conclusion

SPMA is a specific biomonitoring marker for benzene exposure of 0.03 ppm (0.1 mg/m³) and higher using appropriate techniques. The 95 percentile for the non-smoking general population is 0.5 µg SPMA/g creatinine. 0.1 ppm benzene (0.33 mg/m³) correlates to about 4 µg SPMA/g creatinine and 0.05 ppm benzene (0.16 mg/m³) to about 2 µg SPMA/g creatinine. To monitor benzene exposures below 0.1 ppm (0.33 mg/m³) smoking habits need to be taken into consideration.

Applying the correlation between SPMA in urine and benzene in air to values of SPMA in urine observed in smokers without occupational benzene exposure indicates that smokers attain an internal dose corresponding to air borne concentration of roughly 0.05 ppm with a standard deviation of about 0.05 ppm.

t,t-muconic acid (ttMA)

trans,trans-muconic acid (ttMA) is the oxidized product of *trans,trans*-mucondialdehyde, which results from the oxidative ring opening of benzene. The excreted amount of ttMA (2–25% of the total benzene uptake) in urine shows an inverse dose relationship (i.e. the higher the dose of benzene, the lower the relative excreted amount of ttMA). The half-life of ttMA is estimated to be 5.1±2.3 hours (Arnold *et al* 2013; Boogaard and van Sittert 1995, 1996).

Human genetic factors, primarily polymorphisms in benzene metabolizing enzymes, can influence the levels of ttMA excreted in urine. Furthermore, in case of occupational co-exposure to toluene, ttMA urinary levels are suppressed.

Background exposure

ttMA is also a metabolite of sorbic acid and sorbates can be present in various food at concentrations up to 800 mg/kg (Arnold *et al* 2013). Weaver *et al* (2000) identified that in volunteers who consumed two sorbic acid-preserved foods, a large increases in ttMA concentrations were observed with individual peaks ranging as high as 705 µg/g creatinine.

Also smoking habits significantly influence ttMA levels. Smokers had 1.4 to 4.8 times higher urinary ttMA concentrations than non-smokers. The mean or median urinary ttMA concentrations range from 30 to 300 µg/g creatinine among non-occupationally benzene exposed populations (Arnold *et al* 2013). In a more recent publication, ttMA concentration in the general population was identified with 85±108 µg/g creatinine in 336 non-smokers and with 144±137 µg/g creatinine in smokers (Tranfo *et al* 2017).

DFG has derived a reference value (BAR; 95 percentile for general population) of 150 µg ttMA/g creatinine (DFG 2017a) based on data from Schettgen *et al* (2010), Scherer *et al* (2007), and Aprea *et al* (2008). Aprea *et al* (2008) reported for 264 non-smokers a 95 percentile for the elimination of ttMA in urine of 143 µg/g greatinine, Schettgen *et al* (2010) for 33 non-smokers a 95 percentile of 135 µg ttMA/g creatinine, and Scherer *et al* (2007) for 100 non-smokers a 90 percentile of 228 µg ttMA in urine collected within 24 hours, which is about 143 to 228 µg ttMA/g creatinine considering a creatinine excretion of 1.0 to 1.6 g/24 hours.

Occupational exposure to benzene

Table 37 in Appendix 1 lists studies in which ttMA was measured in the urine of workers exposed to benzene concentrations below 0.5 ppm (Campagna *et al* 2012; Carrieri *et al* 2010; Ciarrocca *et al* 2012a, b; Fracasso *et al* 2010; Fustinoni *et al* 2005; Mansi *et al* 2012; Manini *et al* 2006, 2008). The results of those studies confirm that all measured ttMA values are below 150 µg/g creatinine and are therefore within the concentration found in the urine of the general population.

Table 10 below shows the correlation between benzene concentrations in the air and ttMA concentrations in urine as published by DFG (2017a, b) which is based on the same studies as listed in Table 37.

Table 10: Correlation between benzene in the air and ttMA excretion in urine (DFG 2017a, b)

Benzene in air		**tt-Muconic acid in urine**
mL/m^3 (ppm)	mg/m^3	µg/g creatinine
		150 (95 percentile for general population)
0.03	0.1	-
0.06	0.2	-
0.15	0.5	-
0.3	1.0	300
0.6	2.0	500
1.0	3.3	750
2.0	6.5	1200

Conclusion

To monitor occupational benzene exposure, ttMA is used as a biomarker for benzene air concentrations of greater than 0.3 ppm which corresponds to about 300 µg ttMA/g creatinine. The 95 percentile for the non-smoking general population is 150 µg ttMA/g creatinine. Dietary uptake of sorbic acid contributes to ttMA excretion and could be as high as 700 µg/g creatinine. Hence this parameter cannot be used to monitor benzene exposures in air of 0.1 ppm or below.

Phenol, catechol and hydroquinone

In humans, phenol is the primary metabolite of benzene excreted in the urine accounting for 70–88% of the total urinary metabolites. Both catechol and hydroquinone are formed by enzymatic hydroxylation of the intermediate phenol. Catechol is also generated from benzene dihydrodiol. Phenol and its metabolites are conjugated with either sulphate or glucuronic acid. Elimination half-lives have been estimated to be around 13, 15 and 16 hours for hydroquinone, catechol and phenol, respectively (Arnold *et al* 2013).

Phenol has a number of non-benzene sources that confound the interpretation of air benzene exposure up to a concentration of approximately 5 ppm. Phenol is detected in cigarette smoke and over the counter medicines have been shown to increase phenol excretion in the urine up to 40-fold. Catechol and hydroquinone are present in many foodstuffs and are also formed in the human metabolism of amino acids. Hydroquinone occurs naturally in plants as a glucose conjugate, arbutin. Therefore, the base-line excretion of these substances in urine of unexposed persons is relatively high. Furthermore, considerable human exposure to these substances can also result from cigarette smoking.

Conclusion

Phenol, hydroquinone and catechol are not useful biomarkers for the low benzene exposure range (Arnold *et al* 2013).

DNA and protein adducts

It is well established that benzene is metabolized to reactive intermediates that are able to covalently bind to nucleophilic sites of cellular macromolecules including nucleic acids in DNA. Benzene metabolites that bind to DNA are benzene oxide, benzoquinones, hydroquinone and muconaldehyde.

To date, DNA adducts of benzene metabolites cannot be used as biomarkers mainly due to the lack of sensitive and specific analytical methods to measure such adducts. In contrast, adducts of benzene oxide with haemoglobin or plasma proteins, regarded as surrogates of the DNA adducts, are potential markers of exposure. Adducts of benzene metabolites other than benzene oxide are diagnostically unspecific. The correlations between air benzene concentrations and blood adduct levels of benzene oxide in investigations of benzene exposed workers and controls suggest that haemoglobin adducts might be diagnostically less sensitive than adducts of plasma proteins, and support the use of protein adducts of benzene oxide as biomarkers. In addition, serum albumin adducts have a relatively long half-life (about 21 days) compared to benzene in blood or urine and SPMA in urine. Unfortunately, the analytical methods for the determination of haemoglobin and plasma protein adducts are not sensitive enough to monitor environmental exposures. Moreover, reproducibility and reliability data of these analytical methods only exist for one laboratory.

Conclusion

Taken together, these considerations suggest that the determination of haemoglobin and protein adducts of benzene is not viable for routine use in environmental medicine or as exposure biomarkers (Arnold *et al* 2013).

6.1.2 Recommendation with regard to biomonitoring

Benzene and SPMA in urine are suitable biomarkers for benzene exposure in the air of 0.03 ppm and above. 0.1 ppm benzene in the air corresponds to biological limit values (BLV) of about 1 µg benzene/L urine and 4 µg SPMA/g creatinine, 0.05 ppm benzene in the air to BLVs of about 0.7 µg benzene/L urine and 2 µg SPMA/g creatinine.

ttMA cannot be recommended as a reliable biomarker for benzene exposures below 1 ppm due to possible dietary contributions.

The 95 percentiles in the non-smoking general population can be used to set biological guidance values(BGV) as follows:

- 0.3 µg benzene/L urine
- 0.5 µg SPMA/g creatinine
- [150 µg ttMA/g creatinine]

6.2 Monitoring methods

Benzene in air

There are several methods that allow the determination of benzene in air even in low concentrations including concentrations below any proposed limit value. Air sampling can be performed by passing air actively through a sorbent tube or by using diffusive sampling with badge or sorbent tube. The retained benzene is then extracted for analysis by either thermal desorption or desorption on CS_2 (depending on the sorbent tube used) followed by analysis via gas chromatography with different detectors. The table below shows some of the available validated methods for measurement of benzene in air. The methods included in the table have validation data that show compliance with the requirements of the standard EN 482 “Workplace exposure. General requirements for the performance of procedures for the measurement of chemical agents” or potential to meet these requirements for the proposed OEL. Validation data can be consulted in the “methods sheets” provided by the Gestis – Analytical methods database available at: (http://www.dguv.de/ifa/gestis/gestis-analysenverfahren-fuer-chemische-stoffe/index-2.jsp) and/or in the actual analytical method. The calculations of the LOQ take into account the sampling times recommended in the method. However, for concentrations in the range of the limit value the sampling time could be further extended if the duration of the activity allow it.

Table 11: Methods measuring benzene in the air

Standardized method	Analytical technique	LOQ; sampling volume; sampling time	Reference
IFA 6265 Methods	Thermal desorption; GC-MS	Thermal desortion tube: 0.002 mg/m^3 (0.0006 ppm); 2 L; 1 hour	IFA (2013)
ISO 16000-6:2011 ISO 16017-1 and 2[2)]	Thermal desorption; GC-MS or MS-FID	Thermal desortion tube: 0.004 mg/m^3 (0.001 ppm); 2 hours; active sampling 0.020 mg/m^3 (0.006 ppm); 8 hours; passive sampling	Finland Ministry of Social Affairs and Health 2016
OSHA Method 1005	Desorption with CS_2; GC-FID	Sorbent tube: 0.01 mg/m^3 (0.003 ppm); 12 L; 4 hours Passive samplers: 0.011-0.014 mg/m^3 (0.003 – 0.004 ppm); 4 hours	OSHA 2002
MétroPol M40	Desorption with CS_2; GC-FID	Sorbent tube: 0.02-1.7 mg/m^3 (0.006-0.5 ppm) 96 L; 8 hours	INRS 2017

Standardized method	Analytical technique	LOQ; sampling volume; sampling time	Reference
DFG Solvent mixtures, Method No. 1	Desorption with CS_2; GC-FID	Sorbent tube. 0.05 mg/m^3 (0.015 ppm); 25 L; 8 hours	DFG (2014)

Abbreviations: GC: gas chromatography; MS: mass spectrometry; FID: flame ionization detector

Benzene in blood and urine

A critical point for the measurement of benzene in blood and urine is its short half-life and its high volatility. Therefore, sampling should be performed at the end of exposure or end of shift and the samples should be kept cold and hermetically sealed.

Arnold *et al* (2013) reviewed the analytical methods for benzene. For the determination of benzene in blood, urine and expired air, the analytical techniques are able to detect concentrations in the low part-per-trillion (ng/L) range. For the analysis of blood and urine, dynamic headspace (purge and trap) is generally the technique of choice. For enrichment purposes, the analyte (i.e. benzene) is trapped on solid phases like Tenax or charcoal which, in most cases, are cooled. Thereafter, desorption takes place at higher temperature, and the analyte is transferred to a capillary column for gas chromatographic separation. Similarly, for the analysis of expired air, benzene is enriched on a solid phase material and transferred to a capillary column by elevating the temperature of the sorbent. Flame ionization or mass spectrometry (MS) can be used for the detection and quantification of benzene. In recent years, extraction techniques other than purge and trap, such as solid phase micro-extraction, have been used for the determination of benzene and other volatile aromatic hydrocarbons in blood. These data demonstrate that very sensitive analytical methods exist to measure trace levels of benzene in blood, expired air or urine and that the analytical results are comparable among suitably equipped and highly skilled laboratories in various countries. Nevertheless, no ‘‘standardized’’ analytical methods exist. Furthermore, there is only one external quality assessment scheme applicable to benzene in blood (DFG 1993); proficiency testing, which assesses the accuracy of laboratories in conducting a particular measurement is not available for the determination of benzene in expired air and in urine (Arnold *et al* 2013).

Lovreglio *et al* (2017) considered that measurement of benzene in urine is less sensitive than measurement of SPMA in urine.

In the studies reviewed measuring benzene in urine as biomarker (see Table 35), determination of benzene was performed by solid-phase microrextraction (SPME) followed gas chromatography and mass spectrometry (GC-MS; see Table 12 below).

Table 12: Methods to determine benzene in urine

Standardized method	Sample preparation	Analytical technique	LOD / LOQ (µg/L)	Reference
	solid-phase microrextraction	GC-MS	LOD: 0.005-0.01	Andreoli *et al* 1999
	solid-phase microrextraction	GC-MS	LOQ: 0.015	Fustinoni *et al* 1999, 2010a;
	solid-phase microrextraction	GC-MS/ion trap detection method	LOQ: 0.078 (1 nmol/L)	Bråtveit *et al* 2007; Kirkeleit *et al* 2006;

Abbreviations: GC: gas chromatography; LOD: limit of detection; LOQ: limit of quantification; MS: mass spectrometry;

S-phenylmercapturic acid (SPMA)

Arnold *et al* (2013) reviewed the analytical methods for SPMA. Several analytical methods for the determination of SPMA in urine exist. Extraction of SPMA from the urine matrix can be accomplished by liquid–liquid extraction (LLE) with ethyl acetate or by solid phase extraction (SPE). Then, after derivatization (methylation, butylation or silylation), SPMA can be detected by GC-MS with LOD generally in the range 1 to 5 mg/L. A highly sensitive method (LOD ≈60 ng/L) using electron-capture detection after derivatization with pentafluorobenzylbromide has also been reported (Einig *et al* 1996). A standardized GC/MS approach for the determination of urinary SPMA was published in Analyses of Hazardous Substances in Biological Materials by the DFG (DFG 1995c). Here, SPMA is methylated after extraction with ethyl acetate and subsequently detected by GC coupled to high-resolution MS; the LOD was 1 mg/L. In addition to the GC approach, several high-performance liquid chromatography (HPLC) methods in combination with ultraviolet (UV) absorption detection, diode array detection, fluorescence detection and MS or tandem MS have been developed and successfully applied. In many cases, SPE is used for pre-concentrating SPMA from the urine; some methods are designed to determine SPMA and other benzene metabolites in one run. The LODs are often below 1 mg/L, although the most sensitive methods reached LODs of ≤0.2 mg/L. Besides analytical methods, SPMA can also be measured using a sensitive (LOD¼ 0.2 mg/L) competitive enzyme-linked immunosorbent assay (Arnold *et al* 2013).

A critical point in the determination of urinary SPMA is the conversion of pre-SPMA to SPMA under acidic conditions. Therefore, the amount of measured SPMA may change as a function both of pH and of storage conditions of the urine specimens. Previous hydrolysis procedure can increase SPMA urinary concentrations (Arnold *et al* 2013). Sterz et al (2010) reported that complete conversion was found upon treatment of urine with HCl (37%) at pH 1.1.

It is to be noted that the correlation between benzene concentration in the air and SPMA concentration in urine as published by DFG (2017a, b) is based on acidification of the urine sample and an appropriate analytical method (DFG 1995c). Hence, this correlation cannot be applied for results in which an enzyme-linked immunosorbent assay was used or for results in which the urine sample was not acidified.

In the studies reviewed measuring SPMA in urine as biomarker (Table 36), determination of SPMA was performed either by liquid extraction or by solid-phase extraction followed gas chromatography and mass spectrometry or by (high pressure) liquid chromatography and tandem mass spectrometry. In Table 13 below some of the used methods are listed.

Table 13: Methods to determine S-phenylmercapturic acid (SPMA) in urine

Standard method	Sample preparation	Analytical technique	LOD / LOQ (µg/L)	Reference
	Acidification (HCl); solid phase extraction	HPLC-MS/MS	LOD: 0.026 LOQ: 0.078	Tranfo *et al* 2017
	Acidification (HCl); solid phase extraction	HPLC-MS/MS	LOD: 0.03 LOQ: 0.09	Sterz *et al* 2010
	Acidification (H_2SO_4); solid phase extraction	HPLC-MS/MS	LOD: 0.05 LOQ: 0.1	Paci *et al* 2007
	Acidification (formic acid); solid phase extraction	LC-MS/MS	LOD: 0.1	Manini *et al* 2008
	Acidification ('Parma laboratory'); solid phase extraction	HPLC-MS/MS	LOQ: 0.1	Fustinoni *et al* 2010b
NMAM 8326	solid phase extraction	HPLC-MS/MS	LOD: 0.2 ; 0.5 (lowest standard level)	US NIOSH 2014
	solid phase extraction	HPLC-MS/MS	LOD: 0.20	Sabatini *et al* 2008
	Acidification (HCl); solid phase extraction; derivatisation	HPLC, fluorimetric detector	LOD: 0.22 LOQ: 0.68	Mendes *et al* 2017
DFG method	Acidification (HCl); liquid extraction; derivatisation	GC-MS	LOD: 1	DFG 1995c
	Acidification (HCl); liquid extractions	HPLC-MS	LOD: 10	Lv *et al* 2014

Abbreviations: HPLC: high-performance liquid chromatography; GC: gas chromatography; LOD: limit of detection; LOQ: limit of quantification; MS: mass spectrometry; MS/MS: tandem mass spectrometry;

t,t-muconic acid (ttMA)

Arnold *et al* (2013) reviewed the analytical methods for ttMA. For determination of low levels of ttMA, most analytical methods are based on either GC/MS or HPLC/UV detection. Drawbacks of the HPLC/UV methods may be the non-specific detection and the resulting need for a precise chromatographic separation. On the other hand, a limitation of the GC/MS methods is the need for derivatization procedures, which can be an additional source of error. Recently, capillary electrophoresis and LC/MS techniques have been used. The sample preparation techniques include mainly liquid-liquid extraction (LLE) or solid phase extraction (SPE). For LLE, the urine is acidified and ttMA is extracted with an organic solvent (e.g. diethyl ether). Most extractions using SPE techniques rely on (strong) anion exchange sorbent materials. The LLE extract or SPE eluate is evaporated to dryness and reconstituted before derivatization (for GC) or adjusted to a defined volume prior to injection (for HPLC). The LODs range from 0.1 mg/L to 0.005 mg/L. ttMA is stable in urine over a period of 9 months if stored at <20°C in the dark. For application in environmental medicine, the use of the more specific GC/MS and HPLC/ MS/MS methods are advisable. A standardized method, mainly applicable to occupational settings, was published by the German Research Foundation (DFG 1995b). ttMA is separated from acidified urine by anion

exchange chromatography, followed by HPLC/UV detection (LOD¼ 0.1 mg/L) (Arnold *et al* 2013).

In the studies reviewed measuring ttMA in urine as biomarker, determination of ttMA was performed by solid-phase extraction followed by (high pressure) liquid chromatography and for very sensitive methods also followed by mass spectrometry. In Table 14 below some of the used methods are listed.

Table 14: Methods to determine t,t-muconic acid (ttMA) in urine

Standard method	Sample preparation	Analytical technique	LOD / LOQ (µg/L)	Reference
	solid phase extraction	LC-MS/MS	LOD: 0.1	Manini *et al* 2008
	solid phase extraction	HPLC-MS/MS	LOD: 0.55 LOQ: 1.68	Tranfo *et al* 2017
	solid phase extraction	HPLC-UV	LOD: 5	Campagna *et al* 2012
	solid phase extraction	HPLC-UV	LOD: 5-10	Aprea *et al* 2008
DFG method	solid phase extraction	HPLC-UV	LOD: 100	DFG 1995b
MTA/MB – 026/A06	solid phase extraction	HPLC-UV	LOD: 130	INSHT 2015

Abbreviations: HPLC: high-performance liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; MS: mass spectrometry; MS/MS: tandem mass spectrometry;

Summary

For the measurement of airborne benzene well established methods are available that detect benzene in concentrations below 0.01 ppm (0.03 mg/m^3). Analytical methods are available to determine benzene and its metabolites SPMA and ttMA in urine.

Several biomonitoring parameters are available to measure benzene or its metabolites in the body.

- Benzene in blood is usually not used as a biomonitoring parameter.
- Benzene in urine is a suitable biomonitoring parameter for which sensitive analytical methods are available. The reference value (95 percentile for the non-smoking general population) is 0.3 µg benzene/L urine. 0.7 µg benzene/L urine corresponds to about 0.05 ppm benzene in the air (0.16 mg/m^3).
- S-phenylmercapturic acid (SPMA) in urine is a suitable biomonitoring parameter for which sensitive analytical methods are available. However, for reliable results that can be correlated with benzene exposure in the air, acidification of the urine sample is required and a detection with appropriate chromatographic methods like LC/MS/MS. The reference value is 0.5 µg SPMA/g creatinine. A concentration of 2 µg SPMA/g creatinine corresponds to about 0.05 ppm benzene in the air (0.16 mg/m^3).
- *trans,trans*-muconic acid (ttMA) in urine is a biomonitoring parameter for which sensitive methods are available. However, because sorbic acid consumption with the diet contributes to ttMA excretion, ttMA is suitable only for benzene exposures higher than 0.5 ppm. The reference value is 150 µg ttMA/g creatinine.

7. Health Effects

Benzene is a data-rich substance and many assessments of its toxicity are available; e.g.: AGS (2012), ANSES (2014), ATSDR (2007, 2015), Concawe (1999, 2002, 2006, 2012), DECOS (2014), EPA (1998), EU RAR (2008), IARC (2004, 2012).

Data on the toxicity of benzene in experimental animals are summarized in detail in ATSDR (2007) and in the EU Risk Assessment Report (EU RAR 2008). The current report focusses mainly on human data published since the year 2000.

7.1 Toxicokinetics (Absorption, distribution, metabolism and excretion-ADME)

7.1.1 Human data

Absorption

Benzene is readily absorbed by all physiological routes (inhalation, dermal and oral), of which inhalation is the most important for occupational exposure. Mean inhalation absorption has been reported in humans ranging from approximately 50 to 80% (DECOS 2014).

Dermal absorption of **benzene vapour** is possible; however, the uptake is small compared to the uptake via inhalation (Rauma *et al* 2013).

Liquid benzene can be absorbed through human skin, although this is not as substantial as absorption following inhalation or oral exposure. Under normal conditions the contribution of the dermal component to the total uptake may be low, as evaporation from the skin surface will decrease the dermally absorbed amount. However, the dermal route can be an important contributor to total benzene exposure in certain situations, such as immersion of the skin in solution or when the airborne concentration of benzene is very low (Williams *et al* 2011).

Jakasa *et al* (2015) calculated the dermal uptake with 5.85% at an OEL of 1 ppm (3.2 mg/m^3). Williams *et al* (2011) analysed the experimental skin absorption data of benzene (both human and animal; *in vitro* and *in vivo*), and concluded that the steady state absorption rate of benzene ranges from 200 to 400 µg/cm^2*h (DECOS 2014). Considering an OEL of 0.05 ppm (0.16 mg/m^3) this value exceeds by far the critical absorption value (CAV) calculated according to the ECETOC methodology (1998) of 0.08 µg/cm^2*h (with (10 [m^3] x OEL [mg/m^3] x f x 0.1)/2,000 [cm^2], in which 10 m^3 is the human inhalation volume per 8-hour 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).

Kalnas and Teitelbaum (2000) found that for **solvents used for cleaning that contained benzene at concentrations of less than 0.1%**, the amount of benzene absorbed through the skin over a long period was significant, depending on exposure time and exposed skin surface areas.

Distribution

Upon absorption, benzene is distributed throughout the body. Benzene has been detected in various biological fluids and tissues of humans, the highest levels occur in lipid-rich tissues.

Benzene has also been shown to cross the human placenta, and has been found in the cord blood in amounts equal to or greater than those in maternal blood (DECOS 2014).

Metabolism

The metabolism of benzene is inherently complex and occurs principally in the liver and the lungs, with secondary metabolism occurring in the bone marrow (McHale *et al* 2012). It has been intensively investigated (see Figure 3 below) and reviewed.

The first step in the metabolism of benzene is its oxidation to benzene oxide by cytochrome P-450, mainly CYP2E1. This enzyme is mainly expressed in the liver (DECOS 2014). For inhalation exposure, the lung would be a major site of benzene metabolism (Chancy and Carlson 1995). Furthermore, since CYP2E1 is also expressed in the bone marrow of mice (Bernauer *et al* 1999) and in human bone marrow stem cells (Bernauer *et al* 2000) it can be assumed that benzene will also be metabolised directly in bone marrow stem cells to toxic metabolites.

Smith (2010) considers that CYP2E1 is the primary enzyme responsible for mammalian metabolism of benzene and that it is reasonable to assume that it is a low-affinity enzyme responsible for benzene metabolism mainly at higher levels of exposure. Smith (2010) further assumes that CYP2F1 and CYP2A13 are reasonable candidates for high-affinity metabolic enzymes, which are active at environmental levels of exposure below 1 ppm. However, there is lack of scientific evidence for such enzymes (Boogaard 2017).

Several pathways are involved in the metabolism of benzene oxide:

- Benzene oxide can undergo conjugation with glutathione (GSH), resulting in the eventual formation and urinary excretion of S-phenylmercapturic acid (SPMA) (Monks *et al* 2010). The responsible enzyme is glutathione-S-transferase (GST), specifically GSTT1 and GSTM1 for which relevant polymorphisms are reported (see below).
- Benzene oxide may be further metabolized by epoxide hydrolase (EH) to benzene dihydrodiol and catechol (Meek and Klauning 2010).
- Benzene oxide spontaneously rearranges to phenol, which subsequently undergoes either conjugation (glucuronic acid or sulfate) or oxidation. The oxidation reaction is catalyzed by CYP2E1 and gives rise to 1,4-hydroquinone, 1,2-hydroquinone (catechol) and further to 1,2,4-benzene triol (DECOS 2014; Monks *et al* 2010). The enzyme myeloperoxidase (MPO), which is most abundantly expressed in neutrophil granulocytes, a sub-type of white blood cells, metabolises the hydroquinones to their respective benzoquinones. Within this reaction, highly reactive oxygen species (ROS) are formed. In addition, those benzoquinones are very reactive. The conversion from benzoquinones back to the hydroquinones is catalysed by NAD(P)H:quinone oxidoreductase 1 (NQO1) which can lead to further redox cycling (Hartwig 2010). 1,4-Hydroquinone was demonstrated to be clastogenic and aneugenic *in vivo* and in addition mutagenic *in vitro* (see DECOS 2014).
- Benzene oxide equilibrates spontaneously with the corresponding oxepine valence tautomer, which can lead to ring opening to yield a series of six carbon dienes, the most reactive of which is the alpha,beta-unsaturated aldehyde, *trans,trans*-muconaldehyde (Monks *et al* 2010), further aldehyde metabolites (Meek and Klauning 2010) and finally *trans,trans*-muconic acid (ttMA) which is eliminated in the urine. *Trans,trans*-muconaldehyde is a highly reactive di-aldehyde demonstrated *in vitro* to lead to mutations (Nakayama *et al* 2004), DNA-protein crosslinks and DNA strand breaks (Amin and Witz 2001). It was also found to induce cross-linking of the gap junction protein connexin43, which seemed to be responsible for inhibition of gap junction intercellular communication (Rivedal *et al* 2010).

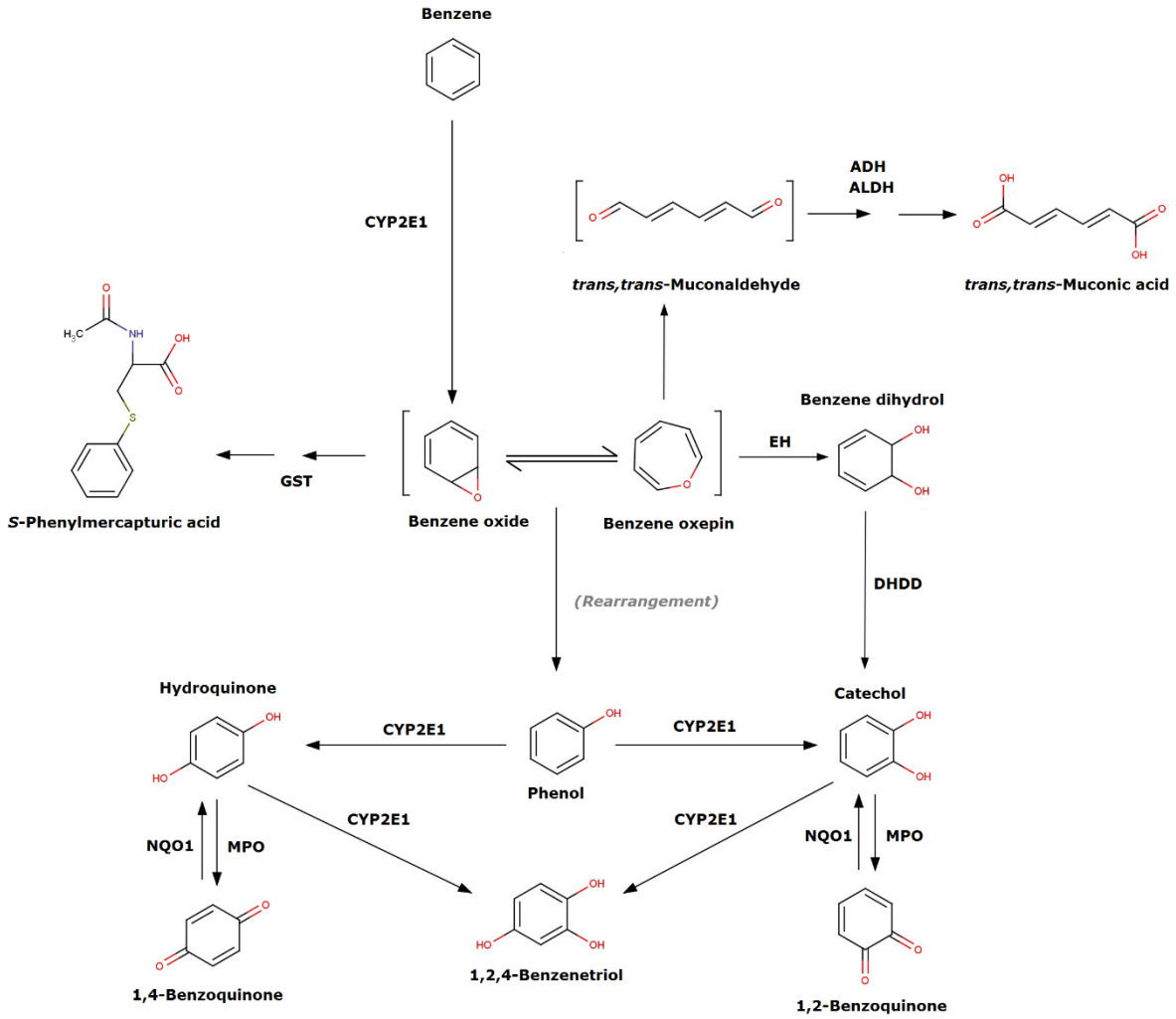

Figure 3: Metabolism of benzene (simplified).

Abbreviations: ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; CYP2E1: Cytochrome P-450 2E1; DHDD: dihydrodiol dehydrogenase; EH: epoxide hydrolase; GST: glutathione-S-transferase; MPO: myeloperoxidase; NQO1: NAD(P)H:quinone oxidoreductase

Elimination

Exhalation is the main route for excretion of unmetabolized benzene (ATSDR 2007). Most of the absorbed benzene however, is metabolised and the metabolites are excreted after phase-II-conjugation predominantly in the urine (DECOS 2014).

In IPCS (1999) it is reported that after inhalation exposure, benzene elimination in humans appears to follow a two compartment model, with half-lives of around 1 hour and 24 hours. The half-life of exhaled benzene in humans varies depending on the benzene exposure concentration and duration. Exposure to 99 ppm for 1 hour resulted in an initial phase half-life of 42 minutes, and exposure to 6.4 ppm for 8 hours resulted in an initial phase half-life of 72 minutes, with a terminal phase half-life (from 10 to 100 hours after exposure) of 23 to 31 hours.

Enzyme Polymorphism

There is evidence from the literature that polymorphic genes involved in benzene metabolism influence susceptibility to leukaemia. More than one type of GST and CYP2E1 polymorphism seem to be associated with a higher susceptibility of developing leukaemia whereas the C609T NQO1 polymorphism seems to show strong correlation with the risk of disease. Although gene polymorphisms may influence the individual metabolism of benzene, the genetic background is not sufficient to explain complex diseases such as leukaemias (Carbonari *et al* 2016).

CYP2E1 is involved in the first step of the benzene biotransformation pathway. Oxidation of benzene by CYP2E1 to reactive intermediates is a prerequisite of cellular toxicity as well as a limiting step in the excretion of metabolites. In benzene-exposed Chinese workers, Ye *et al* (2015) found a significant correlation between reduced white blood counts and genotypes with variant alleles of CYP2E1 in the promotor region (rs3813867, rs2031920). CYP2E1 polymorphisms in different genes have been reported with conflicting results. Those might be due to the rare frequency of allele variants that is very low among Caucasian (1–5%) and much higher in Oriental populations (19–28%) (Carbonari *et al* 2016).

The **glutathione-S-transferase** (GST) super gene family consists of several gene subfamilies including GSTM1, GSTT1 and GSTP1. Both GSTT1 and GSTM1 are involved in the detoxification of benzene oxide to SPMA. Genetic variants of GSTM1 and GSTT1 consist of the complete deletion of the genes and the loss of the corresponding enzyme activity (Carbonari et al 2016). Dougherty et al (2008) in their literature review found that GSTM1 and GSTT1 showed some consistent associations with both biomarkers of exposure and effect. Ye *et al* (2015) found a significant correlation in benzene-exposed Chinese workers between reduced white blood counts and GSTM1 and GSTT1 null genotypes.

Two **epoxid hydrolase** (EPHX1) genotypes, Tyr113His and His139Arg, were also studied in relation to their effect on the benzene metabolism since it has been shown that these polymorphisms influence the corresponding enzyme activity. However, the results of the available studies were mostly inconsistent (Carbonari *et al* 2016). In benzene-exposed Chinese workers Ye *et al* (2015) found no statistically significant relationship between microsomal epoxid hydrolase mEH (rs1051740, rs2234922). However, by comparing a group with slow mEH to a group with fast mEH acitivity, the fast mEH group had lower white blood cell counts.

Myeloperoxidase (MPO) is most abundanly expressed in neutrophil granulocytes, a sub-type of white blood cells, to produce hypohalous acids for antimicrobal activity. It metabolises the benzene-metabolites hydroquinone and catechol to toxic quinones and free radicals leading to the specific toxicity of benzene in white blood cells. Reduction in white blood cells was less severe in subjects with AG or AA genotypes that in GG homozygous subjects (Lan *et al* 2004).

NAD(P)H:quinone oxidoreductase 1 (NQO1) catalyzes the two-electron reduction and detoxification of quinones and their derivatives, avoiding the formation of free radicals (semiquinones) and ROS, hence protecting cells against the adverse effects of quinones and their derivatives. NQO1*2 (C609T) is the more prominent polymorphism, in terms of both frequency and phenotypic consequences (Carbonari *et al* 2016).

Table 15: Frequency of genetic polymorphisms in different populations (Carbonari *et al* 2016)

Enzyme	Genotype	Population (%)		
		Caucasian	Asian	African
CYP2E1	CYP2E1*5B			
	CC	92.4	59.5	97.0
	CT	7.5	35.9	3.0
	TT	0.1	4.6	0.0
	CYP2E1*6			
	TT	85.4	48.3	64.0
	TA	13.8	42.3	35.0
	AA	0.8	9.4	1.0
EPHX1	EPHX1 -28 T>C Tyr113His			
	TT	47.5	25.1	45.2
	TC	34.7	44.2	42.8
	CC	17.8	30.6	12.0
	EPHX1+52 A>G His139Arg			
	AA	60.0	66.9	62.7
	AG	35.0	28.3	34.9
	GG	4.0	4.8	2.4
NQO1	NQO1*2 C609T			
	CC	57.0	35.7	62.6
	CT	36.0	44.4	30.8
	TT	7.0	19.9	6.6
MPO	MPO G463A			
	GG	62.0	56.9	Not available
	GA	35.0	37.1	
	AA	4.0	6.0	
GST-T1	GST-T1			
	Pos	79.0	47.1	58.0
	Null	21.0	52.9	42.0
GST-M1	GST-M1			
	Pos	51.0	35.3	64.2
	Null	49.0	64.7	35.8
GST-A1	GST-A1*A/B (▪ two different alleles)			
	AA▪	33.0	81.0	Not available
	AB▪	55.0	17.0	
	BB▪	12.0	2.0	
GST-P1	GSTP1 Ile105Val			
	AA	45.6	72.9	39.7
	AG	43.9	23.7	44.0
	GG	10.5	5.4	16.3

Abbreviations: CYP2E1: cytochrome P450 2E1; EPHX: epoxidhydrolase; GST: glutathione-S-transferase; MPO: myeloperoxidas; NQO1: NAD(P)H:quinone oxidoreductase 1

Carbonari *et al* (2016) summarised the available data on genetic polymorphisms (see Table 15). The data show variability in polymorphic gene frequencies exist within ethnic groups of Caucasians, Asians, Africans and between those goups. The authors highlight the frequencies of GSTT1, GSTM1 and GSTA1 which are very different between the three ethnic groups. Asians and Africans show an increased frequency of null genotypes compared with

Caucasians, especially for the GSTT1 genotype, whose enzymatic activity appears to be more important than others in determining the overall ability to detoxify benzene.

In summary, variability in polymorphic gene frequencies exist within and between Caucasian, Asian and African populations. Polymorphisms are involved in toxification and in de-toxicfication of benzene. Studies investigating benzene exposed workers confirm that individual polymorphism plays a role in personal sensitivity to benzene (e.g., Manini *et al* 2010). However, the available data do not allow to draw a general conclusion on the overall sensitivity of ethnic groups with respect to their gene polymorphisms related to the toxicity of benzene.

7.1.2 Animal data

Absorption and distribution

Benzene is readily absorbed by all routes (inhalation, dermal and oral), of which inhalation is considered to be the most important route of exposure. 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/m^3 (8-812 ppm) (DECOS 2014).

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 to 400 µg/cm^2*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) (DECOS 2014).

Upon absorption, benzene is distributed throughout the body. 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/m^3 (500 ppm) (DECOS 2014).

Metabolism

See section 7.1.1.

Elimination

Animal data show that, similar to humans, exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in faeces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase. The half-life for the slow phase of benzene elimination suggests the accumulation of benzene (ATSDR 2007).

7.1.3 *In vitro* data

Relevant *in vitro* data are discussed within the other sections on metabolism.

7.1.4 Toxicokinetic modelling

Watanabe *et al* (1994) fitted a three compartment physiologically based toxicokinetic model to human data on benzene disposition. The relation between cumulative quantity of metabolites produced by the bone marrow and continuous benzene exposure was investigated in detail for simulated inhalation exposure concentrations ranging from 0.0039 ppm to 150 ppm. A 32 ppm exposure for 15 minutes predicted consistently higher values than a 1 ppm exposure for eight hours for the total exposure of bone marrow to

benzene and the cumulative quantity of metabolites produced by the bone marrow. The general relation between the cumulative quantity of metabolites produced by the bone marrow and the inhalation concentration of benzene was not linear but slightly S shaped. At levels of 0.0039 to 10 ppm the curve bended upward, and saturates at high experimental exposures (greater than 100 ppm).

Kim *et al* (2006) used natural spline (NS) models to investigate nonlinear relationships between levels of benzene metabolites (ttMA, SPMA, phenol, hydroquinone, and catechol) and benzene exposure among 386 exposed and control workers in Tianjin, China. After adjusting for background levels (estimated from the 60 control subjects with the lowest benzene exposures), expected mean trends of all metabolite levels increased with benzene air concentrations from 0.03 to 88.9 ppm. Molar fractions for phenol, hydroquinone, and ttMA changed continuously with increasing air concentrations, suggesting that competing CYP-mediated metabolic pathways favored ttMA and hydroquinone below 20 ppm and favored phenol above 20 ppm. Mean trends of dose-specific levels of ttMA, phenol, hydroquinone, and catechol all decreased with increasing benzene exposure, with an overall 9-fold reduction of total metabolites. Surprisingly for the authors, about 90% of the reductions in dose-specific levels occurred below about 3 ppm for each major metabolite. Using generalized linear models with NS-smoothing functions, the authors detected significant effects upon metabolite levels of gender, age, and smoking status. Metabolite levels were about 20% higher in females and decreased between 1% and 2% per year of life. In addition, levels of hydroquinone and catechol were greater in smoking subjects. Overall, the author conclude that the results indicate that benzene metabolism is highly nonlinear with increasing benzene exposure above 0.03 ppm, and that current human toxicokinetic models do not accurately predict benzene metabolism below 3 ppm.

Rappaport *et al* (2009) analysed levels of urinary benzene metabolites and the corresponding air concentrations for 263 non-smoking Chinese female shoe factory workers in Tianjin. Benzene exposure ranged from 0.001 ppm to 299 ppm. The authors used values obtained from two Michaelis-Menten-like models and found strong statistical evidence that an unknown high-affinity pathway is responsible for most metabolism of benzene at sub-part per million air concentrations favouring two metabolic pathways, with respective affinities (benzene air concentrations analogous to *Km* values) of 301 ppm for the low-affinity pathway (probably dominated by cytochrome P450 enzyme 2E1) and 0.594 ppm for an unknown high-affinity pathway. The authors assume that a non-smoking woman would metabolize about three times more benzene from the ambient environment under the two-pathway model than under the one-pathway model and that 73% of the ambient benzene dose would be metabolized via the unidentified high-affinity pathway. The authors conclude that the true leukaemia risks at ambient levels of exposure could be about 3-fold higher than currently thought among non-smoking females in the general population.

Rappaport *et al* (2010) used the same Michaelis–Menten-like kinetic models as used in their previous publication (Rappaport *et al* 2009) to individually analyze urinary levels of benzene metabolites from the 263 non-smoking Chinese women (179 benzene-exposed workers and 84 control workers) with estimated benzene air concentrations ranging from less than 0.001 to 299 ppm. One model depicted benzene metabolism as a single enzymatic process (1-enzyme model) and the other as two enzymatic processes which competed for access to benzene (2-enzyme model). According to the authors, the results indicate that the earlier findings from models of total metabolites were driven largely by ttMA, representing the ring-opening pathway, and by phenol, representing the ring-hydroxylation pathway. The predicted percentage of benzene metabolized by the putative high-affinity enzyme at an air concentration of 0.001 ppm was 88% based upon urinary ttMA and was 80% based upon urinary phenol. As benzene concentrations increased, the respective percentages of benzene metabolized to ttMA and phenol by the high-affinity enzyme decreased successively to 66 and 77% at 0.1 ppm, 20 and 58% at 1 ppm, and 2.7 and 17% at 10 ppm. This indicates that the putative high-affinity enzyme was active primarily below 1 ppm and favoured the ring-opening pathway.

Price *et al* (2012) have re-analysed the data from the 263 female shoe factories in Tianjin (Kim *et al* 2006) and suggested that the rate of metabolism at very low exposures has been over-estimated. The authors expressed particular concern at the treatment of the control population in the original analysis (Price *et al* 2012). An exchange of letters to the editor followed the publication of the re-analysis (Price *et al* 2013; Rappaport *et al* 2013 a,b).

Thomas *et al* (2014) used data from 125 benzene exposed workers (Tianjin, China) for a novel nonparametric, data-adaptive model selection method to estimate the change with dose in the expression of investigated genes. The authors describe non-parametric approaches to model pathway responses and used these to estimate the dose responses of the acute myeloid leukaemia (AML) pathway and 4 other pathways of interest. The response patterns of majority of genes as captured by mean estimates of the first and second principal components of the dose-response for the five pathways and the profiles of 6 AML pathway response-representative genes (identified by clustering) exhibited similar apparent supra-linear responses. Responses at or below 0.1 ppm benzene were observed for altered expression of AML pathway genes and CYP2E1. The authors conclude that these data show that benzene alters disease-relevant pathways and genes in a dose-dependent manner, with effects apparent at doses as low as 100 ppb in air. Studies with extensive exposure assessment of subjects exposed in the low-dose range between 10 ppb and 1 ppm are needed to confirm these findings.

McNally *et al* (2017) undertook an independent reanalysis of the date analysed by Price *et al* (2012) is data with a focus on the evidence for an increase in the rate of metabolism of benzene exposures of less than 1 ppm. The analysis dataset consisted of measurements of benzene and toluene from personal air samplers, and measurements of unmetabolised benzene and toluene and five metabolites (phenol hydroquinone, catechol, trans, trans-muconic acid and s-phenylmercapturic acid) from post-shift urine samples for 213 workers with an occupational exposure to benzene (and toluene) and 139 controls. Measurements from control subjects were used to estimate metabolite concentrations resulting from non-occupational sources, including environmental sources of benzene. Data from occupationally exposed subjects were used to estimate metabolite concentrations as a function of benzene exposure. Correction for background (environmental exposure) sources of metabolites was achieved through a comparison of geometric means in occupationally exposed and control populations. The molar fractions of the five metabolites as a function of benzene exposure were computed. The authors report that a supra-linear relationship between metabolite concentrations and benzene exposure was observed over the range 0.1 to 10 ppm benzene. However over the range of 0.1 and 1 ppm only a modest departure from linearity was observed. The molar fractions estimated in this work were near constant over the range 0.1 to 10 ppm. No evidence of high affinity metabolism at these low level exposures was observed. The author conclude that their reanalysis brings in to question the appropriateness of the dataset for commenting on low dose exposures and the use of a purely statistical approach to the analysis.

Cox *et al* (2017) have also re-analysed the data from the shoe factories in Tianjin using non-parametric methods and concluded that low-concentration metabolism can be linear, with metabolite concentrations proportional to benzene concentration in air, and yet dose-specific metabolism ratios can still decrease with benzene concentrations.

Boogaard (2017) commented that the limit of detection of airborne benzene concentration in the Tianjin cohort was 0.2 ppm. All exposure values lower than this limit of detection were calculated from measured urinary benzene concentrations using a linear correlation between airborne benzene and urinary benzene for which the lowest measured value was 0.1 ppm benzene. In the Tianjin cohort, non-linearity was reported between 0.01 and 0.1 ppm benzene. Boogaard (2017) considers that it is wrong to use a linear equation subsequently to demonstrate non-linearity in metaboliesm for low exposure levels.

Knutsen *et al* (2013) developed a physiologically-based pharmacokinetic (PBPK) model of benzene inhalation based on a recent mouse model adapted to include bone marrow (target organ) and urinary bladder compartments. Empirical data on human liver microsomal protein levels and linked CYP2E1 activities were incorporated into the model, and metabolite-specific conversion rate parameters were estimated by fitting to human biomonitoring data and adjusting for background levels of urinary metabolites. Human studies of benzene levels in blood and breath, and phenol levels in urine were used to validate the rate of human conversion of benzene to benzene oxide, and urinary benzene metabolites from Chinese benzene worker populations provided model validation for rates of human conversion of benzene to ttMA and SPMA, phenol, catechol, hydroquinone, and benzenetriol.

Conclusion

Physiologically-based pharmacokinetic (PBPK) models have been developed for benzene exposure. Re-analyses of results from PBPK models based on human data from two Chinese shoe factory workers in Tianjin indicate for the metabolism of benzene only a modest departure from linearity at benzene concentrations below 1 ppm.

7.1.5 Biological monitoring

Biological monitoring of benzene effects are addressed under Section 7.3. 'Specific target organ toxicity / repeated dose toxicity' for haematological effects of benzene and under Section 7.6 'Genotoxicity' for clastogenic effects of benzene.

7.1.6 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. Mean inhalation absorption has been reported in humans ranging from approximately 50 to 80% (DECOS 2014).

The metabolism of benzene is inherently complex and occurs principally in the liver and also in the lung, with secondary metabolism occurring in the bone marrow (McHale *et al* 2012). In the first step benzene is oxidized to benzene oxide mainly by cytochrome P450 2E1 (CYP2E1). Several pathways are involved in the metabolism of benzene oxide:

- Benzene oxide can undergo conjugation with glutathione (GSH), resulting in the formation and urinary excretion of S-phenylmercapturic acid (SPMA) (Monks *et al* 2010).
- Benzene oxide may be further metabolized to benzene dihydrodiol and catechol (Meek and Klauning 2010).
- Benzene oxide spontaneously rearranges to phenol, which subsequently undergoes either conjugation (glucuronic acid or sulfate) or oxidation. The oxidation reaction gives rise to 1,4-hydroquinone, 1,2-hydroquinone (catechol) and 1,2,4-benzene triol (DECOS 2014).
- Benzene oxide equilibrates spontaneously with the corresponding oxepine valence tautomer, which can lead to ring opening to yield a reactive alpha,beta-unsaturated aldehyde, *trans,trans*-muconaldehyde, further aldehyde metabolites and finally *trans,trans*-muconic acid (ttMA) which is eliminated in the urine (Meek and Klauning 2010; Monks *et al* 2010).

Exhalation is the main route for excretion of unmetabolized benzene (ATSDR 2007). Most of the absorbed benzene however, is metabolised and the metabolites are excreted after phase-II-conjugation predominantly in the urine (DECOS 2014).

Studies in humans and animals indicate that both exhalation and urinary excretion occur in several phases, with half-lives of minutes to hours (ATSDR 2007). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene (ATSDR 2007).

Re-analyses of results from PBPK models based on human data from two Chinese shoe factory workers in Tianjin indicate for the metabolism of benzene only a modest departure from linearity at benzene concentrations below 1 ppm.

7.2 Acute toxicity

7.2.1 Human data

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects at levels ranging from 975 to 9,750 mg/m^3 (300-3,000 ppm). 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, haemolysis, and congestion or haemorrhage of organs were reported in the cases for which there were autopsy reports (DECOS 2014).

7.2.2 Animal data

Acute inhalation toxicity is low with a LC_{50} value of 44,500 mg/m^3 (13,700 ppm) after a 4-hour exposure for rats. 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. Acute oral toxicity data for rats suggest that the oral LD_{50} is above 2,000 mg/kg bw, ranging from 810 to 10,000 mg/kg bw. Depending on the dose, the main clinical signs are sedation and narcosis. Pathological findings include among others hyperaemic and haemorrhagic lungs, adrenals and spine (DECOS 2014).

7.2.3 *In vitro* data

Due to the availability of information on acute toxicity in animals and humans, potential published *in vitro* data are not reported here.

7.2.4 Summary

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects at levels ranging from 975 to 9,750 mg/m^3 (300-3,000 ppm). Very high concentrations of benzene vapours produce narcotic effects and can lead to death by respiratory arrest. In rats, acute inhalation toxicity is low with a LC_50 value of 44,500 mg/m^3 (13,700 ppm) after a 4-hour exposure. Depression of the central nervous system appeared to be related to death.

7.3 Specific target organ toxicity / Repeated dose toxicity

The focus of this section is on recent studies in workers exposed to benzene concentration in the range of 10 ppm and below and for which the benzene concentration in air has been measured.

7.3.1 Human data

Benzene exposure has been reported to lead mainly to neurological, haematological and immunological effects.

Multiple studies are available investigating specific target organ toxicity, mainly haematotoxicity and immunotoxicity, in benzene exposed workers. Of highest relevance are studies investigating a larger group of workers (preferable >100) for which appropriate risk management measures have been in place to prevent excessive dermal exposure, which used an appropriate control group (industrial workers), that considered relevant confounders for the endpoint and method used (e.g., gender, smoking), which used personal exposure sampling to monitor benzene exposure, which excluded workers with previous higher benzene exposure, and in which an appropriate regression analysis was performed with control for confounding factors.

NEUROLOGICAL EFFECTS

Neurological effects have been commonly reported in humans following high-level exposure to benzene. Fatal inhalation exposure has been associated with vascular congestion in the brain. Chronic inhalation exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Oral exposure results in symptoms similar to inhalation exposure. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behaviour. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals (ATSDR 2007).

Conclusion

Neurological effects have been commonly reported in humans following high-level exposure to benzene. However, such effects are not relevant for benzene-related risks at low benzene concentrations.

HAEMATOLOGICAL EFFECTS

Both human and animal studies have shown that benzene exerts toxic effects on various parts of the haematological system (ATSDR 2007). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A reduction in the number of the three major blood components, erythrocytes (anaemia), leukocytes (leukopenia) and platelets (thrombocytopenia), can develop following exposure to benzene (Arnold *et al* 2013). A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective haematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed (ATSDR 2007). Severe damage to the bone marrow involving cellular aplasia is known as aplastic anaemia and can occur with prolonged exposure to benzene. This condition can lead to leukaemia (ATSDR 2007).

Numerous earlier studies of benzene-exposed workers demonstrated that chronic exposure to benzene air concentrations of 10 ppm or more resulted in adverse haematological effects, which increased in severity with increasing benzene exposure levels (ATSDR 2007). In the following sections, more recent studies are described in which workers were exposed to benzene including concentrations below 10 ppm.

Studies investigating haematological effects in workers are summarized in Appendix 1, Table 38.

Data from Health surveillance programmes

Tsai *et al* (2004) evaluated haematology data from 1200 male and female employees who participated in the Shell Benzene Medical Surveillance Program (BMSP) compared to 3227 employees not enrolled in either the benzene or butadiene surveillance programs. Representative exposure monitoring data were available (personal sampling), during normal operations and maintenance activities from January 1, 1978 to December 31, 2002. The exposure monitoring data were examined in aggregate by two different time periods (1977–1987 and 1988–2002). The measured time weighted average benzene concentration in the air was 0.60 ppm (range 0.1-5.7 ppm) from 1977 to 1988 and since 1988 0.14 ppm (range 0.005 -1.3 ppm). Entrance criteria to the BMSP for active employees as of 1988 or hired after 1988 include three overlapping groups: (1) employees who are potentially exposed to benzene at or above 0.5 ppm (8-h time weighted average (TWA-8)) for 30 or more days per year, (2) employees who are potentially exposed to benzene at or above 1.0 ppm (TWA-8) during 10 or more days per year, or (3) employees who are potentially exposed to benzene at or above 5.0 ppm over 15 min during 10 or more days per year. Approximately 25% of the employees participated in that program were also exposed to butadiene, which is also associated with haematotoxicity. Relevant demographic data were obtained from the computerized files of Shell's personnel system and health surveillance system. Six haematological parameters were investigated (white blood cells, lymphocytes, red blood cells, haemoglobin, mean corpuscular volume, and platelets). After adjustment for age, sex, race, length of time between first and last exam, and current smoking status no statistically significant differences were found. **This study provides a NOAEC of 0.6 ppm (range 0.1-5.7 ppm).**

Swaen *et al* (2010) investigated 8532 blood samples of 701 male DOW employees in the Netherlands occupationally exposed to benzene in comparison to 12,173 blood samples of 1059 employees from other departments without occupational benzene exposure for haematological parameters (haemoglobin, haematocrit, white blood cells, lymphocytes, neutrophils, eosinophils, basophiles, monocytes). Mean benzene exposure was assessed as 0.22 ppm (range 0.01-1.85 ppm) based on a job-exposure matrix. A further stratification of the exposed population into three subgroups (<0.5 ppm, 0.5–1 ppm and >1 ppm) was performed. In a regression analysis, data were adjusted for age, smoking and month at blood sampling. A small reduction in eosinophils was noted (exposed 181.58 versus controls 182.61) which was statistically significant in a regression model considering continuous benzene exposure; however, the authors considered this reduction as small and clinically not significant. Furthermore, values for basophils (exposed 46.33 µg/L versus controls 42.08 µg/L) and monocytes (exposed 503.13 µg/L versus controls 478.60 µg/L) were statistically significantly increased. Since benzene exposure was not measured individually but assessed by job-exposure matrix, the results may contain some uncertainty. **This study provides some indications for no relevant haematological effects at 0.22 ppm (range 0.01-1.9 ppm).**

Collins *et al* (1991) assessed data from routine medical examinations of 200 male and female persons working with benzene compared with 268 non benzene workers in the same plant for haematological effects. Exposures measured as 8-hour time weighted average ranged from 0.01 to 1.40 ppm over a 10-year period. Averaging the estimated exposure values provided in the publication results in a mean of about 0.09 ppm. Exposure estimates were constructed using actual exposure monitoring data when monitoring results were available and industrial hygienist's judgement in areas of very low exposure potential and little monitoring data. Benzene exposures were estimated with respect of ever exposed, exposure duration, current exposure, highest exposure, and cumulative exposure. Haematological parameters investigated were red blood cell count, white blood cell count, haemoglobin, platelet and MCV. After controlling for confounders, current benzene exposure was significantly correlated with increased white blood cell count and

MCV. The authors comment that the increase in white blood cell count is the opposite direction associated with excessive benzene exposure, whereas the increase in MCV is in the direction for a benzene effect. The authors, however, indicate that the effects in MCV was very small and was not seen in any other indicators for exposure. Hence, the authors concluded that the results did not show differences in the measured haematological parameters. Several other factors (age, sex, race, and smoking), however, were associated with these outcomes, indicating the importance of considering confounding factors when comparing haematology results. It has to be noted that only for one job description exposure was estimated with 1.4 ppm, for two job descriptions exposure was estimated with 0.19 and 0.12 ppm and for the remaining 23 job descriptions exposure estimates were <0.1 ppm. Furthermore, the number of workers with such a job description were not reported. Hence, it is not clear how robust the data are, especially with respect to the higher benzene exposures >0.1 ppm and >1.0 ppm. **Hence, this study provides some indications for no relevant benzene-related effects at concentration of 0.09 ppm**.

Collins (1997) used routinely collected data from medical/industrial hygiene system to study 387 male and female workers with daily 8-hour time-weighted exposures ranging from 0.01 to 87.69 ppm, averaging 0.55 ppm for the years 1980 to 1993 with significant reductions over the years. Control group consisted of 553 unexposed workers. Exposure assessment was based on personal monitoring samples. Parameters investigated were lymphocyte count, total white blood cell count, hemoglobin levels, platelet levels and increased mean corpuscular volume. The author comment that exposure levels exceeding 2.0 ppm were rare (less than 5%). The cross-sectional repeated survey design included 553 unexposed workers. No increase in the prevalence of lymphopenia (abnormal low levels of lymphocytes) among benzene-exposed workers was observed (odds ratio, 0.6; 95% confidence interval, 0.2 to 1.8), taking into account smoking, age, and sex. There was also no increase in risk among workers exposed 5 or more years (odds ratio, 0.6; 95% confidence interval, 0.2 to 1.9). Examination of other early indicators of haematotoxicity including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets, produced similar results. **This study indicates a NOAEC of 0.55 ppm for lymphopenia**. However, since only one haematological parameter was investigated, this study is not suitable for an overall evaluation of haematological effects of benzene.

In a more recent study, **Koh *et al* (2015a)** extracted data from the Korean Special Health Examination Database on 10,702 benzene-exposed workers. Data on complete blood cell counts, differential white blood cells, red blood cells, platelets, lymphocytes and neutrophils were retrieved. Benzene concentrations were estimated based on 8679 8-h TWA personal benzene measurements taken between 2004 and 2008. 67% of the measurements were below the lower limit of detection of 0.01 ppm. The mean concentrations for the benzene exposure groups were calculated using various combinations of factory code, four-digit standard work process (SWP) code and standard industrial classification (SIC) code. A stepwise exposure assignment algorithm was used. The resulting exposure groups were re-classified into five levels, based on the availability of exposure information. The higher the level of information about the exposure, the more credit the exposure estimate was given. Estimated benzene concentrations were divided into four categories, namely category 1, <0.01 ppm (reference); category 2, 0.01 to 0.1 ppm; category 3, 0.1 to 0.5 ppm; and category 4, 0.5 to 5.95 ppm. In a personal communication the author clarified that the mean concentrations were 0.002, 0.043, 0.205, and 2.610 ppm (Koh et al 2015b). For total workers, mean white blood cells, red blood cells, platelets, neutrophils and lymphocyte counts showed no consistent trend with increased exposure. However, in male workers, red blood cell count showed a significant negative association. Considering highest quality exposure data and adjustiment for age and gender, a logistic regression analysis provided statistically significant results for male workers of the highest exposure group (≥0.5 ppm; range 0.5 – 5.95 ppm) for the

reduction in the number of red blood cells (below the lower limit of normal) with an OR of 2.12 (95% CI 1.52-2.95). The authors concluded that there is the potential for haematotoxicity below 1 ppm, as red blood cell counts in males may be affected by benzene exposure as low as 0.5 ppm. The authors mention further that the study has some limitations. For example, in the low-level exposure environments, benzene exposure from cigarette smoking may be important and was not accounted for. Furthermore, the authors indicate that workers could have been co-exposed to other solvents with haematological effects like formaldehyde and the blood tests were conducted at more than 150 hospitals using different counting devices which may lead to some systematic error in cell counts. **Overall, the study indicates a LOAEC of 2.61 ppm and a NOAEC below 0.5 ppm.**

It should be noted that such health surveillance programs are intended to identify early indications of illness and to take corrective actions if required. Therefore, in case of haematological abnormalities in benzene exposed workers, they might have been transferred to a less exposed or non-exposed department (see also Koh *et al* 2015a).

Shoe manufacturing workers

Qu *et al* (2002, 2003a) examined 130 Chinese workers from the region of Tianjin which were exposed to benzene concentrations between 0.06 and 122 ppm and 51 unexposed control workers from a soybean production plant. Benzene in air was measured by personal sampling (Qu *et al* 2003b). In Qu *et al* (2002) and Qu *et al* (2003a) several tables are presented using different criteria to assign the exposed workers. According to Appendix A, Table 9 of Qu *et al* (2003a), the mean benzene exposure (averaged over 4 weeks) were 0.004±0.003 (controls), 2.26±1.35, 8.67±2.44, 19.9±3.1, and 51.8±43.3 ppm. Also cumulative exposures were calculated. For reduction in red blood cell count, white blood cell count and neutrophil count, statistically significant trend tests were reported with reduced values even at the lowest exposure group (see Table 16). However, the values remained in normal ranges. No significant trend tests were reported for haematocrit, platelets and lymphocytes. **From this study a LOAEC of 2.3±1.4 ppm can be derived**.

Table 16: Peripheral blood cell counts in relation to benzene exposure (Qu et al 2003a)

	Controls	>0-5 ppm	>5-15 ppm	>15-30 ppm	>30 ppm
Mean benzene exposure (ppm; 4 weeks average)	0.004±0.003	2.26±1.35	8.67±2.44	19.9±3.1	51.8±43.3
No. of subjects	51	73	33	8	16
Females (%)	53	55	33	88	63
Smokers (%)	31	36	55	0	38
Red blood cells ($x10^{10}$/L)[C]	463±52	399±59	410±60	387±18	392±50
White blood cells ($x10^6$/L)[C]	6671±1502	6415±1266	6006±1752	5825±1550	4988±615
Lymphocytes ($x10^6$/L)	2205±789	2429±741	2226±691	2248±1152	1890±453
Neutrophils ($x10^6$/L) [C]	4006±1108	3541±944	3315±1408	3116±610	2753±580

[C] $p \leq 0.001$, test for exposure-response test

The Lower Olefins LOA REACH Consortium (LOA 2017b) performed a Benchmark dose calculation based on the neutrophil count. Neutrophil count was selected because neutrophils appeared to show the clearest exposure-response trend and they comprise the majority of circulating white blood cells and have a relatively short biological half-life, making them more likely to be a sensitive indicator of bone marrow dysfunction than other blood cell types and particularly lymphocytes (which have a comparatively longer biological half-life and a multi-organ maturation process).

The BMD analysis used several key assumptions:

1. A benchmark response of 5% is a widely-accepted default for continuous endpoints and well within the range of normal, non-adverse variability for neutrophils.
2. Assuming log-normal distribution. Log scale variation in cell counts is more common in general for haematology endpoints and the resulting analysis.
3. Based on the BMD model outputs it appears the variance among groups may not be equal, and as such it is more appropriate to use models without applying the assumption of equal variance.
4. In earlier BMD analyses it was common to restrict the parameters in some models, but later studies have demonstrated this does not appear necessary, and hence the analysis does not restrict the power to be ≥1 for the Power model and n >1 for the Hill model.

LOA REACH Consortium (2017b) noted that the results appear to vary substantially depending on the statistical model used. Only the exponential models Exponential4 and Exponential5 adequately fitted the data based on significance tests, thus most of the models were rejected. Both exponential model 4 and 5 produced the same results, fitted the data, and had the lowest Akaike Information Criteria (AIC) values. **This BMD analysis identified a BMD of 1.06 ppm and a BMDL of 0.43 ppm for a 5% decrease in neutrophils**, as shown in Figure 4 below.

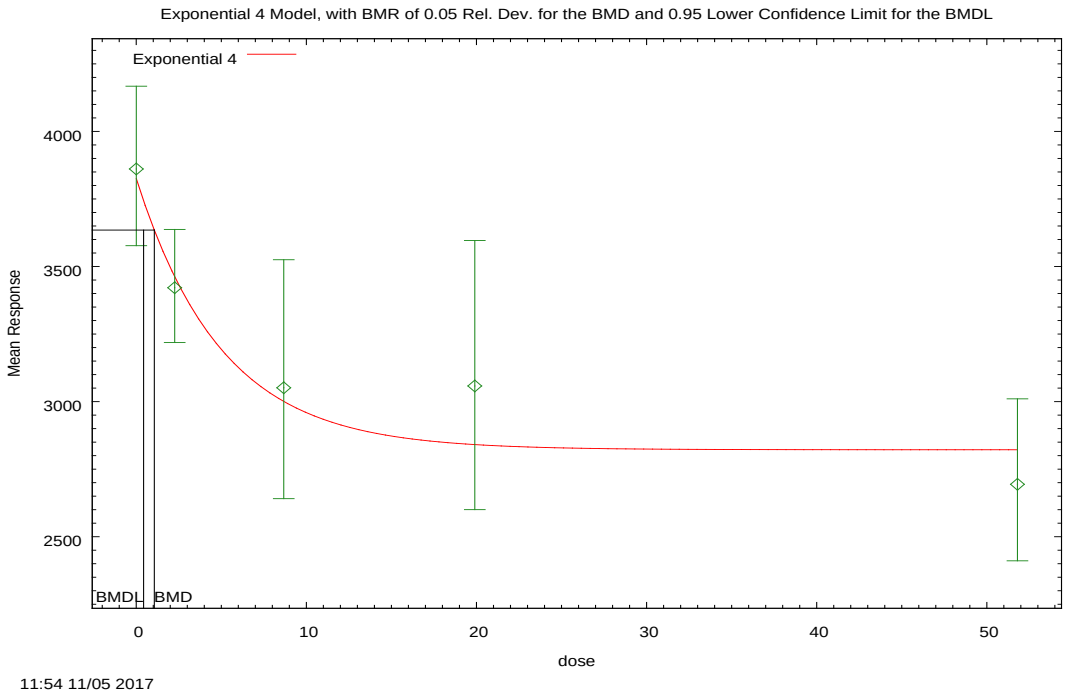

Figure 4 Benchmark dose calculation performed by LOA (2017b) based on reduced neutrophil count as reported in Qu *et al* (2003a)

Qu *et al* (2003a) provided also information on haematological effects at exposure concentrations below 1 ppm. In a subgroup of 16 non-smoking female workers exposed to 0.14±0.04 ppm benzene, red blood cell counts, white blood cell counts and neutrophile cell counts were statistically significant reduced. These associations remained after controling of confounding variables. The lowest detection limit for benzene in personal samplers was about 0.01 ppm. However, this study has relevant limitations. This low exposure group consisted only of 16 individuals, all non-smoking women, whereas about only half of the control subjects were female of which 31% smoked. Since the results have not been corrected either for gender (since women have relatively lower levels of haemoglobin, haematocrit, and red blood cells) or for 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 (see also DECOS 2014).

Lan *et al* (2004) investigated 250 workers in two shoe factories in Tianjin, China, and 140 controls who worked in three clothes-manufacturing factories in the same region. Occupational exposure assessment was published by Vermeulen *et al* (2004). Exposure was measured by personal sampling for factory A on one random day each month (March to June 2000) and for factory B on one to two days per month (March 2000 to June 2001). The mean benzene exposure level was 21.86 ppm (10^{th}–90^{th} percentiles 5.23–50.63 ppm) in the smaller shoe factory (factory A) and 3.46 ppm (10^{th}–90^{th} percentiles 0.20–7.00 ppm) in the larger shoe factory (factory B). Limit of detection was 0.2 ppm benzene. Exposure duration was 6.1±2.9 years. For each subject, individual benzene and toluene exposure was monitored repeatedly up to 16 months before phlebotomy, and post-shift urine samples were collected from each subject. Subjects were categorised into four groups consisting of controls (n=140); <1 ppm (n=109); 1 to <10 ppm (n=110); and ≥10 ppm (n=31) by mean benzene levels measured during the month before blood sampling. The concentration of benzene in urine was 0.382, 13.4, 86.0 and 847 µg/L for controls, <1 ppm, 1 to <10 ppm and >10 ppm, respectively. White blood cell and platelet counts were significantly lower than in the controls, even for exposure below 1 ppm in air (0.57±0.24 ppm) (see Table 17 below). Red blood cell count was not investigated. To exclude the effect of other potential exposures on these associations, the authors identified a group of 30 workers exposed to <1 ppm benzene with negligible exposure to other solvents and reported decreased levels of white blood cells, granulocytes, lymphocytes, and B cells compared to controls. However, no further information is provided in the publication. The authors also investigated progenitor cell colony formation in an *in vitro* colony-forming assay. With increasing benzene exposure progenitor cell colony formation significantly declined and was more sensitive to the effects of benzene than was the number of mature blood cells. Two genetic variants in key metabolizing enzymes, myeloperoxidase and NAD(P)H:quinone oxidoreductase, influenced susceptibility to benzene haematotoxicity. Further investigations performed in this cohort of workers indicated increased immunological alterations (Lan *et al* 2004; see below under 'Immunological effects'), increased chromosomal aberrations and aneuploidy (see 7.6.1), a perturbation of gene expression with the AML pathway most significantly associated with benzene exposure, and immune response pathways associated with most exposure levels (McHale *et al* 2011).

AGS (2012) indicated that exposure assessment and categorisation used in this study (Lan *et al* 2003), especially that of the low exposure group, has to be considered with care. Even if the exposure was monitored for 16 months before investigating the haematological effects, the average exposure duration was 6.1±2.9 years and former exposure concentrations might have been higher. Furthermore, dermal exposure of the glue containing benzene might have contributed to the exposure. According to Vermeulen *et al* (2004) exposure levels in factory B showed a clear seasonal pattern with the lowest exposures during the summer months, followed by the fall and spring and higher exposures during the winter months. In average, the mean benzene concentrations in factory B might

have been higher than 1 ppm. Furthermore, in factory A, benzene exposures were ≥7 ppm at 5 different time points measured. In addition, smoking was not considered as contributing factor. Considering those shortcomings, the LOAEC might be higher than 0.57 ppm.

Table 17: Peripheral blood cell counts in relation to benzene exposure (Lan *et al* 2004)

Subject category	Controls (n=140)	Benzene exposure		
		<1ppm (n=109)	1 to <10 ppm (n=110)	≥10 ppm (n=31)
Benzene, air (ppm)	<0.04	0.57±0.24	2.85±2.11	28.73±20.74
Benzene, urine (µg/l)	0.382±1.24	13.4±18.3	86.0±130	847±1250
White blood cell count§	6480±1710	5540±1220*	5660±1500	4770±892
Granulocytes§	4110±1410	3360±948*	3480±1170	2790±750
Lymphocytes§	2130±577	1960±541*	1960±533	1800±392
$CD4^+$–T cells§	742±262	635±187*	623±177	576±188
$CD8^+$–T cells	553±208	543±212	564±229	549±160
$CD4^+/CD8^+$ ratio§	1.46±0.58	1.26±0.41*	1.22±0.45	1.09±0.35
B cells§	218±94	186±95*	170±75	140±101
NK cells§	586±318	558±299	566±271	415±188
Monocytes	241±92	217±97*	224±93	179±74
Platelets§	230±59.7x10^3	214±48.8x10^3*	200±53.4x10^3	172±44.8x10^3
Haemoglobin (g/dl)	14.5 (1.6)	14.7 (1.5)	14.5±1.7	13.6±1.6

Abbreviations: *P <0.05 for <1ppm vs. control; § significant P trend, all subjects

In addition, as pointed out by Concawe (2013), prior higher levels might indeed be expected within these 6 prior years as the Chinese regulatory occupational exposure level for benzene was significantly lowered in 2002 from 12.3 to 1.9 ppm (Liang *et al* 2006). The exposure measurements of the Lan *et al* (2004) study were taken in the years 2000 to 2001; it is therefore possible that during the 6 years exposure period of the participating workers there had been changes in exposure levels to benzene. The prior exposures might have contributed to lower blood cells counts. Concawe further states that the cumulative exposures indicate that exposures prior to the study may be much higher.

It has to be noted further that the concentrations of benzene measured in urine (0.382, 13.4, 86.0, and 847 µg/L for controls, <1 ppm, 1 to <10 ppm and >10 ppm, respectively) show that endogenous benzene exposure was higher than the measured concentrations of benzene in the air would indicate. Considering the correlation between external and internal benzene exposure as published by DFG (2017a, b; see Table 8), urinary benzene concentration of 13.4 µg/L urine correlates to a benzene concentration in the air of higher than 2 ppm. It might be assumed that dermal uptake might have contributed the endogenous benzene concentrations measured. **Hence, even if this study indicates a LOAEC of 0.57 ppm, exposure might have been higher corresponding to a benzene concentration of >2 ppm.**

Ye *et al* (2015) investigated 385 workers in shoe factories in the Zhejiang Province in China for reduction in white blood cells compared to 220 unexposed indoor workers (teachers, bank clerks). Benzene concentrations were measured at the breathing level of workers by point sampling three times per day. Median measured benzene exposure was 6.4 mg/m^3 (2 ppm) with a range from 2.5 to 57 mg/m^3 (0.7 to 17.8 ppm). Workers were divided into three groups <3.25 mg/m^3 (<1 ppm), <6 mg/m^3 (<1.8 ppm), and ≥6 mg/m^3 (≥1.8 ppm). The cumulative exposure dose was calculated for each worker according to job site, employment duration and work history. Workers were divided into four groups of ≥5.02, >19.90, >31.81, and >59.00 mg/m^3-year, corresponding to 1.5, 6.1, 9.8, and 18.2 ppm-years. In the exposure analysis, the low dose group (<1 ppm) and the high dose group (>1.8 ppm) were associated with a significant decrease in white blood cell count (5.57±1.79 and 5.27±1.54 x 10^9), whereas in the medium dose group (<2 ppm) the decrease was statistically not significant (6.01±1.47 x 10^9). Considering the lack of dose response in the low and medium dose group and considering that the low dose group consisted only of 24 persons, the result of the low dose group needs to be evaluated with care. With respect to cumulative exposure, white blood cell counts significantly declined with increasing benzene cumulative exposure dose with a significant decrease in white blood cell count at >6.1 ppm-year. In the low exposure group with ≥1.5 ppm-year the decreased white blood cell count was only slight and statistically not significant. This finding (no significant effects in the low cumulative exposure group consisting of 96 exposed workers) supports that the finding in the low current exposure group might be an artefact. Decreased white blood cell counts were also found in workers with null-GSTT1 and null-GSTM1 and there was a small but statistically significant association between CYP2E1 (rs3813867 and rs2031920) and white blood cells, although linear regression showed no apparently association between CYP2E1 polymorphism and white blood cells. The authors concluded that individuals with null-GSTT1 and null-GSTM1 genotypes and CYP2E1 (rs2031920 and rs3813867) may have increased susceptibility to haematotoxicity, as evidenced by lower white blood cell counts. **In conclusion, the results of this study indicate a reduction in white blood cell count at 2.0 ppm (range 0.7-18 ppm); however, no other haematological parameters were investigated.**

Zhang *et al* (2016) investigated a group of 317 benzene exposed workers and 102 controls (office employees from local banks and schools) for white blood cell count and micronucleus frequency. The exposed group included 87 smokers (27%) while the original control group included 8 smokers (8%). Due to the benzene exposure history of those 8 persons, they were assigned to a cumulative exposure group. Hence, for the analysis on cumulative benzene exposure, all controls were non-smokers. The measured ambient benzene air concentration ranged from 0.80 to 12.09 ppm with a median of 1.60 ppm. For the sewing department median benzene concentrations were 1.57 ppm (range 0.8-3.78 ppm) with median years of service of 3.0 years (range 1.2-16.9 years). For the molding department median benzene concentrations were 2.60 ppm (range 0.83-12.09 ppm) with median years of service of 2.9 years (range 1.0-18.3 years). For the packaging department median benzene concentrations were 1.79 ppm (range 0.8-4.25 ppm) with median years of service of 3.0 years (range 1.0-21.2 years). Cumulative exposure concentrations of benzene were calculated by ambient air benzene concentration at worksites in conjunction with job type and associated service duration resulting in exposure groups with median cumulative benzene exposures of 3.55, 6.51, 10.72, 20.02, and 40.71 ppm-years. White blood cell count was significantly reduced at cumulative exposures of 10.72, 20.02 and 40.71 ppm-years but not at lower cumulative exposures of 3.55 or 6.51 ppm-years. Workers older than 30 years were more susceptible to abnormal white blood cell count reduction than those younger than 30 years. Other factors (gender, smoking, drinking) did not show a significant impact. Based on the results of the cumulative exposure, the authors estimated lower 95% confidence limits of the benchmark dose (BMDLs) (age-pooled) using log probit model for reduced white blood cell counts considering 40 years of exposure. For reduced white blood cell count (10% excess risk), the benchmark dose (BMD) was

calculated with 6.38 ppm-years and the BMDL with 1.37 ppm-years. The authors also calculated a dose-response model (Hill models) with resulting BMD of 9.57 ppm-years and BMDL of 0.29 ppm-years, corresponding to 10% excess risk. The author observed that the BMDLs for elevated micronucleus frequencies are lower than those for reduced white blood cell count, irrespective of the methods and options used for computation. The authors conclude that this result suggested that the micronucleus frequency is a more sensitive biomarker than reduction of white blood cell count for benzene exposure. According to the authors it also implied that genotoxicity can be a more sensitive endpoint than haematotoxicity. However, it is to be noted that only one parameter related to haematotoxicity and only one parameter related to genotoxicity were measured. Since benzene affects several haematological and genotoxic parameters, such a general conclusion would require further confirmation from other parameters affected by benzene. **In conclusion, the results of this study indicate a reduction in white blood cell count at 1.6 ppm (range 0.8-12 ppm); however, no other haematological parameters were investigated**.

Workers in industries other than shoe factories

Huang *et al* (2014) reported in a group of 121 petrochemical workers in Shanghai, China, which were exposed to benzene in concentrations from 0.25 to 15.7 mg/m^3 (range 0.08-4.8 ppm) no effects on white blood cells, haemoglobin or platelets in comparison to 110 “healthy people”. Benzene exposure was assessed by regular monitoring of benzene in seven sampling points in the workshop. The arithmetic mean of benzene emission was 2.24 mg/m^3 (0.69 ppm) for the reformer, 1.83 mg/m^3 (0.56 ppm) for the sulfolane extraction unit, 2.65 mg/m^3 (0.82 ppm) for the disproportionation/isomerization unit, and 1.71 mg/m^3 (0.53 ppm) for morpholine extraction unit. From those data, a mean of 0.65 ppm can be calculated. After classification of workers on the basis of cumulative benzene exposure (<6 mg/(m^3 a); 6 to 40 mg/(m^3 a); >40 mg/(m^3 a)) no effects were reported on those parameters neither. Exposure was assessed by regular monitoring of benzene in seven sampling points in the workshop of the refinery between January 2008 and June 2012. **This study indicates no haematological effects in four different work unit with mean benzene exposures of 0.65 ppm (between 0.53 and 0.82 ppm).** However, since exposure was measured only stationaly but not personally, the result contains uncertainty.

Kang *et al* (2005) and **Sul *et al* (2005)** obviously investigated the same Korean workers involved in benzene, toluene and xylene process, Carbomer production, methylene di-aniline (MDA) producing process, Shoe manufacture, and offset printing. Sul *et al* (2005) reported that the study consisted of 56 male and 5 female workers and 33 smokers and 28 non-smokers. No controls were included. Exposure was assessed by personal air monitoring and the measurement of *trans,trans*-muconic acid (ttMA) in urine. The geometric mean of benzene in the air was provided with 0.094 ppm (range 0.005–5.3 ppm) (Kang *et al* 2005) and the mean as 0.268±0.216 ppm (range 0.005-2.0 ppm). The mean duration of employment was 8.9±6.7 years. Workers were divided according to their benzene exposure in air: <0.1 ppm (n=26), 0.1 to 1 ppm (n=28) and 1 to 3 ppm (n=7). No effects on haematological parameters were reported for red blood cell count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, white blood cell count, neutrophils, lymphocytes, monocytes in white blood cells and platelet volume distribution width. Differences among the groups were reported with respect to MCHC and platelet number; however, no clear dose-response relationship could be demonstrated. **Overall, this study indicates no relevant haematological effects at mean benzene concentrations of 0.27±0.22 ppm**. However, due to the relative small number of investigated workers and the wide exposure range and since obviously smoking, age and gender were not considered as confounding factors, the results of this study contain relevant uncertainties.

Schnatter *et al* (2010) investigated 928 male and female workers from five factories in Shanghai. Weekly benzene exposure estimated from representative individual monitoring results ranged from 0.07 to 872 mg/m^3 (0.02-269 ppm) with a median value of 7.4 mg/m^3 (2.3 ppm). The authors reported that stronger effects on peripheral blood were seen for red blood cell indices such as anaemia and macrocytosis, albeit at higher (>10 ppm) exposure levels. The most sensitive parameters to benzene appeared to be neutrophils and the mean platelet volume, where effects were seen for benzene air concentrations of 7.8 to 8.2 ppm. To further assess dose-response the authors categorised workers according to their benzene exposure to <1 ppm, 1 to <10 ppm, or >10 ppm. Stronger effects were seen for mean platelet volume and red blood cell count, with highly significant OR's in the >10 ppm exposure category. The mean platelet volume showed a monotonic risk, while red blood cells show an irregular dose–response, making it more difficult to interpret the results. **The results indicate a reduction in neutrophils at benzene concentrations of 7.8 to 8.2 ppm.**

Pesatori *et al* (2009) investigated 153 Bulgarian petrochemical workers exposed to benzene in a range between 0.01 and 23.9 ppm (median 0.46 ppm) and 50 unexposed subjects for haematological outcomes (white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, platelets and mean platelet). Exposure assessment was based on personal monitoring sampling the day before phlebotomy and demonstrated 0.02±0.09 ppm for controls, 0.3±0.2 ppm for the low exposure group (n=106) and 4.9±5.3 ppm for the high exposure group (n=47). All subjects who had worked in the same position for at leat one year were enrolled. Mean length of employment were 13.2±10.9 years, 15.3±8.9 years, and 15.7±8.6 years for controls, low exposure and high exposure group, respectively. Urinary trans-trans-muconic acid (ttMA) was determined at the beginning and end of the work shift. End-shift values were 108±135 µg/L for controls, 801±924 µg/L for the low exposure group and 2917±2993 µg/L for the high exposure group. Mean values of each haematologic outcomes in each exposure category were compared with the referent group using a multiple linear regression model adjusted for age, gender, current smoking habits and environmental toluene level. The influence of the CYP2E1 (RsaI and DraI) and NQO1 609C>T genetic polymorphisms on differential haematological parameters was also investigated. No dose-response effect was observed for most of the examined haematological outcomes. The authors mentioned that the eosinophil count was inversely related to benzene exposure only among smokers and conversely, that basophils increased with increasing exposure. According to the authors no effect on benzene haematotoxicity was found for any of the investigated polymorphisms. Taking into account that in the high exposure group (4.9 ppm) only 47 persons, whereas in the lower exposure group (0.3 ppm) 106 workers were included, **a NOAEC of 1.7 ppm seems to be appropriate** [4.9 ppm x 47 + 0.3 ppm x 106) / 153=1.7 ppm].

Seow et al (2012) examined in 158 Bulgarian petrochemical workers and 50 unexposed office workers the association between benzene exposure and DNA methylation and also investigated haematological parameters (basophils, eosinophils, monocytes, lymphocytes and neutrophils). Exposure assessment included personal monitoring of airborne benzene and provided median levels of airborne benzene of 0.46 ppm (range 0.19-23.9 ppm). Concentrations of urinary biomarkers of benzene metabolism were measured with 15.5 µg SPMA/L (median; range 0.24-349.4 µg/L) and 711 µg ttMA/L (median; range 25-9961 µg/L) respectively. The proportion of basophiles of the various leukocyte cell types was significantly increased compared to the control group. The authors do not discuss this effects. The proportion of the other leukocyte cell types (eosinophils, monocytes, lymphocytes or neutrophils) were not changed. This investigation has limited value for the evaluation of the dose-response for haematological effects of benzene.

Workers exposed to gasoline

Moro *et al* (2015) investigated early haematological and immunological alterations in 60 male fuel filling station attendants and 28 male controls (no occupational benzene exposure) in Brazil. Benzene exposure was in median 0.144 mg/m^3 (0.045 ppm) with a range of 0.058 to 2.207 mg/m^3 (0.018-0.680 ppm). Duration of occupational exposure was 119.8±12.8 months. The attendants showed decreased δ-aminolevuline dehydratase activity, reduced red blood cell counts and haemoglobin content, and increased neutrophil counts but within the reference values. Individual toluene exposure, age and cigarette smoking did not show significant influences on the multiple regression models. Biomonitoring measurements of exposed works showed relevant internal exposure with a median of 334 µg ttMA/g creatinine (range 190-600 µg/g creatinine). The corresponding values in the controls were 90 µg ttMA/g creatinine (range 50-120 µg/g creatinine). Comparing those data with the correlation published by DFG (2017a, b) indicates that internal benzene exposure would correlate to benzene concentrations in air of 0.3 ppm and higher. It cannot be excluded that ttMA from diet might have also contributed to the measured ttMA value. However, it can be assumed that dermal uptake of benzene contributed in large extend to total benzene exposure.

In a later study, **Moro *et al* (2017)** investigated 20 male and 20 female fuel filling station attendants compared to 20 male and 20 female controls (no occupational benzene exposure) in Brazil. Benzene exposure for males was 0.139 mg/m^3 (0.043 ppm), for females 0.124 mg/m^3 (0.038 ppm). Exposure ranges for males were 0.068 to 2.207 mg/m^3 (0.021-0.68 ppm) and for females 0.064 to 0.670 mg/m^3 (0.02-0.2 ppm). Duration of occupational exposure was 48.8±10.9 months for males and 44.0±9.8 months for females. In male, but not in female attendant, red blood cell count and haemoglobin were decreased and neutrophil count increased. In female attendants, but not in males, higher white blood cell count, and reduced counts in lymphocytes and eosinophils were observed. All mean haematological parameters were still within reference values, although individually, some workers showed levels outside the normal clinical range. It has to be noted that the control males and females were indicated as non-smokers, whereas in the male group were 40% smokers and the female group 10% smokers. Furthermore, similar to the study by Moro *et al* (2015), biomonitoring measurements of exposed works showed relevant internal exposure with a median of 330 µg ttMA/g creatinine (range 204-449 µg/g creatinine) for males and 461 µg ttMA/g creatinine (range 210-1070 µg/g creatinine) for females. The corresponding values in the controls were 89 µg ttMA/g creatinine (range 55-128 µg/g creatinine) for males and 204 µg/g creatinines (range 70-271 µg/g creatinine) in females. Comparing those data with the correlation published by DFG (2017a, b; see Table 10) indicates that internal benzene exposure would correlate to benzene concentrations in air of 0.3 ppm and higher, for females even higher than 1 ppm. It cannot be excluded that ttMA from diet might have also contributed to the measured ttMA value. However, it can be assumed that dermal uptake of benzene contributed in large extend to total benzene exposure.

In summary, information on haematological effects in workers exposed to gasoline are limited to one working group investigating fuel filling station attendants in Brazil. The biomonitoring data are indicating more than 10-fold higher internal benzene concentrations than air concentrations would result. Therefore, working conditions might have been insufficient. Furthermore, there were significant differences in smoking habits between exposed and controls. Consequently, those data are not suitable for a quantitative assessment of benzene effects.

Workers exposed to engine emissions

Maffei *et al* (2005) did not find a significant difference in haematological parameters (haemoglobin, haematocrit, platelets, white blood cell count, lymphocytes and neutrophils) in 49 traffic policemen (59% males; 35% smokers) exposed to 24.32±14.38 µg benzene/m^3 (0.0075±0.0044 ppm) compared to 36 indoor workers (58% males; 36% smokers) exposed to 4.39±0.99 µg benzene/m^3 (0.001±0.0003 ppm).

Casale *et al* (2016) investigated 215 workers (137 men and 78 women), including 112 traffic policemen, 69 police drivers and nine police motorcyclists plus 25 other external police activities, all of which occupationally exposed to urban pollutants. Benzene concentrations for 8 traffic policemen were measured with 0.017±0.010 mg/m^3, (0.005±0.003 ppm) and for 4 police drivers with 0.010±0.007 mg/m^3 (0.003±0.002 ppm). Blood benzene levels for those groups showed means and standard deviations of 337±287 and 304±365. In the publication, the dimension is not mentioned. However, limit of detection for the analysis was <150 ng/L. Multiple linear regression was performed on the total sample and the subgroups after confounding after the main confounding factors were evaluated. Blood benzene levels were reported by the authors to be significantly and inversely correlated with white blood cell, lymphocytes and neutrophil counts in subgroups with the exception of police drivers. No significant correlations were reported between the blood-benzene concentration and age or length of service, or between the blood-benzene concentration and other parameters of the blood counts. Although significant, changes in white blood cell count were still within the normal range. It is to be noted that no control group was included and the measured concentration of benzene in blood lies slightly above the 95 percentile for the general non-smoking population of 0.3 µg/L (Campagna *et al* 2014) and below the median benzene concentrations in blood for smokers of 0.8 µg/L (Campagna et al 2012). The authors noted that the blood benzene level was not correlated with smoking habits because the workers did not smoke or were not exposed to passive smoking for 5 days before and during the sampling. However, smoking before that time could have contributed to the increase in blood benzene and the effects. To appropriately investigate haematological effects of benzene at such low benzene concentrations, only non-smokers should have been investigated. Overall, this study is not suitable to investigate haematological effects at environmental benzene concentrations (0.005 ppm) because smoking habits were not controlled appropriately and smoking could have interfered with the effects observed.

Summary of haematological effects in workers

The studies reviewed investigating haematological effects in filling station attendants and studies in traffic personal are not considered in the overall evaluation. More specifically, results from a cohort of Brazilian fuel filling station attendants (Moro *et al* 2015, 2017) are not considered due to assumed additional dermal absorption. The results for traffic personnel (Casale *et al* 2016; Maffei *et al* 2005) cannot be considered as relevant to identify a dose-response for benzene because of the significant contribution of the complex mixtures of traffic/engine exhausts to the total exposure.

It is to be noted that all available studies have one or more shortcomings. None of the available studies controlled for co-exposure to other substances. Considering the individual shortcomings, the more reliable studies reviewed provided evidence for effects on haematological parameters at benzene concentrations of 1.7 ppm (Pesatori *et al* 2009), above 2 ppm (Lan *et al* 2004), at 2.3 ppm (Qu *et al* 2003a), and at 2.6 ppm (Koh *et al* 2015a, b). Other studies provide some evidence of effects at 1.6 ppm (Zhang *et al* 2016), at 2.0 ppm Ye *et al* (2015), at 7.6 ppm (Rothman *et al* 1996), and at 7.8 ppm (Schnatter *et al* 2010). Taking into account those results, a LOAEC in the order of 2 ppm seems to be plausible.

It has to be noted that it is more difficult to identify an appropriate benzene concentration leading to no statistically significant effects on haematological parameters than identifying statistically significant effects. One approach could be to apply the standard assessment factor 3 for extrapolation from LOAEC to NOAEC which would result in a NOAEC of 0.67 ppm. A more scientific approach is to perform a benchmark analysis. Based on the data from Qu *et al* (2003a) on neutrophils, The Lower Olefins and Aromatics REACH Consortium (LOA 2017b) calculated a BMDL of 0.43 ppm for a benchmark response of 5% using an exponential model. Furthermore, data in workers provide an indication for a NOAEC. A health surveillance study investigating data from 1200 benzene exposed workers (Tsai *et al* 2004) did not report adverse effects on haematological parameters at a mean benzene exposure of 0.6 ppm. Data from another health surveillance program reported no significant effects among 10,702 workers exposed up to 0.5 ppm (Koh *et al* 2015a). Taking into account those results, a NOAEC of 0.5 ppm seems to be plausible.

In the following table, the results of studies investigating workers exposed to benzene concentrations below 10 ppm are listed according to decreasing benzene concentrations.

Table 18: Summary of studies investigating haematological effects in workers

Benzene (ppm)	Result	Effects	Cohort, study characteristics, major shortcomings	Reference
7.6 (1-20)	(+)	Red. LYM (no other parameters investigated)	11/44 BZ exposed workers, 44 controls (workers, sewing machine factory), Shanghai, China, personal exposure sampling	Rothman *et al* 1995
7.8-8.2	(+)	Red. NEU;	928 BZ exposed workers; in total 1046 workers, Shanghai, China, no external controls; representative personal exposure samplings	Schnatter *et al* 2010
3.5	(−)	no effects on WBC, LYM, NEU, EOS, RBC, MCV, Hb, PLT		
2.61 (0.5-<6.0)	+	Red. RBC in males;	In total 10,702 BZ exposed workers, Korea, no controls; health surveillance data; job exposure assessment based on personal and air measurements; 67% measurements <0.01 ppm; not controlled for smoking; blood tests performed in more than 150 hospitals	Koh *et al* 2015a, b
0.001-**<0.5**	−	no effects on WBC, RBC, PLT, NEU, LYM		
2.0 (0.7-17.8)	(+)	Red. WBC (no other parameters investigated)	385 BZ exposed shoe factory workers, 220 controls (teachers, bank clerks), Zhejiang, China; personal exposure sampling, only WBC measured	Ye *et al* 2015
1.7* [0.3±0.2 4.9±5.3] *calculated	−	No effects on WBC, NEU, LYM, MONO, EOS, RBC, Hb, HCT, MCV, PLT, MVP	153 BZ exposed petrochemical workers, 50 controls (white collar workers, same plant), Bulgaria; personal exposure sampling	Pesatori *et al* 2009
1.6 (0.8-12.1)	(+)	Red. WBC (no other parameters investigated)	317 BZ exposed shoe factory workers, 102 controls (office personal, schools and banks), Zhejiang, China; personal exposure sampling, only WBC measured	Zhang *et al* 2016

Benzene (ppm)	Result	Effects	Cohort, study characteristics, major shortcomings	Reference
≥0.6 **(≥2[1)])**	+	Red. WBC, GRA, LYM, MONO, PLT; no effects on Hb	250 BZ exposed shoe factory workers, 140 controls (workers, clothes factories), Tianjin, China; personal exposure sampling; biomonitoring data indicate higher endogenous exposure, presumably due to dermal absorption	Lan *et al* 2004
2.26±1.35 8.67±2.44 19.9±3.1 51.8±43.3	+	Red. RBC, WBC, NEU; no effects on HCT, PLT, LYM	130 BZ exposed shoe factory workers, 51 controls (soybean production plant), Tianjin, China; personal exposure sampling	Qu *et al* 2003a, b
0.14 (0.0-0.5)	?	Red. RBC, WBC, NEU	Sub-group of 16 BZ exposed non-smoking female workers; result not reliable due to inappropriate controls (51 controls consisting of 53% females and 31% smokers)	
0.43	BMDL	BMDL (5% resp., exponential 4 model) for red. NEU	130 BZ exposed shoe factory workers, 51 controls (soybean production plant), Tianjin, China; personal exposure sampling	LOA 2017b (Qu *et al* 2003a, b)
0.65* 0.08-4.8 *calculated	(–)	No effects on WBC, Hb, HCT	121 BZ exposed petrochemical workers, 110 controls (“healthy people”), Shanghai, China; only stationary exposure sampling	Huang *et al* 2014
0.6 (0.1-5.7)	–	No effects on WBC, LYM, RBC, Hb, HCT, MCV, PLT	1200 BZ exposed workers, 3227 controls (workers, same plant), Shell, USA, health surveillance data, representative personal exposure samplings	Tsai *et al* 2004
0.55 (0.01-88) >2 (<5%)	(–)	No effect on lymphopenia; no other parameters investigated	387 BZ exposed workers, 553 controls (workers, same plant), Monsanto, USA; health surveillance data, personal exposure sampling, only lymphopenia investigated	Collins *et al* 1997
0.46 (0.19-23.9)	(–)	no effects on the proportion of EOS, MONO, LYM, NEU among LEU	158 BZ exposed petrochemical workers, 50 controls (office workers, same plant), Bulgaria; personal exposure sampling; investigated parameters have limited value for the evaluation of the dose-response for haematological effects of benzene	Seow *et al* 2012
	?	Incr. proportion of BAS among LEU;		
0.27±0.22 (0.005-2.0)	(–)	No effects on RBC, HCT, Hb, MCV, WBC, NEU, LYM, MONO, PVD; effects on MCHC and PLT (no dose-resp.)	61 BZ exposed workers, Korea, no external controls; personal exposure sampling	Kang *et al* 2005; Sul *et al* 2005
0.22 (0.01-1.9)	(–)	No effects on Hb, HCT, WBC, LYM, NEU;	701 BZ exposed workers, 1059 controls (workers, other departments), Dow, Netherlands, health surveillance data, job	Swaen *et al* 2010

Benzene (ppm)	Result	Effects	Cohort, study characteristics, major shortcomings	Reference
	?	red. EOS (small effect), incr. BAS, MONO	exposure matrix based only on air measurements	
0.09* (0.01-1.4) *calculated	(−)	No effects on RBC, Hb, PLT;	200 BZ exposed workers, 268 controls (workers, same plant), Monsanto, USA; exposure assessment only based on stationary sampling	Collins *et al* 1991
	?	incr. WBC, MCV (small effect)		
0.045 (0.018-0.68) (**≥0.3**[1)])	?	Red. WBC, Hb	60 BZ exposed male fuel filling station attendants (27% SM), 28 male controls (no occup. BZ exposure, all NS), Brazil, personal exposure sampling, biomonitoring data indicate higher endogenous exposure (≥0.3 ppm), presumably due to dermal absorption and insufficient working conditions	Moro *et al* 2015
0.043 (0.021-0.68) (**≥0.3**[1)])	?	Red. RBC, Hb	20 BZ exposed male fuel filling station attendants (40% SM), 28 male controls (no occup. BZ exposure, only NS), Brazil, personal exposure sampling, biomonitoring data indicate higher endogenous exposure (≥0.3 ppm), presumably due to dermal absorption and insufficient working conditions, relevant differences in smoking habits	Moro *et al* 2017
0.038 (0.02-0.2) (**≥1**[1)])	?	Inc. WBC, red. LYM, EOS	20 BZ exposed female fuel filling station attendants (10% SM), 28 female controls (no occup. BZ exposure, only NS), Brazil, personal exposure sampling, biomonitoring data indicate higher endogenous exposure (≥1 ppm), presumably due to dermal absorption and insufficient working conditions, relevant differences in smoking habits	
0.008± 0.004	(-)	No effects on Hb, HCT, PLT, WBC, LYM, NEU	49 traffic policemen (59% M; 35% SM), 36 controls (indoor workers; 58% males; 36% smokers), Italy, personal exposure sampling	Maffei et al 2005
0.005± 0.003 (n=8); 0.003± 0.002 (n=4)	?	Blood BZ level negatively correlated with WBC, LYM, NEU	112 traffic policemen (69% M; 25% SM), 69 police drivers (43% M; 38% SM), 9 police motorcyclists (100% M; 22%SM), 26 policemen with other outdoor activities (72% M; 40% SM) no external controls; WBC, LYM, NEU correlated with blood BZ levels but smoking might have interfered with the result	Casale *et al* 2016

Abbreviations: ?: questionable, not relevant or not reliable; +: positive; (+): positive with relevant uncertainties; −: negative; (−): negative with relevant uncertainties; BAS: basophiles; BZ: benzene; F: females; GRA: granulocytes; Hb: haemoglobin; HCT: haematocrit; Incr.: increased; LEU: leukocytes; LYM: lymphocytes; M: males; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MONO: monocytes in WBC; MPV: mean platelet volume, NEU: neutrophils; NS: non-smoker; PLT: platelet count; RCB: red blood cell count; Red.: reduced; SM: smoker; WBC: white blood cell count

[1)] considering the biomonitoring results, total benzene exposure could have been higher (possibly due to dermal uptake); the indicated value is based on a correlation.

IMMUNOLOGICAL EFFECTS

Benzene has been shown to have adverse immunological effects (humoral and cellular immunological suppression) in humans following inhalation exposure for intermediate and chronic durations. Adverse immunological effects in animals occur following both inhalation and oral exposure for acute, intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular (leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene causes decreases in the levels of circulating leukocytes in workers at levels of 30 ppm and decreases in levels of circulating antibodies in workers exposed to benzene at 3 to 7 ppm. Other studies have shown decreases in human lymphocytes and other blood elements after exposure; these effects have been seen at occupational exposure levels as low as 1 ppm or less. Animal data support these findings. Both humans and rats have shown increases in leukocyte alkaline phosphatase activity. No studies regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene through ingestion or dermal contact could cause immunological effects similar to those seen after inhalation exposure in humans and inhalation and oral exposure in animals (ATSDR 2007).

Lan *et al* (2004) investigated 250 workers in two shoe factories in Tianjin, China, and 140 controls who worked in three clothes-manufacturing factories in the same region (see also description of this study under “Haematotoxicity”). A statistically significant trend was observed with increasing benzene concentration in the air for the reduction in $CD4^+$-T cells, $CD4^+/CD8^+$ ratio, and NK cells. Already in the lowest exposure group the reduction in $CD4^+$-T cells and the $CD4^+/CD8^+$ ratio were statistically significant. In this lowest exposure group, concentration of benzene in air was given with 0.57±0.24 ppm. However, it has to be noted that the concentrations of benzene measured in urine for this group (13.4 µg/L) shows that endogenous benzene exposure was higher than the measured concentrations on benzene in the air would indicate. Considering the correlation between external and internal benzene exposure as published by DFG (2017a, b; see Table 8), urinary benzene concentration of 13.4 µg/L urine corresponds to a benzene concentration in the air of higher than 2 ppm.

Huang *et al* (2014) investigated 121 petrochemical in China for platelet parameters and platelet-associated antibodies compared to 110 controls (“*healthy people*”) matched for age, sex, nationality, education level, alcohol consumption, smoking and history of blood transfusion. Exposure to benzene was reported with 0.25 to 15.7 mg/m^3 (0.08-4.8 ppm). No statistically significant effects were reported on white blood cell count, haemoglobin, and platelet count. Significant effects on platelet-associated (PA)IgA and PAIgM were observed at the two higher exposure groups 6 to 40 mg/m^3-years and ≥40 mg/m^3-years, but not at the lower exposure group <6 mg/m^3-years. PAIgA, but not PAIgM levels were also significantly affected by smoking and dringking. Since stratification was not performed with regard to current exposure levels, the result cannot be used to derive a NOAEC or LOAEC.

Uzma *et al* (2010) examined 428 fuel filling station attendants in India exposed 12 hours per day to 0.345 ppm benzene (range 0.118–0.527 ppm) and 78 unexposed controls matched for socioeconomic status, age and gender. Concentration of benzene was increased in blood and urine and signs of oxidative stress were observed (e.g., increased reactive oxygen species and malondialdehyde formation, decreased glutathione and total superoxide dismutase concentrations). A statistically significant decrease in the immunoglobulin levels, CD4-T cells, CD4/CD8 ratio was observed in workers compared to the controls, whereas no significant difference was observed for CD8-T cells. P53 gene expression was markedly higher in workers than in controls. Benzene concentrations in urine for exposed workers were reported post-shift with 42.6 nmol/L (3.3 µg/L) and pre-shift with 9.8 nmol/L (0.8 µg/L). The shift-related increase in benzene concentration in mean of 2.5 µg/L correlates to about 0.3 ppm benzene in air (see Table 8) and hence,

confirms the measurement of benzene in air. However, 0.345 ppm has to be considered as 12-h TWA; the respective 8-h TWA would be 0.52 ppm. It is to be noted that the control group consisted of a significant lower number (n=78) compared to the exposed group (n=428) and was matched only for socioeconomic status, age and sex. Furthermore, co-exposure to other substances has not been considered.

Moro *et al* (2015) investigated 60 males fuel filling station attendants in Brazil exposed to median benzene concentrations of 0.044 ppm (range 0.018-0.680 ppm) and reported decreased CD80 and CD86 expression in monocytes and increased IL-8 levels compared to 28 non-smoking male control subjects. Furthermore, according to multiple linear regression analysis, benzene exposure was associated with a decrease in CD80 and CD86 expression in monocytes. Biomonitoring measurements of exposed workers showed relevant internal exposure with a median of 334 µg ttMA/g creatinine (range 190-600 µg/g creatinine). The corresponding values in the controls were 90 µg ttMA/g creatinine (range 50-120 µg/g creatinine). Comparing those data with the correlation published by DFG (2017a, b) indicates that internal benzene exposure would correlate to benzene concentrations in air of 0.3 ppm and higher. It cannot be excluded that ttMA from diet might have also contributed to the measured ttMA value. However, it can be assumed that dermal uptake of benzene contributed in large extend to total benzene exposure.

It is to be noted that reduction of immune function can have various reasons and may be related not only to benzene exposure, but also to exposure to other substances occurring at the workplaces like diesel exhaust particulates (Siegel *et al* 2004), smoking and alcohol drinking (Huang *et al* 2014), or stress (e.g., Yoon *et al* 2014). Without proper matching exposed persons with appropriate controls, no meaningful conclusion would be possible.

In the following table, the results of studies investigating workers exposed to benzene concentrations below 10 ppm are listed according to decreasing benzene concentrations.

Table 19: Summary of studies investigating immunological effects in workers at lower benzene concentrations

Benzene (ppm)	Result	Effects	Cohort, study characteristics, major shortcomings	Reference
≥0.6 (**≥2**[1)])	(+)	Red. $CD4^+$-T cells, $CD4^+/CD8^+$ ratio, and NK cells	250 BZ exposed shoe factory workers, 140 controls (workers, clothes factories), Tianjin, China; control for age and sex; personal exposure sampling, biomonitoring data indicate higher endogenous exposure, presumably due to dermal absorption	Lan *et al* 2004
0.65* 0.08-4.8 *calculated	(+)	Incr. in PAIgA and PAIgM; no effects on PAIgG	121 BZ exposed petrochemical workers, 110 controls (“healthy people”), Shanghai, China; only stationary exposure sampling; control for age, sex, nationality, education level, alcohol consumption, smoking and history of blood transfusion; stationary exposure sampling	Huang *et al* 2014
12-h TWA: 0.35 (0.12-0.53) calculated as 8-h TWA: 0.52	(+)	Red. IgG, $CD4^+$-T cells, $CD4^+/CD8^+$ ratio	428 BZ exposed male non-smoking fuel filling station attendants, 78 non-smoking controls (no further information); personal exposure sampling, control for socioeconomic status, age, sex; personal exposure sampling	Uzma *et al* 2010

Benzene (ppm)	Result	Effects	Cohort, study characteristics, major shortcomings	Reference
0.045 (0.018-0.68) (**≥0.3**[1)])	(+)	Red. CD80 and CD86 expression in monocytes; incr. IL-8 levels	60 male fuel filling station attendants (27% SM), 28 male controls (all NS), Brazil, personal exposure sampling, biomonitoring data indicate higher endogenous exposure (≥0.3 ppm), presumably due to dermal absorption and insufficient working conditions, control for smoking questionable	Moro *et al* 2015

Abbreviations +: positive; −: negative; BZ: benzene; PAIgA/M/G: platelet-associated immunoglobulin A/M/G;

[1)] The measured concentration of benzene in the air was 0.57 ppm; however, based on the measured benzene concentration in urine, total benzene exposure was higher correlating to a benzene concentration in the air of higher than 2 ppm.

Conclusion

The studies reviewed show effects on the immune system (see Table 19) at similar benzene exposure levels, for which also haematological effects have been reported. However, the studies reviewed are not suitable to derive NOAECs or LOAECs for immunological effects of benzene mainly due to insufficient control groups.

7.3.2 Animal data

Haematological effects

Animal studies support the findings in humans. Significantly reduced counts for all three blood factors (white blood cells, red blood cells, and platelets); and other evidence of adverse effects on blood-forming units (reduced bone marrow cellularity, bone marrow hyperplasia and hypoplasia, granulocytic hyperplasia, decreased numbers of colony-forming granulopoietic stem cells and erythroid progenitor cells, damaged erythrocytes and erythroblast-forming cells) have been observed in animals at benzene concentrations in the range of 10 ppm to 300 ppm and above (ATSDR 2007).

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. 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 benzene concentrations above 10 ppm (DECOS 2014).

Immunological effects

Animal studies have shown that benzene decreases circulating leukocytes and decreases the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been demonstrated in animals exposed for acute, intermediate, or chronic periods via the inhalation route. This decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice following intermediate inhalation exposure to 100 ppm of benzene. The impaired cellular immunity after benzene treatment was observed both *in vivo* and *in vitro*. Mice exposed to 100 ppm for a total of 100 days were challenged with 104 polyoma virus-induced tumour cells (PYB6). Nine of 10 mice had reduced tumour resistance resulting in the development of lethal tumours. In the same study, lymphocytes were obtained from spleens of benzene-treated mice and tested for their immune capacity *in vitro*. The results showed that two other immune functions,

alloantigen response (capacity to respond to foreign antigens) and cytotoxicity, were also impaired. Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods, and in rats and mice exposed for chronic time periods. A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed. Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours/day for 30 days. The acute-duration inhalation MRL was based on a study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure of mice to benzene vapours at a concentration of 10 ppm, 6 hours/day for 6 days. The intermediate-duration inhalation MRL was based on a study showing delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapours at a concentration of 10 ppm, 6 hours/day, 5 days/week for a total of 20 exposures. Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system (ATSDR 2007).

7.3.3 *In vitro* data

Due to the availability of information on systemic effects in human and animals, potential published *in vitro* data are not reported here.

7.3.4 Summary

As regards toxic effects, high exposure levels cause central nervous system depression followed by cyanosis, haemolysis, and congestion or haemorrhage of organs. At chronic exposure to lower levels (in the order of 10 ppm), haematological and immunological effects are observed in experimental animals.

Also in humans, benzene affects these systems, and haematological and immunological effects have accordingly been observed in workers in several studies which have their strengths and weaknesses.

The studies reviewed investigating haematological effects in filling station attendants and studies in traffic personal are not considered in the overall evaluation. More specifically, results from a cohort of Brazilian fuel filling station attendants (Moro *et al* 2015, 2017) are not considered due to assumed additional dermal absorption. The results from one study in traffic personnel (Casale *et al* 2016; Maffei *et al* 2005) cannot be considered as relevant to identify a dose-response for benzene because of the significant contribution of the complex mixtures of traffic/engine exhausts to the total exposure.

Several studies are available investigating haematological effects in benzene exposed industrial workers. Of highest evidence are studies investigating all relevant haematological parameters in a larger group of workers (preferable >100) for which appropriate risk management measures have been in place to prevent excessive dermal exposure, which used an appropriate control group (industrial workers, considering relevant confounders like gender and smoking), which used personal exposure sampling to monitor benzene exposure, which excluded workers with previous higher benzene exposure, and in which an appropriate regression analysis was performed with control for confounding factors. It is to be noted that all available studies have one or more shortcomings. None of the available studies controlled for co-exposure to other substances. Considering the individual shortcomings, the more reliable reviewed studies provided evidence for effects on haematological parameters at benzene concentrations of 1.7 ppm (Pesatori *et al* 2009), above 2 ppm (Lan *et al* 2004), at 2.3 ppm (Qu *et al* 2003a), and at 2.6 ppm (Koh *et al* 2015a, b). Other studies provide some evidence of effects at 1.6 ppm (Zhang *et al* 2016), at 2.0 ppm Ye *et al* (2015), at 7.6 ppm (Rothman *et al* 1996),

and at 7.8 ppm (Schnatter *et al* 2010). Taking into account those results, a LOAEC in the range of 2 ppm seems to be plausible.

It has to be noted that it is more difficult to identify an appropriate benzene concentration leading to no statistically significant effects on haematological parameters than identifying statistically significant effects. One approach could be to apply the standard assessment factor 3 for extrapolation from LOAEC to NOAEC which would result in a NOAEC of 0.67 ppm. A more robust approach is to perform a benchmark analysis. Based on the data from Qu *et al* (2003a) on neutrophils, LOA (2017b) calculated a BMDL of 0.43 ppm for a benchmark response of 5%. A health surveillance study investigating data from 1200 benzene exposed workers (Tsai *et al* 2004) did not report adverse effects on haematological parameters at a mean benzene exposure of 0.6 ppm. Data from another health surveillance program reported no significant effects among 10,702 workers exposed up to 0.5 ppm (Koh *et al* 2015a). Taking into account those results, a NOAEC in the range of 0.5 ppm seems to be plausible.

Only limited immunological studies are available to draw conclusions on effect levels of benzene immunotoxicity. It seem however plausible that adverse effects on the immune system, e.g. an altered CD4/CD8 cell ratio, are caused by similar concentrations of benzene as the observed haematological suppression, as indicated by available studies (Uzma *et al* 2010, Lan *et al* 2004).

7.4 Irritancy and corrosivity

7.4.1 Human data

Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapours (ATSDR 2007).

In humans, benzene is a skin irritant. By defatting the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis. Acute fatal exposure to benzene vapours caused second degree burns on the face, trunk, and limbs of the victims. Fifteen male workers were exposed to benzene vapours (>60 ppm) over several days during the removal of residual fuel from shipyard fuel tanks. Exposures to benzene range from 1 day to 3 weeks (mean of 5 days), 2.5 to 8 hours/day (mean of 5.5 hours). Workers with more than 2 days (16 hours) exposure reported mucous membrane irritation (80%), and skin irritation (13%) after exposure to the vapour (ATSDR 2007).

Solvent workers who were exposed to 33 ppm benzene (men) or 59 ppm benzene (women) exhibited eye irritation while being exposed to the vapours (ATSDR 2007).

7.4.2 Animal data

Benzene has been shown to be irritating to the skin of rabbits, inducing moderate erythema, oedema, and moderate necrosis following application. Benzene can also cause irritation of the mucous membranes (eye, respiratory tract and mouth, oesophagus and stomach) (DECOS 2014).

7.4.3 *In vitro* data

Due to the availability of information on skin and eye irritation in animals and humans, potential published *in vitro* data are not reported here.

7.4.4 Summary

Benzene has been shown to be irritating to the skin of rabbits, inducing moderate erythema, oedema, and moderate necrosis following application. Benzene can also cause irritation of the mucous membranes (eye, respiratory tract and mouth, oesophagus and stomach) (DECOS 2014).

In humans, dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapours (ATSDR 2007).

Benzene has a harmonised classification for skin irritation 2 (H315) and eye irritation 2 (H319).

7.5 Sensitisation

7.5.1 Human data

Benzene exposure is not associated with skin or respiratory sensitisation in humans.

7.5.2 Animal data

The skin sensitisation potential of benzene was assessed in a mouse ear swelling test (MEST) and a reduced guinea pig maximisation test (GPMT) using neat benzene. None of the mice and none of the guinea pigs showed any evidence of sensitisation (Gad *et al* 1986).

7.5.3 *In vitro* data

No relevant *in vitro* data on sensitisation are available.

7.5.4 Summary

Benzene did not show a skin sensitizing potential in mice.

7.6 Genotoxicity

There is evidence that benzene and its metabolites induce the following genotoxic effects in humans and in experimental animals (DECOS 2014; Whysner *et al* 2004):

- Micronuclei (MN);
- Structural chromosomal aberrations (CA);
- Numerical chromosomal aberrations (aneuploidy);
- Sister chromatid exchange (SCE);
- DNA strand breaks;
- Mutations (primary mechanisms and secondary mechanisms via oxidative damage and error-prone DNA repair).

7.6.1 Human data

The focus of this section is on studies investigating genotoxic effects in workers exposed to benzene concentrations in the range of 1 ppm and below and for which the benzene concentration in air has been measured.

Multiple studies are available investigating genotoxic effects in benzene exposed workers. Of highest evidence are studies investigating a larger group of workers (preferable >100) for which appropriate risk management measures have been in place to prevent excessive dermal exposure, which used an appropriate control group (industrial workers), that considered relevant confounders for the endpoint and method used (e.g., gender, smoking

or age), which used personal exposure sampling to monitor benzene exposure, which excluded workers with previous higher benzene exposure, and in which an appropriate regression analysis was performed with control for confounding factors.

Workers in shoe and glue factories

Studies investigating genotoxic effects in shoe factory workers are described in Appendix 1, Table 38.

Shoe factories in Tianjin, China

Qu *et al* (2003a) investigated 130 workers in two shoe factories in Tianjin, China, among others for chromosomal aberrations and aneuploidy. Benzene exposure ranged from 0.06 to 122 ppm Limit of detection for benzene in air was 0.01 ppm. Workers with blood cell counts below the normal range were not included in the study. From each participant of the study, current-day exposure level and 4-week mean exposure levels were measured using personal samplers and lifetime cumulative exposure levels were estimated. 51 workers from a nearby soybean production plant were used as controls (47% males, 31% smokers). Exposure was categorised according to a 4-week mean benzene exposure with 2.26±1.35 ppm (n=73; 45% males, 36% smokers); 8.67±2.44 ppm (n=33; 67% males, 55% smokers); 19.9±3.1 ppm (n=8; 22% males, 0% smokers); 51.8±43.3 ppm (n=16; 37% males, 38% smokers)and according to lifetime cumulative exposure with 16.0±8.0 ppm-years; 40.8±6.0 ppm-years; 73.9±14.4 ppm-years; 187±117 ppm-years. The authors report significant exposure-response trends for chromatid breaks, total chromatid-type aberrations, total chromosomal-type aberrations, and total aberrations compared to controls. These were primarily due to an increase in chromatid breaks, although other aberration categories such as chromosomal breaks appeared to contribute to the trend. The author also observed a modest increase in the mitotic index (metaphases per 1000 cells) with increasing benzene exposure. This was almost entirely due to an increase in the number of metaphases observed in the samples from workers with the highest exposure (see Table 20 below). When adjustments were made for possible confounding variables (sex, age, toluene exposure and smoking based on cotinine levels) and benzene exposure was treated as a continuous variable, the authors found moderate associations of benzene exposure with chromatid gaps and chromosomal breaks, but not for any of the grouped categories of total chromatid aberrations, total chromosomal aberrations, or total aberrations. The aneuploidy data revealed that only hypodiploidy (45 chromosomes) had a positive association with benzene exposure (see Table 28 of Qu *et al* 2003a). Further analyses showed that both hypodiploidy (45 chromosomes) and aneuploidy (45 or 47 chromosomes) were strongly associated with exposure intensity (mean benzene exposure level per year), but not with exposure duration (see Table 16 of Appendix A of Qu *et al* 2003a). **This study demonstrates a significant exposure-response trend for clastogenic and aneugenic effects with 2.3±1.4 ppm as the lowest investigated concentration**.

Table 20: Chromosomal aberrations and aneuploidy in relation to benzene exposure (Table 27 of Qu et al 2003a)

	Controls	>0-5 ppm	>5-15 ppm	>15-30 ppm	>30 ppm
Mean benzene exposure (ppm; 4-week mean)	0.004±0.003	2.26±1.35	8.67±2.44	19.9±3.1	51.8±43.3
No. of subjects	51	73	33	8	16
Females (%)	53	55	33	88	63
Smokers (%)	31	36	55	0	38

	Controls	>0-5 ppm	>5-15 ppm	>15-30 ppm	>30 ppm
Total chromatid aberrations (excluding gaps)[B]	1.20±1.4	2.01±1.5	2.00±1.4	1.43±1.3	2.25±1.8
Total chromosomal aberrations (excluding gaps)[A]	0.59±1.3	0.97±1.2	0.74±1.0	0.71±1.3	1.44±1.3
Total aberrations (excluding gaps)[B]	1.78±2.1	2.99±2.1	2.74±2.0	2.14±2.1	3.69±2.5
Chromatid breaks[B]	1.20±1.4	1.99±1.5	2.00±1.4	1.43±1.3	2.25±1.8
Hypodiploidy – 45 chromosomes	0.22±0.54	0.12±0.33	0.39±0.84	0.29±0.49	0.38±0.72
Total aneupolidy (≤45, 47 chrom.)	3.0±2.7	7.3±7.2	8.1±9.5	5.8±5.1	3.9±3.5
Metaphases/1000 cells[B]	45±26	49±25	47±20	45±22	72±27

[A] p≤0.05, test for exposure-response trend
[B] p≤0.01, test for exposure-response trend

Qu *et al* (2003a) also investigated a small group exposed to 0.14±0.04 ppm benzene and reported positive associations for total chromatid aberrations, total chromosomal aberrations, total aberrations, chromatid breaks, and acentric fragments. No changes were observed for any type of aneuploidy. However, this group consisted of only 16 individuals, all non-smoking women, whereas only about half of the control subjects were female of which 31% smoked. Since there has not been a correction for gender and smoking, this effect level needs to be interpreted with caution and cannot be used for the evaluation (see also comments on Qu *et al* 2003a in section 7.3.1. of this report).

Further studies have been published that investigate smoking and non-smoking workers from factories in Tianjin, China.

Xing *et al* (2010) and **Ji *et al* (2012)** investigated 33 male workers exposed to either 1.0±2.6 ppm (n=17) or 7.6±2.3 ppm benzene (n=16) for aneuploidies compared to 33 matched controls working in other factories. Geometric mean and standard deviation for benzene in urine was 4.2±2.5 µg/L and 50±3.1 µg/L for the low and high exposure groups, respectively. Numerical abnormalities were examined in chromosomes X, Y and 21 using multicolour fluorescence *in situ* hybridization (FISH) in sperm and peripheral lymphocytes. Exposed workers and controls were compared for several general characteristics (e.g., body mass index, alcohol, tea and cola drinking habits, chronic diseases, education, smoking, fruit and vegetable) and characteristics with potential impact on sperm (e.g., abstinence, hot baths, bicycle riding). Benzene exposure was measured in the air by personal samplers for one 8-hour workday shift. Benzene and ttMA were measured in the urine one month before examination. A second air and urine sample was taken before the examination. Xing *et al* (2010) applied adjusted negative binomial regression models and reported that rates of overall hyperhaploidy, hypohaploidy, disomy X, disomy Y, and other anomalies were significantly higher among exposed men than unexposed men. The authors further report that the incidence rate of hyperhaploidy was 1.6 times higher for men in the low-exposed group (p=0.03) and 2.3 times higher for men in the high-exposed group (p <0.001) after adjusting for age, smoking, hot baths, tea drinking, fruit and vegetable intake, and history of chronic disease (p_{trend} across three exposure groups, <0.001). This finding was driven by the strong association between benzene exposure and disomy X and to a lesser extent by disomy Y. Ji *et al* (2012) further reported a gain of

chromosome 21 in peripheral blood lymphocytes at both exposure groups but not of chromosomes X or Y. According to the authors the findings suggest that benzene exposure induces aneuploidies in both blood cells and sperm within the same individuals, but selectively affects chromosome 21 in blood lymphocytes and the sex chromosomes in sperm. **This study indicates a LOAEC of 1.0±2.6 ppm**.

In a further publication of the same group **Marchetti *et al* (2012)** investigated 30 benzene exposed male workers (77% smokers) and 11 male controls (73% smokers) for structural aberrations in chromosome 1 in sperm (partial chromosomal duplications or deletions of 1cen or 1p36.3 or breaks within 1cen-1q12) using fluorescence *in situ* hybridization (FISH) technique. Duration of abstinence before semen collection was similar between exposed (6.6±3.7 days) and control group (6.4±5.4 days). Benzene exposure was measured in the air by personal samplers for one 8-hour workday shift. Benzene and ttMA were measured in the urine one month before examination. A second air and urine sample was taken before the examination. Workers were grouped based on benzene exposure: 1.0±2.6 ppm (n=10), 3.0±3.4 ppm (n=10), and 7.6±2.2 ppm (n=10). The population characteristics of the exposed and unexposed men was published in a supplement document which was not accessible. However, it seems that similar criteria as described by Xing *et al* (2010) were applied. Adjustments were made with respect to age, smoking, alcohol consumption and history of chronic diseases. Adjusted incidence rate ratios (IRRs) and 95% confidence intervals (CI) for all structural aberrations combined were 1.42 (95% CI: [1.10-1.83]), 1.44 (95% CI: [1.12-1.85]), and 1.75 (95% CI: [1.36-2.24]) for men with low, moderate, and high exposure, respectively, compared with unexposed men. For deletion of 1p36.3 alone respective IRRs were 4.31 (95% CI: 1.18-15.78), 6.02 (95% CI: 1.69-21.39), and 7.88 (95% CI: 2.21-28.05). Chromosome breaks were significantly increased in the high-exposure group with IRR 1.49 (95% CI: 1.10-2.02). The author conclude that the sperm findings point to benzene as a possible risk factor for *de novo* 1p36 deletion syndrome. **This study indicates a LOAEC of 1.0±2.6 ppm.**

Zhang *et al* (2011) examined 47 benzene exposed workers in comparison to 27 controls for aneuploidies in peripheral blood lymphocytes by using a novel OctoChrome fluorescence *in situ* hybridization (FISH) technique that simultaneously detects aneuploidy in all 24 chromosomes. Workers were grouped according to their current benzene exposure with either <10 ppm (4.95±3.61 ppm; n=22) or ≥10 ppm (28.3±20.1 ppm; n=25). Gender, current smoking, current alcohol drinking and recent respiratory infections were reported for exposed workers and controls. In the control group, age of the subjects was slighly lower (31.7 versus 35.3 and 35.2 years for control, low dose and high dose groups, respectively), the percentage of male subjects was higher (44 versus 23 and 36%), and the percentage of smokers was higher (37 versus 9 and 28%). Personal air full shift monitoring was performed once a month for 3 to 4 months before blood sampling. The authors reported heterogeneity in the monosomy and trisomy rates of the 22 autosomes when plotted against continuous benzene exposure. In addition, statistically significant, chromosome-specific increases in the rates of monosomy (5, 6, 7, 10, 16 and 19) and trisomy (5, 6, 7, 8, 10, 14, 16, 21 and 22) were found to be dose dependently associated with benzene exposure. Furthermore, significantly higher rates of monosomy and trisomy were observed in a priori defined 'susceptible' chromosome sets compared with all other chromosomes. The authors conclude that these findings confirm that benzene exposure is associated with specific chromosomal aneuploidies in haematopoietic cells, which suggests that such aneuploidies may play roles in benzene-induced leukemogenesis. The authors discuss that many of the significant aneuploidies have been demonstrated in leukemia patients. Loss of whole chromosomes 5 or 7 (—5/—7) or of the long arms of the two chromosomes (5q—/7q—) is the most common unbalanced aberrations in de novo and therapy-related myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). Trisomy 8 is the most frequent numerical aberration in AML and MDS, occurring at a

frequency of 10 to 15%. Trisomy 21 is the second most common trisomy in AML and MDS, occurring at a frequency of about 3%. Trisomies 6, 10, 14 and 16 have all been reported to be non-random numerical anomalies of myeloid disorders. The authors consider that these benzene-induced and leukemia-relevant aneuploidies occur in healthy workers with current exposure to benzene as demonstrated in the present study, as well as in benzene-related leukemia and preleukemia patients, which suggests that aneuploidy precedes and may be a potential mechanism underlying benzene-induced leukemia. Aneuploidy of chromosomes 5 and 7 may also be a mechanism underlying therapy-related AML cases, which arise after treatment with the alkylating drugs such as melphalan. **This study indicates a LOAEC of 5.0±3.6 ppm.**

Zhang *et al* (2012) investigated monosomy and trisomy of chromosomes 7 and 8 by fluorescence *in situ* hybridization (FISH) in interphase colony-forming unit–granulocyte-macrophage (CFU-GM) cells cultured from otherwise healthy benzene-exposed (n=28) and unexposed (n=14) workers. Workers were grouped according to their current benzene exposure with either <10 ppm (2.64±2.70 ppm; n=18) or ≥10 ppm (24.19±10.6 ppm benzene; n=10). Benzene concentrations in urine were 0.25±0.61 µg/L for controls and 66±138 µg/L and 897±874 µg/L for the low and high exposure groups. Gender, current smoking, current alcohol drinking and recent respiratory infections were reported for exposed workers and controls. In the control group, age of the subjects was slighly higher (32.3 versus 28.9 and 31.2 years for control, low dose and high dose groups), the percentage of male subjects was different (36 versus 11 and 60%), and the percentage of smokers was different (21 versus 11 and 50%). For each subject, individual benzene and toluene exposure was monitored repeatedly for up to 16 months before blood sampling, and post-shift urine samples were collected from each subject during the week before blood sampling. In workers exposed to 2.64±2.70 ppm and 24.19±10.6 ppm benzene, monosomy 7 and 8 (but not trisomy) in the myeloid progenitor cells was significantly increased. The author conclude that those findings provide a mechanistic basis for leukaemia induction by benzene. Further, the degree of monosomy induction was greater than the proportionate decline in peripheral blood cell counts, suggesting that it may be a more sensitive biomarker of benzene exposure. **This study indicates a LOAEC of 2.6±2.7 ppm**. However, considering the biomonitoring results, total benzene exposure could have been higher, possibly due to dermal uptake. According to the correlation as published by DFG (2017a, b; see Table 8), the measured urinary benzene concentration of 66±138 µg/L, correlates to a benzene concentration of much higher than 2.6 ppm.

Shoe factories in Wenzhou, China

Zhang *et al* (2014) investigated 385 workers (49% males; 24% smokers; 42% >30 years old) in shoe factories in Wenzhou, China, and 197 healthy controls (“indoor” workers and teachers; 49% males, 10% smokers; 55% >30 years old) on the association between inheritance of certain benzene metabolizing genes and the induction of micronuclei determined in the cytokinesis-block micronucleus assay in lymphocytes. Benzene exposure was measured using stationary air samples in different worksites of the plant. It is mentioned in the publication that such sampling in the different worksites was done three times during the study. However, no information is given on how these sampling times corresponded to the entire career duration of the exposed workers in the plant, i.e. if exposures in the past could also be estimated based on measurements. Median benzene concentrations were reported to be 6.4 mg/m^3 (2.0 ppm) with a range of 2.6 to 57.0 mg/m^3 (0.8-18 ppm). Calculation of the cumulative exposure dose for each worker was estimated based on the work history, work location and work duration at the plant, and current exposure levels. Workers were allocated to groups according to their current exposure of <3.25 mg/m^3 (<1.0 ppm; n=24), <6.0 mg/m^3 (<1.8 ppm; n=149), and ≥6.05 mg/m^3 (≥1.8 ppm; n=212). Benzene-exposed workers in all exposure groups had significantly increased micronucleus frequency compared with the controls. A dose-response with cumulative exposure (covering the entire exposure duration in the plant) was observed. The presence of two promoter polymorphisms in the CYP2E1 gene were correlated with the induction of micronuclei. It is to be noted that the mean micronucleus frequency was

quite different between the exposed and controls. While the controls had a mean frequency of 1.92±1.44 ‰, the frequency was 3.29±1.40 ‰, 3.11±1.92 ‰, and 3.45±1.91 ‰ for the current benzene exposure groups of <1 ppm, <1.8 ppm, and ≥1.8 ppm, respectively. I.e. there was only a modest increase, if any, in the micronucleus frequency according to increasing exposure while there was a much more pronounced difference between the exposed and the controls. Hence, this might be due to insufficient exposure assessment (only stationary but no personal air or biomonitoring measurements), or there might have been a systematic confounder between exposed and controls, or within the exposure groups, or confounding by other substances that could lead to micronucleus formation. Furthermore, the number of workers in the lower exposure groups (<1 ppm) was limited with 24. Due to the insufficient exposure assessment and the unclear dose-response for micronuclei, the statificantion into the different exposure groups cannot be considered as reliable. **Overall, this study indicates a LOAEC of 2.0 ppm (range 0.8-18 ppm).**

In a further publication of the same group, **Zhang *et al* (2016)** investigated a group of 317 exposed workers and 102 controls (office employees from local banks and schools) for white blood cell count and micronucleus frequency determined in the cytokinesis-block micronucleus assay in lymphocytes. The exposed group included 87 smokers (27%) while the original control group included 8 smokers (8%). Due to the benzene exposure history of those 8 persons, they were assigned to a cumulative exposure group. Hence, for the analysis on cumulative benzene exposure, all controls were non-smokers. The measured ambient benzene air concentration ranged from 0.80 to 12.09 ppm with a median of 1.60 ppm. For the sewing department median benzene concentrations were 1.57 ppm (range 0.8-3.78 ppm) with median years of service of 3.0 years (range 1.2-16.9 years). For the moulding department median benzene concentrations were 2.60 ppm (range 0.83-12.09 ppm) with median years of service of 2.9 years (range 1.0-18.3 years). For the packaging department median benzene concentrations were 1.79 ppm (range 0.8-4.25 ppm) with median years of service of 3.0 years (range 1.0-21.2 years). Cumulative exposure concentrations of benzene were calculated by ambient air benzene concentration at worksites in conjunction with job type and associated service duration resulting in exposure groups with median cumulative benzene exposures of 3.55, 6.51, 10.72, 20.02, and 40.71 ppm-years. The micronucleus frequency was significantly increased in all cumulative exposure groups and showed an increase with increasing cumulative exposure, whereas the reduction in white blood cell count was statistically significant only in the 3 higher cumulative exposure groups. Workers older than 30 years were more susceptible to abnormal micronucleus frequency and to white blood cell count reduction than workers younger than 30 years. According to the authors, other factors (gender, smoking, drinking) did not show a significant impact. Based on the results of the cumulative exposure, the authors estimated BMDLs (age-pooled) using log probit model for elevated micronucleus frequencies and reduced white blood cell counts considering 40 years of exposure. For elevated micronucleus frequency (10% excess risk), the BMD was calculated with 0.72 ppm-years and the BMDL with 0.06 ppm-years. The authors also calculated a dose-response model (Hill model) with resulting BMD of 1.85 ppm-years and BMDL of 0.22 ppm-years, corresponding to 10% excess risk. Taking as point of departue (PoD) the estimated BMDL derived from log-probit models for elevated micronucleus frequencies and dividing by 40 year of employment, the authors calculated a threshold limit value (age pooled) of 0.0015 ppm. However, it is to be noted that the median duration of employment was only 3 years. Hence, it might not be appropriate to use an exposure metric based on cumulative exposure during the entire career when assessing micronucleus induction and to then extrapolate this exposure metric to 40 years when estimating the BMD. Due to the insufficient exposure assessment and the unclear dose-response for micronuclei, the statificantion into the different cumulative exposure groups cannot be considerd as reliable. **Overall, this study indicates a LOAEC of 1.6 ppm (range 0.8-12 ppm).**

Other shoe factories in China

Liu *et al* (1996) investigated in total 87 benzene exposed workers in China and 30 controls (university staff) for oxidative damage and micronucleus formation in peripheral blood lymphocytes. 35 workers were shoe factory workers with low benzene exposure. Mean benzene exposure measured for this group by personal sampling was 2.46±2.42 mg/m^3 (0.75±0.73 ppm). The medium and high exposure groups consisted of 24 car paint workers and 28 workers from another shoe factory. Their benzene exposures were 103±50 mg/m^3 (31.7±15.5 ppm) and 424±181 mg/m^3 (131±56 ppm), respectively. The number of white blood cell count was not changed, whereas the frequency of micronuclei was significantly increased in all groups with 2.64±1.67 ‰ , 3.98±1.77 ‰ , 7.89±1.28 ‰ , and 8.15±1.45 ‰ , for controls, low, medium and high benzene exposure groups, respectively. The authors noted that the average age in all 4 groups was 21 years and there were only two smokers. However, further information is not provided and biomonitoring by mesuring ttMA in urine showed only a slight increase of ttMA in the low exposure group compared to control with 190±10 versus 140±0 mg/g creatinine. Considering this small increase, which is much lower than expected from the correlation as published by DFG (2017a, b), the insufficient control for confounders and also that the white blood cell was not significantly changed even at the highest benzene exposure group, raises questions about reliability of the results and potential co-exposure.

Conclusion (shoe factory workers)

Increased frequencies of chromosomal aberrations and aneuploidies in lymphocytes and in sperm of Chinese shoe factory workers were observed at benzene concentrations of 1.0±2.6 ppm (Ji *et al* 2012; Marchetti *et al* 2012; Xing *et al* 2010), 2.0 ppm (range 0.8-18 ppm; Zhang et al 2014), 1.6 ppm (range 0.8-12 ppm; Zhang et al 2016), 2.0 ppm (range 0.8-18 ppm; Zhang et al 2014), 2.3±1.4 ppm (Qu et al 2003a), >2.6±2.7 ppm (Zhang *et al* 2012) and 5.0±3.6 ppm (Zhang *et al* 2011).

Industrial workers other than shoe factories

Studies investigating genotoxic effects in workers employed in industries other than shoe factories are described in Appendix 1, Table 40.

Testa *et al* (2005) investigated 25 male car painters (48% smokers; mean age 46 years; range: 25-55 years) working in different automobile paint-shops in Italy and 37 male control subjects (usual blood donors; 38% smokers; mean age 46 years; range 30-57 years) for chromosomal aberrations and micronuclei in lymphocytes. Air monitoring of benzene was conducted by stationary sampling. Mean concentrations of ethyl acetate, ethyl benzene, xylene, dichloropropane and n-butylacetate were below the permissible Threshold Limit Values (TLVs) as defined by the American Conference of Governmental Industrial Hygienists 1998. Conversely, the mean concentrations of benzene and toluene in the air obtained during repeated measures in the paint-shops were 9.99±17.6 mg/m^3 (3.1±5.4 ppm) and 212.4±308 mg/m^3, respectively, which were higher than the corresponding TLVs. The exposed group had higher frequencies of chromosomal aberrations (both chromosome- and chromatid-type), micronuclei, and SCE; similar results were obtained when only non-smokers were investigated. However, exposure was not measured personally but stationary and hence, includes some uncertainty with respect to individual exposure. **This study indicates a LOAEC of 3.1±5.4 ppm.**

Major *et al* (1994) investigated 42 benzene distillers of an oil refinery in Hungary compared to 42 controls of similar gender distribution (80.9% males), mean age (34 years) and cigarette consumption for chromosomal aberrations in lymphocytes. Benzene was measured by personal sampling and varied between 3 and 20 mg/m^3 (0.3 and 15 ppm) with a mean of 7 mg/m^3 (2.2 ppm). The frequencies of chromosomal aberrations and sister-chromatid exchanges were significantly increased. **This study provides a LOAEC of 2.2 ppm (range 0.3-15 ppm).**

Surrallés *et al* (1997) investigated 38 benzene and coke oven plant workers in Estonia (Kohtla-Järve; 82% males, 71% smokers) and 13 controls (age-matched volunteers; 62% males, 31% smokers) for numerical abnormalities of chromosome 9 in lymphocytes and 18 male workers (50% smokers) and 15 male controls (33% smokers) for numerical abnormalities of chromosome 9 in buccal cells. Fluorescence *in situ* hybridization (FISH) was used to examine the content of micronuclei (whether whole chromosomes or acentric chromosomal fragments) using the pan-centromeric DNA probe SO-aAllCen. Blood samples of 38 benzene exposed workers and 13 controls were collected in March 1994 for FISH analysis in the cytokinesis-blocked micronucleus analysis in lymphocytes. At that time mean exposure measured for 25 benzene plant workers by personal sampling was 4.06±4.75 mg/m^3 (1.25±1.46 ppm). No measurements were performed for the coke oven plant workers; however, later measurements show lower exposures for coke oven plant workers than for benzene plant workers. Buccal cell sampling was performed in March 1995 and included 15 control and 18 exposed persons. At that time exposure for coke oven workers was measured with 0.13±0.13 mg/m^3 (0.04±0.04 ppm); no measurements were performed for the benzene plant at that time. No increases in the frequency of total micronuclei, micronuclei harbouring whole chromosomes or acentric chromosomal fragments or chromosome 9 numerical abnormalities were reported in lymphocytes or buccal cells in relation to benzene exposure in the present study. However, when pooling results from controls and exposed persons, the authors mentioned a slight but non-significant correlation between donor age and the frequency of cells with chromosome 9 numerical abnormalities. According to the authors, the lack of positive results was consistent in both buccal cells and lymphocytes, indicating that the benzene exposure levels encountered did not induce detectable clastogenic or aneugenic effects in the exposed workers. It is to be noted that exposure for coke oven plant workers was significant lower (0.04 to 0.30 ppm) than for benzene plant workers (0.8 to 1.2 ppm). However, the authors did not analyse the results with respect to the different benzene exposures. Therefore, the results need to be considered with care and are not suitable to derive a NOAEC.

Marcon *et al* (1999) investigated chromosome alterations in lymphocytes of a group of 17 Estonian (Kohtla-Järve) workers consisting of 5 cokery workers (2.4 pack cigarettes/year; 35.7 years old), and 12 benzene factory workers (6.1 pack cigarettes/year; 32.6 years old) compared to 8 controls (rural; 3.6 pack cigarettes/year; 39.1 years old). The cokery workers were exposed to benzene concentrations (geometric mean) of 1.0 mg/m^3 (range 0.5-1.7 mg/m^3), corresponding to 0.31 ppm benzene (range 0.15-0.52 ppm) and the benzene factory workers to concentrations of 1.3 mg/m^3 (range 0.1-28.6 mg/m^3), corresponding to 0.40 ppm (range 0.03-8.8 ppm). Exposure was assessed by personal sampling over three consecutive full shifts with additional stationary samplings. The methodology employed (multicolour tandem-labelling fluorescence in situ hybridization (FISH) procedure) allowed the simultaneous detection of both chromosome breakage and hyperploidy (i.e. presence of extra chromosomes) in interphase cells of chromosomes 1 and 9. According to the authors no significant difference in the incidence of breakage of chromosomes 1 and 9 was detected in the nucleated cells of blood smears of exposed vs. control subjects. In contrast, modest but significantly increased frequencies of breakage affecting both chromosomes 1 and 9 were observed in the cultured lymphocytes of the benzene-exposed workers compared to the unexposed controls, suggesting an expression of premutagenic lesions during the S-phase in vitro. Across the entire study group, the frequencies of breakage affecting chromosomes 1 and 9 in the stimulated lymphocytes were highly intercorrelated ($p < 0.001$). No significant difference was found in the incidence of hyperploidy among the study groups, although a tendency to higher values was observed in benzene-exposed workers. Although the relatively small size of the study groups does not allow firm conclusions on the role of occupational exposure, the authors concluded that the observed patterns are suggestive of effects in the benzene-exposed workers. However, it is to be noted that the number of workers

investigated was very low and for benzene factory workers, for which effects have been reported, smoking was more severe the range of benzene exposure was up to 8.8 ppm. Hence, this study does not allow to conclude on a NOAEC or LOAEC. This study was also described by Eastmond *et al* (2001).

Jamebozorgi *et al* (2016) investigated 50 male workers and 31 male controls (from administration) from a petrochemical plant in Iran for micronuclei and nuclear abnormalities. Benzene exposure was mentioned to be below 1 ppm. After the authors excluded 3 samples due to insufficient quality, peripheral blood lymphocytes of the remaining 47 exposed and 31 unexposed workers were analysed. No significant difference was found in the frequencies of micronuclei, nucleoplasmic bridge, and nuclear budding by cytochalasin-blocked MN technique. With respect to benzene exposure, the authors only noted that the workers have been exposed to benzene concentrations below 1 ppm, confirmed based on periodic environmental monitoring, for at least five years. However, since no further information was provided on benzene concentrations, this study cannot be used for a quantitative evaluation of benzene-related effects.

Kim *et al* (2004) reported an increased frequency of chromosomal aberrations, aneuploidy and translocations in lymphocytes of 82 coke oven plant workers in Korea exposed to benzene at concentrations (geometric mean) of 0.56 ppm (range 0.014-0.743 ppm) compared to 76 controls ("healthy people"). The exposed group consisted of 87% males and 49% smokers of which 40% had medium and high cigarette consumption (pack-year). The control group consisted of 66% males and 38% smokers with small consumption of cigarettes. The exposed group was slighly older (41.5±6.3 years) compared to the controls (37.3±7.6 years). Chromosome aberration was performed according to a method published 1986 by the International Atomic Energy Agency and the metaphases were processed by using fluorescence in situ hybridization (FISH) of chromosomes 8 and 21. Exposure was assessed using personal samplers for the entire work shift on five separate days over a period of two weeks. An increased frequency of monosomy and trisomy of chromosomes 8 and 21 was associated with benzene exposure and with polymorphism in the metabolic enzyme genes. A particular subset of genotypes, which included GSTM1-null and GSTT1-null genotypes, the slow acetylator type of NAT2, a variant of the NQO1 genotype and the CYP2E1 DraI and RsaI genotypes, were either separately, or in combination, associated with increased frequencies of aneuploidy among the benzene-exposed individuals after adjustment for age, alcohol consumption and smoking. Translocations between chromosomes 8 and 21 [t(8:21)] were eight-fold more frequent in the high-level exposure group compared to the control group. However, the authors did not provide quantitative information on the high-level exposure benzene concentrations. The authors noted that after adjustment for age, alcohol intake and smoking in the multiple regression analyses, the frequencies of aneuploidy and translocation increased significantly relative to the benzene concentration. The authors discuss that the emissions from coke ovens contain chemicals other than benzene that may act as aneugens or clastogens. They consider it likely that the workers in this study were exposed simultaneously to "*several complex chemicals*". However, considering the different behaviours of ambient chemicals once released, the authors are of the opinion that significant dose-response relationship observed with respect to benzene concentration, as opposed to exposure duration, indicates a specific role for benzene in this association. Although smoking behaviour differed between the benzene exposed and control groups in this study, this difference was taken into account in the multiple regression analysis, which showed no significant effect of smoking. The authors conclude that the results of this study indicate that benzene exposure within a very low concentration range is specifically associated with significant increases in the frequencies of both aneuploidy of chromosomes 8 and 21 and of translocations between chromosomes 8 and 21. With respect to past exposures, it cannot be excluded that past exposures to benzene have been higher. Kang *et al* (2005) reported that the Korean OEL value for benzene was reduced from 10 ppm to 1 ppm in 2002 and was effective since July 2003. **In conclusion, this study indicates a LOAEC of 0.56 ppm (range 0.01 – 0.74 ppm)**.

However, it cannot be excluded that co-exposure to polycyclic aromatic hydrocarbons, differences in smoking habits and unknown previous benzene exposure might have contributed to the clastogenic and aneugenic effects.

Kim *et al* (2008) investigated 108 petroleum refinery workers in Korea compared to 33 office workers for chromosomal aberrations and micronuclei in lymphocytes. Mean age of exposed workers was lower than controls (36.9±6.9 versus 43.2±8.8) and smoking was less extensive (10.5±9.0 versus 12.3±12.9 pack-years). Benzene exposure was estimated for workers in 15 job categories. Mean benzene exposure was calculated with 0.51 ppm with a range from 0.004 to 4.25 ppm. The frequency of chromosomal aberrations and micronuclei measured in the cytokinesis-block micronucleus assay were significantly higher than in the control group. **The study indicates a LOAEC of 0.51 ppm (range 0.004-4.3 ppm).** Since exposure was not measure personally, the concentration of 0.51 ppm benzene contains relevant uncertainties.

Kim *et al* (2010) observed an increased frequency of aneuploidy of chromosomes 7 and 9 using a micronucleus assay in lymphocytes in a group of 30 Korean petroleum refinery workers (48% smokers) compared to 10 controls (office workers, 50% smokers). The micronucleus-centromere assay was used that combines the cytokinesis-blocked micronucleus (CBMN) assay with a fluorescence *in situ* hybridization (FISH) technique to detect aneuploidy. Frequency of aneuploidy of chromosomes 7 and 9 was significantly higher among workers compared to the unexposed control group. Poisson regression analysis revealed that aneuploidy frequency of chromosome 7 or 9 was significantly associated with benzene level after adjusting for confounding variables such as age, smoking, and alcohol intake. Benzene exposure was indicated with 0.51 ppm with 8.5% of the measurements in total population above 0.5 ppm. Cumulative exposure was 0.14 to 4.77 ppm-years. The workers were grouped according to their cumulative exposure <1.5 ppm-years or ≥1.5 ppm-years. According to the authors environmental exposure measurements were available only for limited time periods and locations. Details on exposure measurements was not published. The degree of benzene exposure was estimated using a job-exposure matrix. Based on available measured data and post work practices, cumulative exposure were calculated and the authors conclude that past exposures were low. However, benzene exposures were available only for limited time periods. **This study indicates increased aneugenic effects at 0.51 ppm**; however, due to the small number of investigated workers and shortcoming with respect to exposure assessment, the concentration of 0.51 ppm contains relevant uncertainties.

Yang *et al* (2012) investigated 219 benzene-exposed Chinese workers (50% smokers) for micronucleus frequency in lymphocytes compared to 93 controls (30% smokers) by using the cytokinesis-blocked micronucleus assay. Benzene exposure was measured stationary at 23 monitoring sites in the working environment of benzene-exposed workers for three times per day and was reported with <0.17 ppm. The workers were grouped according to their white blood cell (WBC) count as low (65 workers with WBC count $<4.5x10^9$/L in two tests), unstable (72 workers with WBC count $<4.5x10^9$/L in one of the two tests), and normal (82 workers with normal WBC count $\geq 4.5x10^9$/L). Micronuclei frequency was increased in all WBC groups compared to the control group, with 2.75±1.95 ‰, 2.49±1.85 ‰ and 2.02±1.63 ‰ for the low, unstable and normal WBC group respectively compared to 1.22±1.12 ‰ for the control. A higher average in workers with low and unstable white blood cell count was observed. However, this study cannot be used to quantify benzene-related effects because exposure assessment is insufficient and no correlation was performed between benzene exposure and white blood cell count or micronucleus frequency. Furthermore, 50% of the exposed workers were smokers compared to 30% controls. Considering the low benzene exposure, the difference in smokers percentage might be relevant. Therefore, the results cannot be used to derive a LOAEC for clastogenic effects of benzene.

Basso et al (2011) investigated 79 male workers (38.6 years old; 33% smokers) in petroleum refineries in Italy and 50 male controls (administrative office; 37.1 years old; 16% smokers) for chromosome damage identified with the cytokinesis block micronucleus (CBMN) assay in human peripheral blood lymphocytes. Mean personal benzene exposure for the petroleum refinery workers was 0.093±0.11 mg/m^3 (0.029±0.034 ppm) with a range of 0.0002 to 0.81 mg/m^3 (0.0001-0.25 ppm). Benzene exposure for the controls was not measured. Smoking was identified as a confounding factor. For non-smoking benzene exposed workers the frequency of micronuclei (MN) and binucleated cells with micronuclei (BNMN) was statistically significant increased compared to controls. However, in a multiple regression analysis BNMN and MN frequencies were significantly correlated with age (p=0.0023 and 0.0010, respectively), length of employment (*p*=0.0107 and 0.007) and smoking (*p*=0.0334 and 0.0489), but the correlation with benzene exposure was statistically not significant (*p*=0.6356 and 0.5040). It is not clear to which extend age has been taken into consideration as major confounder for length of employment. **This study indicates no significant benzene-related effects on micronuclei formation at a benzene concentration of 0.03±0.03 ppm.**

Sha *et al* (2014) investigated in China 132 decorators and 129 painters exposed to benzene, toluene and xylene (BTX) compared to 130 unexposed controls for DNA methylation, micronucleus formation and haematological parameters. Mean age rage, gender distribution and smoking habits were comparable between groups. "*Gas masks*" were used during exposure. Personal exposure to BTX was measured by air samplers placed near the subjects breathing zones. For decorators median external benzene exposure was 0.03 mg/m^3 (0.009 ppm) with a range of 0.02 to 0.04 mg/m^3 (0.006-0.012 ppm) and for painters 0.21 mg/m^3 (0.06 ppm) with a range of 0.12 to 0.32 mg/m^3 (0.04-0.10 ppm). The lymphocyte cytokinesis block micronucleus (CBMN) assay was used. No statistically significant effects were reported on haematological parameters (white blood cells, red blood cells, haemoglobin and platelets) and micronucleus formation. However, an effect on DNA methylation was observed (down-regulation of DNMTs and poly(ADP-ribosyl)ation) that reproduce the aberrant epigenetic patterns found in benzene-treated cells. From the information provided in the publication, it is not clear if the measured benzene concentration reflects the exposure outside or inside the masks. However, considering the low concentrations measured, it might reflect the actual concentrations the workers have been exposed to. **Hence, this study indicates no effects at 0.06 ppm (range 0.006-0.012 ppm) and 0.009 ppm (range 0.04-0.10 ppm).**

Three publications are available in which DNA damage (comet assay) was investigated in factory workers.

Sul *et al* (2005) examined 61 workers (54% smokers) from five companies in Korea exposed to benzene for DNA damage measured by the comet assay in lymphocytes and for haematological parameters. Workers were from six different work sites: printing (n=4), shoe making (n=7), and production of nitrobenzene (n=9), methylene di-aniline (MDA; n=18), carbomer (n=17), and benzene (n=6). No external controls were used. Mean benzene concentrations determined by personal sampling were 0.268±0.216 ppm with a range from 0.005 to 2.032 ppm. Highest benzene concentrations were measured in the carbomer production plant. In urine, concentrations of phenol and ttMA were measured. The authors report a significant correlation between benzene exposure and DNA damage and between ttMA excretion and DNA damage. It is to be noted that the comet assay was performed within 3 hours after blood sampling. The authors presented the results for DNA damage according to the workplace. The levels of DNA damage in workers in printing, shoe-making, nitrobenzene, MDA, carbomer and benzene production were: 1.41±0.41, 1.34±0.53, 1.82±1.10, 1.19±0.29, 2.05±0.54 and 1.98±0.29, respectively. The authors report that DNA damage was significantly different between carbomer production site and the other sites. At this site also the highest levels of benzene occurred which were seven time higher than the other sites. The authors report that DNA damage and haematological parameters were not significantly correlated. Due to the relative small number of investigated workers and the wide exposure range, since no external controls were used,

and since smoking, age and gender and co-exposure to other substances were not considered as confounding factors, the results of this study cannot be used to conclude on a NOAEC or LOAEC for DNA damaging effects of benzene.

Li *et al* (2017) investigated 96 non-smoking workers in a petrochemical plant in China in comparison to 100 non-smoking controls for DNA damage in blood by the comet assay. Median benzene exposure was 0.11 mg/m^3 (range 0.01-0.89 mg/m^3) corresponding to 0.034 ppm benzene (range 0.003-0.27 ppm). Benzene concentrations were measured using stationary samplers. The concentration of the biomarker S-phenylmercapturic acid (SPMA) in urine (measured with LC-MS/MS after acid hydrolysis) was significantly increased with 1.76 µg/g creatinine compared to the controls with 0.68 µg/g creatinin. Haematological parameters did not significantly differ to those of the controls. However, DNA damage in blood was significantly increased in workers with 6.51±2.03% tail DNA compared to the controls with 5.84±2.24% tail DNA. Also a positive association between urinary SPMA level and extent of DNA damage was postulated (ß=0.081; 95% CI: 0.032-0.131). It is to be noted that blood samples were taken at the end of shift. However, it is not reported how and how long the samples were stored before the comet assay was performed. This information is relevant because storage, extraction, and assay workup of blood samples are associated with a risk of artifactual formation of damage (Al-Salmani *et al* 2011). Furthermore, the exposure range was wide with 0.003 to 0.27 ppm and no stratification of the workers according to the benzene exposure was performed. Overall the correlation observed between SPMA in urine and DNA damage in the study population was not strong. Furthermore, co-exposure to other substances that could induce DNA damage was not considered. Hence, the information on DNA damage has to be evaluated with care and cannot be used to derive a LOAEC for DNA-damaging effects of benzene.

Fracasso *et al* (2010) investigated 15 non-smoking and 18 smoking petrochemical industry operators compared to 26 non-smoking controls and 25 smoking controls for DNA damage (comet assay) in lymphocytes. Benzene exposure measured with personal samplers was 0.033 mg/m^3 (range 0.002–0.594 mg/m^3) for non-smokers and 0.023 mg/m^3 (range 0.006–0.482 mg/m^3) for smokers corresponding to 0.010 ppm (range 0.001-0.183 ppm) for non-smokers and 0.007 ppm (range 0.002-0.148 ppm) for smokers. SPMA was measured in urine as biomarker (using an immunoassay) with 8.6 µg/g creatinine for smokers and non-smokers but with a higher range for smokers (up to 35.6 µg/g creatinine) compared to non-smokers (up to 13 µg/g creatinine). DNA damage in lymphocytes was increased in smokers and in non-smokers compared to the respective controls. It is to be noted that the authors did not report how long the samples were stored before the comet assay was performed. Furthermore, the exposure range was wide, e.g. for non-smokers with 0.002 to 0.148 ppm and no stratification of the workers according to the benzene exposure was performed. In addition, co-exposure to other substances that could induce DNA damage was not considered. Hence, the result of this publication has to be considered with care and cannot be used to derive a LOAEC for DNA-damaging effects of benzene.

Conclusion (Industrial workers other than shoe factory workers)

Increased frequencies of chromosomal aberrations and micronuclei were observed in lymphocytes of Italian car painters exposed to 3.1±5.4 ppm benzene. However, exposure was not measured personally but stationary and hence, includes some uncertainty.

Increased frequencies of chromosomal aberrations were observed in a group of 42 oil refinery workers in Hungary at mean benzene concentrations of 2.2 ppm (range 0.3 to 15 ppm; Major et al 1994).

Increased frequencies of chromosomal aberrations, aneuploidy and translocations were observed in 82 Korean coke oven workers exposed to 0.56 ppm benzene (Kim *et al* 2004). However, it cannot be excluded that co-exposure to polycyclic aromatic hydrocarbons,

differences in smoking habits and unknown previous benzene exposure might have contributed to the effects observed.

Kim *et al* (2008) reported clastogenic effects in 108 petroleum refinery workers and Kim *et al* (2010), aneugenic effects in 30 petroleum refinery workers exposed to 0.5 ppm benzene. However, the positive result may have been due to exposures higher than 0.5 ppm because the measured range was up to 4.3 ppm (Kim *et al* 2008). Furthermore, exposure assessment was not based on personal measurements but on limited number of air measurements and hence, includes some uncertainties.

In a group of 79 male petroleum refinery workers exposed to 0.029±0.034 ppm benzene, no statistically significant benzene-related effect on micronucleus formation was found (Basso *et al* 2011). However, due to the relative small number of investigated workers (n=79) and the very low range of benzene exposure, the study might not have the statistical power to detect benzene-related effects at such low benzene exposure.

In Chinese 132 decorators and 129 painters waring face masks, no increased micronucleus formation was observed. Median benzene exposures were 0.009 ppm (range 0.006-0.012 ppm) for decorators and 0.06 ppm (range 0.04-0.10 ppm) for painters (Sha *et al* 2014).

Several other studies investigating clastogenic or aneugenic effects of benzene in industrial workers other than shoe factory workers were reviewed but are not suitable to be used for a quantitative evaluation of benzene effects due to relevant shortcomings (Jamebozorgi *et al* 2016; Marcon *et al* 1999; Surrallés *et al* 1997; Yang *et al* 2012).

Furthermore, some studies investigated DNA damage as measured in the comet assay (Fracasso *et al* 2010; Li *et al* 2017; Sul *et al* 2005). However, since the comet assay is only an indicator test and the DNA damage may be repaired, the result might no necessarily be adverse. Furthermore, relevant shortcomings in the performance of the comet assay cannot be excluded. Hence such studies cannot be used for the evaluation of adverse clastogenic and aneugenic effects of benzene.

Workers exposed to petrol

Studies investigating genotoxic effects in workers exposed to gasoline are described in Appendix 1, Table 41. For such workers, co-exposure to toluene, ethylbenzene, xylenes and other substances found in gasoline as well as co-exposure to the complex emissions from vehicle exhausts are to be expected.

Studies performed in Europe

Carere *et al* (1995) investigated the frequency of micronuclei and chromosomal aberrations in 23 Italian non-smoking males exposed to petroleum fuels compared to age-paired, healthy non-smoking blood donors. Benzene exposure was measured by personal sampling (6.5 samplings per year and subject) and resulted in a mean of 1.5±0.7 mg/m^3 (0.46±0.14 ppm) and a range of 0.1 to 13.1 mg/m^3 (0.03-4.0 ppm). Benzene exposure of control was not measured. Mean length of employment was 22.4 years. The correlation analysis highlighted a significant positive correlation between age and micronucleus formation but not with benzene exposure. For chromosomal aberrations a slight increase of borderline significance (p=0.066) was observed. **This study indicates borderline but no statistically significant effects at 0.46±0.14 ppm.**

Pitarque *et al* (1996) found no increase in the frequency of micronuclei in lymphocytes of 50 Spanish male fuel filling station attendants (66% smokers; 43.32±1.84 years old) compared to 43 controls (from the university campus 40% smokers; 40.53±1.28 years old). Benzene exposure was measured by personal sampling and provided a mean benzene concentration of 0.91±0.14 mg/m^3 (0.28±0.04 ppm). Benzene exposure of control was not measured. **This study indicates no effects at 0.28±0.04 ppm.**

Carere *et al* (1998) investigated 12 male Italian fuel filling station attendants for chromosome loss and hyperploidy in periperal lymphocytes compared to 12 male age- and smoking-matched controls (employees). Average personal benzene exposure was 0.32±0.03 mg/m^3 (0.1±0.01 ppm) for the fuel filling station attendants. Benzene exposure of controls was not measued. Fluorescence in situ Hybridization (FISH) methods was performed using probes for chromosomes 7, 11, 18 and X. Also micronuclei with the FISH method were investigated. No statistically significant effects were reported. **This study indicates no effects at 0.1±0.1 ppm.**

Bukvic *et al* (1998) examined 21 Italian male fuel filling station attendants and 19 controls for micronucleus formation in lymphocytes. Benzene exposure measured by personal sampling was 0.072 ppm (geometric mean); no range was provided. Benzene exposure of control was not measured. Micronucleus frequencies were significantly increased in relation with length of employment. However, in a multiple regression analysis no relation with benzene was observed when age and smoking habits were taken into consideration. **This study indicates no effects at 0.072 ppm.**

Fracasso *et al* (2010) investigated in total 28 fuel filling station attendants (46% smokers) and 21 pump maintenance workers (43% smokers) for DNA damage (comet assay) and a subgroup of 19 fuel filling station attendants (42% smokers) also for chromosomal aberrations. A control group consisted of 51 persons (49% smokers). Benzene exposure was measured with personal samplers. For fuel filling station attendants exposure was 0.040 mg/m^3 with a range of 0.008 to 0.260 mg/m^3 (0.012 ppm; range 0.003-0.080 ppm) and for pump maintenance workers 0.024 mg/m^3 with a range of 0.008 to 0.165 mg/m^3 (0.007 ppm; range 0.002-0.051 ppm). Benzene exposure for controls was 0.0054 mg/m^3 with a range of 0.002 to 0.016 mg/m^3 (0.002 ppm; range <0.001–0.05 ppm). Chromosomal aberrations were investigated in 19 fuel filling station attendants and no significant increase was observed compared to 16 controls either for smokers or for non-smokers; an slight increase for smokers was statistically not significant. **Hence, this study provides indications for no clastogenic effects at benzene concentrations of 0.01 ppm.** Significant increased DNA damage (tail moment in the comet assay) was reported for smoking and non-smoking fuel filling station attendants but not for gasoline pump maintenance workers. Biomonitoring measurement of SPMA in urine (using an immunoassay) indicated a higher exposure of fuel filling station attendants compared to controls and gasoline pump maintenance workers. The author commented that the higher values for 'tail moment' suggests that small DNA fragments were produced that are known to be induced in a prevalent manner as a consequence of an increased reactive oxygen production. It is to be noted that the authors did not report how long the samples were stored before the comet assay was performed. Furthermore, the exposure ranges were wide and no stratification of the workers according to the benzene exposure was performed. In addition, co-exposure to other substances that could induce DNA damage was not considered.

Lovreglio *et al* (2014) investigated 24 male fuel filling station attendants (50% smokers) and 19 male fuel tank drivers (58% smokers) for chromosomal aberrations and micronucleus frequency. Mean benzene was 23.3±17.0 µg/m^3 (0.007±0.005 ppm) for fuel filling station attendants and 306.7±266.7 µg/m^3 (0.1±0.1 ppm) for fuel tank drivers. 31 male control persons (52% smokers) living in the same geographical area were included. Mean benzene exposure for controls was 0.0046±0.0026 mg/m^3, corresponding to 0.0014±0.0008 ppm. Benzene exposure was measured with passive personal samplers and through biological monitoring, i.e. by measurement of urinary ttMA, SPMA and benzene. No increased frequency of chromosomal aberrations was observed in either group. By using the cytokinesis-block technique, the frequency of micronuclei was significantly dependent on age in all subjects examined as a single group. Only in fuel-tanker drivers the frequency of micronuclei was found in a multiple stepwise regression analysis to depend not only on age, but also on exposure to benzene. Mean micronucleus

frequencies were 7.3±2.7% for controls, 8.0±3.0% for fuel filling station attendants and 8.6±2.7% for fuel tank drivers. However, the median micronucleus frequencies for fuel filling station attendants and fuel-tank drivers were similar with 8.0% (controls 7.0%) and the maximum values were even higher for fuel filling station attendants and controls than for fuel tank drivers. Hence, the mean increase in micronucleus frequency cannot be considered as a relevant positive result. **Hence, this study provides no indications for clastogenic effects at 0.007 ppm and questionable effects at 0.1±0.1 ppm**.

Lovreglio *et al* (2016) investigated 18 male fuel tanker drivers (55% smokers) and 13 male fuel filling-station attendants (54% smokers) in comparison to 20 males with no occupational exposure for DNA damage and repair capacity. Mean benzene concentrations were 0.280±0.249 mg/m^3 (0.086±0.077 ppm), 0.020±0.016 mg/m^3, (0.006±0.005 ppm), and 0.005±0.003 mg/m^3 (0.002±0.001 ppm) for fuel tank divers, fuel filling station attendants, and controls, respectively. Exposure to airborne benzene was measured using passive personal samplers, and internal doses were assayed through the biomarkers ttMA, SPMA and urinary benzene. No differences in DNA damage (comet assay, tail intensity) or excretion of 7-hydro-8-oxo-2'deoxiguanosine as biomarker for oxidative damage was observed.

Studies performed in North America

Krieg *et al* (2012) assessed DNA damage (comet assay) in the leukocytes of archived blood specimens from U.S. Air Force personnel exposed to jet propulsion fuel 8 (JP-8). No external controls were investigated. Exposure was measured using personal sampling for approximately 4 hours. Furthermore, benzene in breath, napthalene in air and breath and (2-methoxyethoxy) acetic acid concentrations were measured. The blood samples were frozen 24 hours after sampling (mean), minimum 11 hours and maximum 43 hours after blood sampling. No differences in mean comet assay measurements were found in low benzene (0.004±0.006 mg/m^3; 0.001±0.002 ppm benzene; n=139), moderate benzene (0.137±399 mg/m^3; 0.042±0.12 ppm benzene; n=38), and high benzene (0.875±1.479 mg/m^3; 0.33±0.46 ppm benzene; n=115) exposure groups after a 4 hour work shift, whereas increased DNA damage was observed in samples taken before the shift.

Studies performed in South America

Moro *et al* (2013) investigated 43 male Brazilian fuel filling station attendants and 28 controls for DNA damage. Smokers were excluded. Benzene exposure was measured by personal sampling at the end of the work shift after three consecutive days of exposure. In addition, ttMA was measured in urine. The concentration of benzene in the air was provided with 0.076 mg/m^3 (0.023 ppm) with a range of 0.050 to 1.285 mg/m^3 (0.015-0.396 ppm). ttMA in urine was 326 µg/g creatinine compared to the controls with 74 µg/g creatinine. The authors found in the gasoline station attendants higher DNA damage indices and micronucleus frequencies in buccal cells, increased oxidative protein damage, and decreased antioxidant capacity relative to the control group. Duration of benzene exposure was correlated with DNA and protein damage.

Göthel *et al* (2014), from the same working group as Moro *et al* (2013), also investigated 43 male Brazilian fuel filling station attendants for DNA damage (comet assay) in whole blood and for micronuclei in buccal cells. Benzene exposure in the air was not measured. ttMA in urine was determined with 439.8±97 µg/g creatinine for exposed attendants compared to the controls with 117±439 µg/g creatinine. Consistent with the findings from Moro *et al* (2013), DNA damage index was significantly higher for the attendants compared to the controls and showed a positive correlation with 8-hydroxy-2-deoxyguanosine, an indicator for oxidative damage. However, in contrast to the findings from Moro *et al* (2013), no increased frequency in micronuclei in buccal cells was reported.

Moro *et al* (2017) investigated 20 male and 20 female Brazilian fuel filling station attendants for DNA damage in whole blood. Benzene exposure was measured by personal sampling at the end of the work shift after three consecutive days of exposure. In addition, ttMA was measured in urine. The concentration on benzene in the air was 0.139 mg/m^3

(0.043 ppm) for the male attendants with a range of 0.068 to 2.207 mg/m^3 (0.021-0.680 ppm) and 0.124 mg/m^3 (0.038 ppm) for the female attendants with a range of 0.064 to 0.670 mg/m^3 (0.020-0.206 ppm). The authors reported gender differences for benzene-related haematological effects and reported that DNA damage and micronucleus frequency were significantly correlated with ttMA excretion in urine and duration of occupational exposure. It is to be noted that the male and female control groups were obviously non-smokers, whereas the exposed groups consisted of 40% smokers for males and 10% smokers for females.

Studies performed in Asia

Pandey *et al* (2008) examined 100 Indian fuel filling station attendants and an equal number of controls (matched for age, body mass index and smoking) for DNA damage (comet assay) and micronucleus formation (cytokinesis-blocked micronucleus test) in lymphocytes. Benzene concentrations in the air were measured stationary and indicated a range of 0.1 to 0.25 ppm for the exposed workers and of 0.005 to 0.01 ppm for controls. Mean benzene concentrations in blood were reported with 7.94±1.45 ppb for exposed and 2.82±1.45 ppb for controls. The fuel filling station attendants had higher levels of DNA damage and higher frequencies of micronuclei. Comparing the benzene concentration in blood (ca. 8 ppb) with concentrations measured by Rekhadevi *et al* (2010, 2011; see below) with 5 to 13 ppm, **significant dermal uptake has to be assumed**.

Rekhadevi *et al* (2010, 2011) investigated 200 fuel filling station attendants (166 males and 34 females) and 200 controls (matched for gender, age and smoking) for DNA damage and micronucleus formation in peripheral blood lymphocytes. The fuel filling station attendants were slightly older than the controls (37.55±6.37 versus 34.83±6.26 years) and included slighly more smokers (53.5 versus 49.5%). Stationary benzene exposure was measured with 1.322±0.097 mg/m^3 (0.40±0.03 ppm) and personal exposure with 1.500±0.138 mg/m^3 (0.46±0.043 ppm). Personal samples were taken from 10 exposed and controls after 6-day work week at the end of the last 8 to 10 hour shift. In addition, toluene and xylene concentrations were measured. The authors (Rekhadevi *et al* 2010) found a statistically significant increase in mean comet tail length (25.09 versus 10.27 mm) and frequency of micronuclei in lymphocytes (11.83 versus 5.83 ‰) in the service station attendants compared to controls. Multiple regression analysis showed a significant influence of benzene, toluene and xylene exposure on DNA damage but only benzene had a significant influence on micronucleus frequency. Furthermore, the micronucleus frequency was shown to be higher for males than for females and was higher for persons ≥35 years than <35 years. Indicators for oxidative damage were found with significant reduction in superoxide dismutase and glutathion peroxidase levels and increased rates of lipid peroxidation. In the futher publication Rekhadevi *et al* (2011) found a significant increase in micronuclei in buccal cells and in chromosomal aberrations in peripheral blood lymphocytes. The authors indicated that none of the study subjects used facemasks or hand gloves but got frequently dirty as they pumped fuel and worked around oil and grease. Urine analysis for benzene and its metabolites, phenol, ttMA, and SPMA was performed in all the study subjects. According to the authors, the benzene concentrations in the air showed a good correlation especially to SPMA in urine. However, by comparing the concentration of benzene in urine with the correlation between benzene concentration in the air and benzene concentration in urine as published by DFG (2017a, b; see also Table 8), **dermal uptake of benzene most likely contributed significantly to the total body burden**. According to the correlation published by DFG (2017a, b), the endogenous benzene concentration correlates to a benzene concentration in the air of higher than 1 ppm. According to the authors, the results revealed that exposure to petrol vapors induced a statistically significant increase in the frequency of micronuclei in the buccal cells and chromosomal aberrations with increasing benzene exposure. Nuclear anomalies were observed in the mid and the higher exposure groups.

Xiong *et al* (2016) investigated 200 Chinese fuel filling station workers (49% males; 33.1 years old; 29% smokers) exposed to 60 µg/m^3 benzene (0.018 ppm) compared to 52 controls (44% males; 34.3 years old; 31% smokers) for DNA damage (comet assay) in whole blood and micronuclei in buccal cells. Exposure was measured from "occupational air samples" collected from the petrol stations. DNA damage was analysed using the comet assay in whole blood and the micronucleus test in buccal epithelial cells. Tail and Olive tail moments measured in the comet assay were significantly longer compared to the controls, and the micronucleus rate in buccal cells was significantly higher. Furthermore, the antioxidant ability in the workers was reduced (glutathione and superoxide dismutase levels) and markers indicating oxidative stress (malondialdehyde and 8-hydroxydeoxyguanosine levels) were increased.

Conclusion (Workers exposed to petrol)

In the studies reviewed that were performed in **Europe**, a slight increase of chromosomal aberrations of borderline significant but no increase in micronucleus formation was reported for 23 workers with a mean exposure to 0.46±0.14 ppm benzene (Carere *et al* 1995). In a later study with lower benzene concentrations of 0.1±0.01 ppm no aneugenic effects were observed in a group of 12 workers (Carere *et al* 1998). Lovreglio *et al* (2014) reported an increase in the mean micronucleus frequency in a group of 19 workers exposed to 0.1±0.1 ppm benzene of questionable relevance. No effects on micronucleus formation were found in other studies with benzene concentrations of 0.28±0.04 ppm (Pitarque *et al* (1996; n=50), 0.072 ppm (Bukvic *et al* (1998; n=21), 0.012 ppm (Fracasso *et al* (2010; n=19) and 0.007 ppm (Lovreglio *et al* 2014; n=24). No DNA damage (comet assay) could be observed in fuel filling station attanedants with exposure to 0.086 ppm (Lovreglio *et al* 2016; n=18) and 0.006 ppm benzene (Lovreglio *et al* 2016; n=13).

In one study performed in **North America**, no increase in DNA damage (comet assay) was reported in blood samples of workers exposed to low, medium or high concentrations of jet propulsion fuel 8 with benzene exposure up to 0.33±0.45 ppm benzene (Krieg et al 2012).

In several studies performed by one working group on fuel filling station attendants in **South America** (Brazil), DNA damage (comet assay) and/or micronucleus formation in buccal cells was reported at median benzene exposure from 0.023 ppm to 0.042 ppm and maximum ranges up to 0.68 ppm (Moro *et al* 2013, 2017).

Several studies on fuel filling station attendants in **Asia** were reviewed. DNA damage and micronucleus formation in either lymphocytes or buccal cells were reported in China for benzene concentration of 0.018 ppm (Xiong *et al* 2016) and in India for benzene concentrations of 0.1 to 0.25 ppm (Pandey *et al* 2008), 0.40±0.03 ppm (Rekhadevi *et al* 2010) and up to 0.4 ppm (Rekhadevi *et al* 2011).

It has to be noted that specifically for fuel filling station attendants technical measures for exposure reduction, working practice and personal protection equipment are important to control average and peak inhalation exposures and to avoid dermal exposures. Furthermore, the working and environmental conditions in Asia and South America might be less protective compared to Europe and North America. In addition, fuel filling station attendants are also exposed to other substances occurring in gasoline (like toluene, ethylbenzene and xylenes) and to the complex emissions from vehicle exhausts.

For the overall evaluation of benzene-related genotoxic effects, studies investigating clastogenic or aneugenic effects in workers exposed to petrol (mainl fuel filling station attendants) in Europe are further considered.

Workers exposed to engine emissions

Studies investigating genotoxic effects in workers exposed to engine emissions from urban traffic, are described in Appendix 1, Table 42.

Violante *et al* (2003) investigated the micronuclei frequency in peripheral lymphocytes of 15 traffic wardens and 47 chemical laboratory workers with occasional use of benzene. The cytokinesis-block micronucleus (MN) assay in peripheral lymphocytes was used. No external controls were investigated. Environmental benzene exposure was 0.014±0.010 mg benzene/m^3 (0.004±0.003 ppm), urinary benzene 0.66±0.99 µg/L and urinary ttMA 107±123 µg/L urine. Traffic wardens and laboratory workers had similar frequencies of micronuclei. In a multiple regression analysis no significant association was found between micronuclei induction and any of the air or urinary benzene exposure variables.

Leopardi *et al* (2003) compared the micronucleus frequency in peripheral lymphocytes of 134 traffic control personnel with 58 office workers balances for age, gender and smoking habits. The cytokinesis-block micronucleus assay in peripheral lymphocytes was used. Exposure was measured personally in a parallel exposure survey and the 7-h TWA was 0.0095 mg/m^3 (0.003 ppm) for the traffic personnel and 0.0038 mg/m^3 (0.0012 ppm) for controls. Regression analysis of data showed that the micronucleus frequency was mainly modulated by the age and gender of the study subjects (relatively higher in the elderly and females), whereas it was unaffected by the occupational exposure to traffic fumes and smoking habits. A weak association between lower MN frequency and the GSTM1 null genotype was also observed. In order to improve the sensitivity of the method to excision repairable lesions, a modified protocol, with exposure of cells to the repair inhibitor cytosine arabinoside (Ara-C) during the first 16 hr of growth, was applied to 78 subjects (46 exposed and 32 controls). The results confirmed the higher micronucleus frequency in females, but failed to demonstrate any significant effect of chemical exposure (occupational or related to smoking habits). When the frequency of micronucleus induced by Ara-C (i.e., spontaneous values subtracted) was considered, a significant inverse correlation with age was observed, possibly related to the age-dependent decrease in repair proficiency.

Maffei *et al* (2005) investigated haematological parameters and micronuclei frequency in peripheral lymphocytes of 49 traffic policemen (59% males; 35% smokers; 39.5±7.1 years old) in Rome in comparison to 36 indoor workers (indoor workers; 58% males; 36% smokers; 40.1±7.2 years old). The cytokinesis-block micronucleus assay in peripheral lymphocytes was used. Benzene concentrations (personal sampling) were 0.024±0.014 mg/m^3 (0.008±0.004 ppm). Recommended threshold levels for other pollutants like nitrogen oxides, polycyclic aromatic hydrocarbon compounds, total suspended particulate matter, carbon monoxide, or sulphur dioxide did not exceed the maximum atmospheric concentration established for air-quality standards. While no significant difference in haematological parameters was found between the two groups, the micronucleus frequency was significantly higher among the traffic police than in indoor workers. Among the study population, micronucleus frequency was found to increase with duration of employment, but no influence was observed for gender or smoking. As regards to smoking, significantly higher micronucleus frequencies were found in smoking policemen compared to smoking controls (6.94±2.13 versus 5.23±1.42), as well as in non-smoking policemen compared to non-smoking controls (7.12±3.23 versus 4.61±2.04). Since mean age for policemen (39.53±7.14 years) and control (40.13±7.22 years) was comparable, the increased micronucleus frequency that correlated with duration of employment cannot be explained with an age differences to controls. Since no effects in haematological parameters were observed, factors like co-exposure to other clastogenic substances might have contributed to the increased micronucleus frequency.

Angelini *et al* (2011) examined 70 traffic policemen (56% males; 39.1±7.8 years old; 29% smokers) compared to 40 city employees (73% males; 45.0±9.1 years old; 38% smokers) for micronucleus frequency. The cytokinesis-block micronucleus (MN) assay in peripheral lymphocytes was used. Benzene exposure measured by personal sampling for traffic policemen was 0.019 mg benzene/m^3 (0.006 ppm) with a range of 0.013 to 0.031 mg/m^3 (0.004-0.010 ppm) and for the 40 city employees 0.003 mg benzene/m^3

(0.001 ppm) with a range of 0.001 to 0.008 mg/m^3 (0.0003-0.002 ppm). The 6.55-fold higher benzene exposure for traffic policemen was confirmed by a significant, 2.53-fold higher S-PMA excretion in traffic policemen compared with that observed for indoor workers. After adjustment for age and gender, the micronucleus frequency in lymphocytes was significantly higher in policemen compared to indoor workers (median 7.0 ‰ , range 5.50–9.0 ‰ vs median 6.0 ‰ , range 4.0–8.0 ‰). No difference in micronucleus frequency between smokers and non-smokers was reported. With regard to biomarkers of susceptibility, the analysis revealed that high epoxide hydrolase (mEH) (predicted) enzyme activity was significantly correlated with a lower median micronucleus frequency. A gene–gender interaction was observed for the glutathione-S-transferase M1 (GSTM1) genotype. The GSTM1-null genotype was associated with a significantly higher median micronucleus frequency in men, not in women. Statistical analysis did not reveal any association between the presence of the protective allele, pushing the pathway towards benzene detoxification, and micronucleus frequency or S-PMA excretion. Angelini *et al* (2012) reported that the observed increased micronucleus frequency was not associated with polymorphisms in DNA-repair genes (APEX1, hOGG1, NBS1, XPD, XRCC1, and XRCC3). The authors conclude that even though there are some limitations in the study, the results indicate that policemen are exposed to higher levels of benzene than individuals spending most of the time indoors that may contribute to DNA damage, suggesting an increase health risk from traffic benzene emission.

Conclusion (Workers exposed to engine emissions)

Increase micronucleus frequencies were reported in traffic personnel at benzene concentrations of 0.006 and 0.008 ppm (Angelini *et al* 2011, 2012; Maffei *et al* 2005) but not at 0.003 and 0.004 ppm (Leopardi *et al* 2003; Violante *et al* 2003). However, the complex emissions from urban and traffic exhausts contain further substances like polycyclic aromatic hydrocarbons (e.g., Deng *et al* 2014) that have been demonstrated to lead to increased micronucleus freqency. Hence, the effects observed (increased micronucleus frequency) reflect the effect of the combined exposure to traffic exhausts. Therefore, the results are not suitable to quantify the contribution of benzene to the observed effects.

Summary of genotoxic effects

Chromosomal aberrations, aneuploidy and micronucleus formation

Benzene is well known to lead to chromosomal aberrations, aneuploidy, and micronucleus formation.

Micronuclei are formed within proliferating cells after chromosome breakage or chromosome malsegregation (Angelini *et al* 2016). When kinetochore or centromer detection methods are used, e.g. fluorescence *in situ* hybridisation (FISH) it is posible to distinguish between micronuclei caused by chromosomal breakage (clastogenic effect) and micronuclei caused by malsegregation (aneugenic effect). The possibility to automatise scoring of large numbers of cells for micronuclei and the use of the cytokine-block method micronucleus test (CBMN) to improve the detection of micronuclei (Fenech *et al* 1999) has contributed to the frequent use of this test. Micronucleus frequency in human lymphocytes was demonstrated to increase monotonically with age in both genders, with the steepest increase after 30 years of age and to a higher level in females. The micronucleus frequency for 60 year old persons was about twice as high as for 20 year old persons. For females, the micronucleus frequency was about 19% higher than in males. Furthermore, baseline frequencies among laboratories could vary, depending on the methods used. The median of the background was 6.5 ‰ and the interquartine range was between 3 and 12 ‰ (Bonassi *et al* 2001). An increased frequency of micronucleus formation is not necessarily specific for benzene exposure; other substances occurring at the workplace could contribute to the effect (Sram *et al* 2016). Micronucleus formation in lymphocyts due to

heavy exposure to polycyclic aromatic hydrocarbons in coke oven workers has been reported (Pavanello *et al* 2008).

Methods to analyse structural **chromosomal aberrations** are well established, e.g. by the International Atomic Energy Agency (IAEA 2011) and used since decades. However, since such methods require highly experienced personnel and time-intensive manual evaluation, this method is less frequently used nowadays. The analysis of chromosomal aberrations can be combined with fluorescence in situ hybridisation (FISH) to identify translocations and intrachromosomal rearrangements. However, it is not possible to detect numerical aberration. Chromosomal aberrations have been shown to increase with age in a small number of investigated control persons (Roma-Torres et al 2006).

Benzene is one of few prominent substances with occupational exposure that are well known to lead to numerical chromosomal aberrations (**aneuploidy**) in humans. Hence, this effect could be considered as relative specific for benzene exposure which provides a basis to correlate measured benzene exposure with benzene-specific effects. Usually, methods are used to detect aneuploidy of individual chromosomes in interphase binucleated cells. In case of benzene e.g., chromosomes 1, 7, 8, 9, 11, 18, 21, X, Y have been investgated (e.g., Carere *et al* 1998; Ji *et al* 2012; Kim *et al* 2004, 2010; Marchetti *et al* 2012; Qu *et al* 2003a; Xing *et al* 2010; Zhang *et al* 2012). Zhang *et al* (2011) used a novel method to detect aneuploidy in 24 chromosomes and observed that similar aneuploidies occurred in a group of 47 healthy workers with current exposure to benzene (above and below 10 ppm) and in patients with benzene-related leukemia and preleukemia. The authors suggested that aneuploidy precedes and may be a potential mechanism underlying benzene-induced leukemia. Qu *et al* (2003a) concluded from investigations in 130 benzene exposed workers that aneuploidy was strongly associated with exposure intensity (mean benzene exposure level per year), but not with exposure duration.

It is to be noted that the investigations in workers were usually performed in peripheral blood lymphocytes. Most lymphocytes are short-lived, with an average life span of a week to a few months. Considering that the frequency of micronuclei in blood and bone marrow erythrocytes is increasing with increasing benzene exposure in mice (Farris *et al* 1996), also an accumulation of genetic damage in lymphocytes could be expected. Hence, the results may reflect effects of cumulative benzene exposure within the life span of the lymphocytes.

However, the major target organ of benzene relevant for its leukaemic effect is the bone marrow. It is prudent to assume that human bone marrow cells show a higher sensitivity to genetic insult when compared to peripheral cells, e.g. due to higher sensitivity of the long-lived and potentially dividing stem and progenitor cells, or that affected cells might not reach the blood system, e.g. due to apoptosis or altered differentiation. Notably, conversion of benzene to reactive metabolites and accompanied redox cycling is suggested to occur directly in the bone marrow leading to exposure of the various stem and progenitor cells and the bone marrow niche. Thus, measurements in peripheral blood cells may underestimate the severity of the effects to some extent.

Several studies are available investigating clastogenic and aneugenic effects in benzene exposed workers. Of highest relevance are studies investigating aneugenic and clastogenic effects in a larger group of workers (preferable >100) for which appropriate risk management measures have been in place to prevent excessive dermal exposure, which used an appropriate control group (industrial workers, considering relevant confounders like gender, age and smoking), which used personal exposure sampling to monitor benzene exposure, which excluded workers with previous higher benzene exposure, and in which an appropriate regression analysis was performed with control for confounding factors. It is to be noted that all studies reviewed have one or more shortcomings. None of the studies reviewed controlled for co-exposure to other substances.

In the concentration range of 1 ppm benzene and above, clastogenic and aneugenic effects were reported in most studies reviewed.

In 130 Chinese shoe factory workers Qu *et al* (2003a) found a significant exposure-response trend for clastogenic and aneugenic effects with 2.3±1.4 ppm benzene as the lowest investigated concentration. Results from two larger groups of Chinese shoe factory workers (n=385 and 317) showing clastogenic effects at 2.0 ppm (range 0.8-18 ppm) and at 1.6 ppm (range 0.8-12 ppm) contain relevant uncertainties due to missing personal exposure assessment (Zhang et al 2014, 2016). In smaller groups of Chinese shoe factory workers aneugenic effects were found in lymphocytes at benzene concentrations of 7.6±2.3 ppm (Ji *et al* 2012; n=33), 5.0±3.6 ppm (Zhang *et al* 2011; n=47) and >2.6±2.7 ppm (Zhang *et al* 2012; n=28). Aneugenic effects in sperms of Chinese shoe factory workers were also observed at 1.0±2.6 ppm benzene and above (Ji *et al* 2012 and Xing *et al* 2010; n=33; Marchetti *et al* 2012; n=30). Clastogenic effects were also found in Italian car painters exposed to 3.1±5.4 ppm benzene (Testa *et al* 2005) and in Hungarian oil refinery workers at 2.2 ppm benzene (range up to 15 ppm; Major *et al* 1994). No clastogenic and aneugenic effects were reported in 38 Estonian workers (Surrallés *et al* 1997); however, exposure was obviously measured only in the location with higher exposure (1.25±1.46 ppm), but not in the location with much lower benzene exposure.

At concentrations in the range of 0.1 ppm to <1 ppm the results are less consistent and less reliable.

Positive results (aneugenic effects in lymphocytes) were obtained in a study investigating 82 Korean coke oven plant workers at 0.56 ppm (range 0.01-0.74 ppm; Kim *et al* 2004). However, it cannot be excluded that co-exposure to polycyclic aromatic hydrocarbons, differences in smoking habits and unknown previous benzene exposure might have contributed to the clastogenic effect.

Kim *et al* (2008) reported clastogenic effects in 108 petroleum refinery workers and Kim *et al* (2010) aneugenic effects in 30 petroleum refinery workers exposed to 0.51 ppm benzene. However, the positive result may have been due to exposures higher than 0.5 ppm because the measured range was up to 4.3 ppm (Kim *et al* 2008). Furthermore, exposure assessment was not based on personal measurements but on limited number of air measurements and hence, includes some uncertainties.

Clastogenic effects were also reported in 35 shoe factory workers exposed to 0.75±0.73 ppm benzene (Liu *et al* 1996). However, due to insufficient control for confounders, this result cannot be considered as reliable. Also clastogenic effects reported for 219 workers exposed to <0.17 ppm (Yang *et al* 2012) cannot be considered as reliable due to insufficient exposure assessment and relevant differences in smoking habits. Clastogenic and aneugenic effects reported in 24 Estonian workers at 0.3 to 0.4 ppm benzene (Marcon *et al* 1999) cannot be considered as reliable due to different smoking habits and since exposure ranged up to 8.8 ppm.

Clastogenic effects reported in fuel filling station attendants in India (Pandey *et al* 2008; Rekhadevi *et al* 2010, 2011), Brazil (Moro *et al* 2013, 2017) and China (Xiong *et al* 2016) are not considered in the evaluation because insufficient working conditions have been reported or can be assumed.

Several reliable studies are available with appropriate exposure assessment and control for relevant confounders. However, the number of investigated benzene-exposed workers is limited.

For Italian fuel filling station attendants, Carere *et al* (1995) reported at 0.46±0.14 ppm benzene a borderline positive increase of chromosomal aberrations in 23 male fuel filling station attendants but no increase in the micronucleus frequency. Lovreglio *et al* (2014) found in 19 fuel tank drivers exposed to 0.1±0.1 ppm benzene no increase in the frequency of chromsomal aberration but an increase in the mean frequency of micronuclei. However,

since there was no difference for the median micronucleus frequency or the range, the positive results seems to be of questionable relevance.

Negative results have been obtained for 50 male Spanish fuel filling station attendants exposed to 0.28±0.04 ppm benzene (Pitarque *et al* 1996) and for 12 Italian fuel filling station attendants exposed to 0.1±0.1 ppm benzene (Carere *et al* 1998).

At concentrations below 0.1 ppm the results from reliable studies are negative.

No clastogenic effects were reported for 21 Italian fuel filling station attendants exposed to 0.072 ppm benzene (Bukvic *et al* 1998) and for 19 Italian fuel filling station attendants exposed to 0.012 ppm benzene. In a more robust study investigating 79 male Italian petroleum refinery workers exposed to 0.03 ppm benzene (Basso *et al* 2011) also no micronucleus formation was found. In 132 decorators and 129 painters using face maks for which benzene exposure near breathing zone was measured with 0.06 ppm and 0.009 ppm, no clastogenic effects were reported (Sha *et al* 2014).

Results for traffic personnel (Angelini *et al* 2011; Leopardi *et al* 2003; Maffei *et al* 2005; Violante *et al* 2003) cannot be considered as relevant to identify a dose-response for benzene because of the significant contribution of the complex mixtures of traffic/engine exhausts to the total exposure. For example, exposure to polycyclic aromatic hydrocarbons (PAH) was demonstrated to lead to increased micronucleus frequencies (Sram *et al* 2016).

Taking into account all data reviewed, and considering that the positive results obtained in the concentration range below 1 ppm are less reliable, **a LOAEC of 1.0 ppm can be derived for clastogenic and aneugenic effects in peripheral blood lymphocytes.**

Several reliable studies in workers with exposure below 1 ppm benzene are available. At 0.46 ppm, no effects on micronucleus formation but a borderline increase in chromosomal aberrations was observed (Carere et al 1995). No clastogenic effects were reported at 0.28 ppm (Pitarque *et al* 1996; n=50), 0.1 ppm (Carere *et al* 1998, n=12; Lovreglio *et al* 2014, n=19); 0.07 ppm (Bukvic *et al* 1998, n=21); 0.06 and 0.009 ppm (Sha *et al* 2014, n=132 and 129); 0.03 ppm (Basso *et al* 2011, n=79); 0.01 ppm (Fracasso *et al* 2010). Due to the limited number of workers investigated, those studies might not have sufficient statistical power to detect small benzene-related effect. However, the studies were able to detect age and smoking-related effects.

Taking together those data reviewed, **an overall weight-of-evidence NOAEC in the range of ≤0.1 ppm can be considered for clastogenic and aneugenic effects in peripheral blood lymphocytes**.

In the following table, studies are summarized that investigated clastogenic or aneugenic effects in benzene-exposed workers. The studies are listed with regard to decreasing benzene concentrations.

Table 21: Summary of studies in workers investigating clastogenic and aneugenic effects

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
5.0±3.61 28.3±20.1		+ / FISH (24 chrom.) / PBL		47 (22+25) shoe factory workers, 27 controls (workers, other factory, slighly younger, less M, less SM) Tianjin, China; personal exposure measurement	Zhang *et al* 2011

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
3.1±5.4 **stationary**	+ / CA / PBL		+ / MN / PBL	25 male car painters (48% SM), 37 male controls (blood donors, 38% SM, simliar age), Italy, positive result also for non-smokers, **only stationary exposure measurements**	Testa *et al* 2005
2.6±2.7 24.2±10.6		+ / FISH (chrom. 7 and 8) /PBL		28 (18+10) shoe factory workers, 14 controls (workers, other factories, slighly older, less M and SM in low dose group, more M and SM in high dose group) Tianjin, China; personal exposure measurement, **biomonitoring indicates higher endogenous exposure**	Zhang *et al* 2012
0.14±0.04	(+) / FISH (chrom 1 and 7) / PBL			Sub-group of 16 non-smoking female workers; **result not reliable due to inappropriate controls** (51 controls consisting of 53% females and 31% smokers)	Qu *et al* 2003a
2.26±1.4 8.67±2.4 19.9±3.1 51.8±43.3	+ / FISH (chrom 1 and 7) / PBL			130 (73+33+8+16) shoe factory workers (low dose group n=73, 45% M, 36% SM), 51 controls (workers, other factory; 47% M, 31% SM) Tianjin, China; personal exposure measurement	
2.2 (0.3-**15**)	+ / CA / PBL			42 BZ exposed workers, 42 controls (matched for gender, age, smoking), Hungary, personal exposure measurement, **exposure up to 15 ppm**	Major *et al* 1994
2.0 (0.8-18): <1.0 <1.8 ≥1.8 **stationary**			+ / CBMN / PBL	385 shoe factory workers (49% M; 24% SM; 42% >30 years old; n=24+149+212), 197 controls (“indoor” workers and teachers; 49% M, 10% SM; 55% >30 years old), Zhejiang, China; **exposure assessmnent and hence stratification not reliable**	Zhang et al 2014
1.6 (0.8-12): 3.6 ppm-y 6.5 ppm-y 11 ppm-y 20 ppm-y 41 ppm-y **stationary**			+ / CBMN / PBL	317 shoe factory workers (55% M; 27% SM; 60% >30 years old; n=65 per cumulative exposure group), 102 controls (office personal, schools and banks; 49% M; 8% SM; 52% >30 years old), Zhejiang, China; **exposure assessmnent and hence stratification not reliable**	Zhang et al 2016

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
1.25±1.46 (only for 25 BZ plant workers)		(−) / CBMN with FISH; (chrom. 9) / PBL		38 benzene and coke oven plant workers in Estonia (82% M, 71% SM), 13 controls (age-matched volunteers; 62% M, 31% SM), personal exposure sampling, difference in smoking habits; **BZ exposure for coke oven plant workers was presumably lower** but not measured at the time of MN investigation	Surrallés *et al* 1997
1.0±2.6 7.6±2.3		+ / FISH (chrom. X, Y, 21) / sperm		33 (17+16) male shoe factory workers, 33 male matched controls (workers, other factories) Tianjin, China; personal exposure sampling	Xing *et al* 2010; Ji *et al* 2012
1.0±2.6 7.6±2.3		– + / FISH (chrom X, Y, 21) / PBL			Ji *et al* 2012
1.0±2.6 3.0±3.4 7.6±2.2	+ / FISH (chrom. 1) / sperm			30 male shoe factory workers (77% SM; n=10 per group), 11 male controls (workers, other factories; 73% SM) Tianjin, China; personal exposure sampling	Marchetti *et al* 2012
<1 – 0.1					
<1			(−) / CBMN / PBL	47 male petrochemical workers, 31 male controls (administration), Iran, **insufficient exposure assessment**	Jamebozorgi *et al* 2016
0.75±0.73 31.7±15.5 131±56			(+) / MN / PBL	35 shoe factory workers, 24 car paint workers, 28 shoe factory workers, 30 controls (university), China, personal exposure sampling; **result not reliable (especially low exposure group) due to insufficient control for confounders**	Liu *et al* 1996
0.56 (0.01-0.74)		(+) / FISH; (chrom. 8 and 21 and translocat.) / PBL		82 coke oven plant workers (87% M; 49% SM), 76 controls (“healthy people”; 66 M; 38% SM) Korea, personal exposure sampling, past BZ exposure might have been higher, **co-exposure to PAH, BZ exposed workers more and more heavy smokers**	Kim *et al* 2004
0.51 (0.004-4.25) **stationary**	(+) / CA / PBL		(+) / CBMN / PBL	108 petroleum refinery workers, 33 controls (office workers), Korea, only **job exposure matrix based on limited air measurements**	Kim *et al* 2008

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
0.51 (8.5% >0.5) **stationary**		(+) / CBMN with FISH; (chrom. 7 and 9) / PBL		30 petroleum refinery workers, 10 controls (office workers), Korea; only **job exposure matrix based on limited air measurements**	Kim *et al* 2010
0.46±0.14	(+; p= 0.066) / CA / PBL		− / CBMN / PBL	23 male non-smoking fuel filling station attendants, 12 male non-smoking controls (similar age), Italy, personal exposure sampling, MN correlated with age but not with BZ exposure	Carere *et al* 1995
0.46±0.04 **(>1)**	(+) / CA / PBL		(+) / CBMN / PBL; (+) / MN / buccal cells	200 fuel filling station attendants, 200 matched controls (gender, age smoking), India, personal exposure measurements, 8-10 h shifts, biomonitoring data indicate higher endogenous exposure (≥1 ppm), presumably due to dermal absorption; **insufficient working conditions** described in publication	Rekhadevi *et al* 2010, 2011
0.31 (0.15-0.52), 0.4 (0.03-**8.8**)		(+) / CBMN with FISH; (chrom. 1 and 9) / PBL		5 coke oven workers (2.4 pack cigarettes/year), 19 BZ factory workers (6.1 pack cigarettes/year), 8 controls (rural, 3.6 pack cigarettes/year), Estonia, personal sampling, result not reliable due to small numer of workers, **different smoking habits and range up to 8.8 ppm BZ**	Marcon *et al* 1999
0.1-0.25			(+) / CBMN / PBL	100 fuel filling station attendants, 100 matched controls (age, body mass index, smoking), India, only air measurements, **insufficient working conditions to be assumed**	Pandey *et al* 2008
0.28±0.04			− / MN / PBL	50 male fuel filling station attendants (66% smokers; 43.32±1.84 years old), 43 controls (university, 40% smokers; 40.53±1.28 years old), Spain, personal exposure sampling	Pitarque *et al* 1996
<0.17 stationary			(+) / CBMN / PBL	219 BZ exposed workers (50% SM), 93 controls (30% SM), China, **insufficient exposure assessment** (only stationary exposure measurements), **relevant differences in smoking habits**	Yang *et al* 2012
0.1±0.1		− / FISH (chrom. 7, 11, 18, X) / PBL		12 male fuel filling station attendants, 12 controls (matched for sex, age and smoking), Italy, personal exposure sampling	Carere *et al* 1998
		− / CBMN with FISH (centromer) /PBL			

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
0.007± 0.005	– / CA / PBL		– / CBMN / PBL	24 male fuel filling station attendants (50% SM; 40.7±9.6 years old),	Lovreglio *et al* 2014
0.1±0.1	– / CA / PBL		(+?) / CBMN / PBL	19 male fuel tank drivers (58% SM, 42.1±7.5 years old), 31 male controls (52% SM; 41.7±9.1 years old), Italy, personal exposure sampling; only significant difference for mean MN frequency in fuel tank drivers, but not for median frequency and similar range compared to controls; **hence the positive result is questionable**	
				<0.1	
0.072			– / MN / PBL	21 male fuel filling station attendants, 19 controls, Italy, personal exposure sampling, no range provided	Bukvic *et al* 1998
0.06; 0.009			– / CBMN / PBL	132 decorators, 129 painters, 130 controls (similar age range, gender distribution and smoking habits), China, face masks used, personal exposure sampling near breathing zone	Sha *et al* 2014
0.043 (0.021-0.68) (**≥0.3**)			(+) / MN / buccal cells	20 male fuel filling station attendants (40% SM), 28 male controls (no occup. BZ exposure, only NS), Brazil, personal exposure sampling, biomonitoring data indicate higher endogenous exposure (≥0.3 ppm), presumably due to dermal absorption and **insufficient working conditions**, relevant differences in smoking habits	Moro et al 2017
0.029± 0.034			**–** / CBMN / PBL	79 male petroleum refineries workers (33% SM), 50 male controls (office; 16% SM), Italy, personal exposure sampling, correlation MN with age, smoking, length of employment but not with BZ exposure	Basso *et al* 2011
0.023 (0.015-0.396			(+) / MN / buccal cells	43 non-smoking male fuel filling station attendants, 28 non-smoking male controls, Brazil, personal exposure sampling, **insufficient working conditions to be assumed** (see also Moro et al 2017)	Moro *et al* 2013

Benzene (ppm)	Result / test / target			Cohort, charcateristics, major shortcomings	Reference
	CA	aneugen	MN		
0.018 stationary			(+) / MN / buccal cells	200 fuel filling station workers (49% M; 33 years old; 29% SM), 52 controls (44% M, 34 years old; 31% SM), China, stationary sampling, **insufficient working conditions to be assumed**	Xiong *et al* 2016
0.012 (0.002-0.80)	– / CA / PBL			19 male fuel filling station attendants (42% smokers), 16 male controls (56% smokers), Italy, personal exposure sampling	Fracasso *et al* 2010
0.008± 0.004			(+) / CBMN / PBL	49 traffic policemen (59% M; 35% SM, 40 years old), 36 controls (indoor workers; 58% M; 36% SM, 40 years old), Italy, personal exposure sampling, **co-exposure to traffic exhausts (PAH)**	Maffei *et al* 2005
0.006 (0.004-0.010)			(+) / CBMN / PBL	70 traffic policemen (56% M; 39.1±7.8 years old; 29% SM), 40 controls (73% M; 45.0±9.1 years old; 38% SM), Italy, personal exposure sampling, **co-exposure to traffic exhausts (PAH)**	Angelini *et al* 2011
0.004± 0.003			(−) / CBMN / PBL	15 traffic wardens, 47 chemical laboratory workers with occasional use of benzene, no external controls, Italy, stationary exposure sampling, biomonitoring (urinary benzene and ttMA), multiple regression analysis, traffic wardens with **co-exposure to traffic exhausts (PAH)**	Violante *et al* 2003
0.003			(−) / CBMN / PBL	134 traffic control personnel, 58 controls (office workers, balanced for age, gender, smoking habits), Italy, personal exposure measurements, **co-exposure to traffic exhausts (PAH)**	Leopardi *et al* 2003

Abbreviations: ?: questionable; +: positive; (+): positive with relevant uncertainties; −: negative; (−): negative with relevant uncertainties; CA: chromosomal aberrations; CBMN: Cytokinesis-blocked micronucleus assay; FISH: fluorescence in situ hybridization; M: males; MN: micronuclei; PAH: polycyclic aromatic hydrocarbons; PBL: peripheral blood lymphocytes; SM: smokers

DNA damage (comet assay)

Benzene increases DNA damage *in vivo* in mice (Plappert *et al* 1994; Tuo *et al* 1996). DNA damage is a sensitive effects that could be detected already after 3 days exposure of mice to 300 ppm benzene, wheras slight anaemia developed after 4 weeks at 900 ppm and after 8 weeks also at 300 ppm (Plappert *et al* 1994).

Table 22 lists results from publications investigating DNA damage (comet assay) in workers. The studies are listed with decreasing mean (or median) benzene concentration. The studies indicate increased oxidative stress leading to increased DNA damage in most

groups of workers exposed to gasoline ranging from 0.018 ppm (Xiong *et al* 2016) to 0.4 ppm (Rekhadevi *et al* 2010).

It has to be noted that the comet assay has several shortcoming that could potentially lead to either 'false positive' and 'false negative' findings; e.g.,

- Lymphocytes might not be sufficiently sensitive to detect all types of DNA damage compared to whole blood (Bausinger and Speit 2016).
- Storage, extraction, and assay workup of blood samples are associated with a risk of artifactual formation of damage (Al-Salmani *et al* 2011).

Furthermore, for some studies on fuel filling station attendants in Brasil (Moro *et al* 2017) and India (Rekhadevi *et al* 2010) biomonitoring data indicated high endogenous exposure for which dermal exposure might have been the reason.

In addition, co-exposure to other DNA damaging substances at the workplace are expected to contribute to the effects observed. Rekhadevi *et al* (2010) found in a multiple regression analysis a significant influence of benzene, toluene and xylene exposure on DNA damage, whereas only benzene had a significant influence on micronucleus frequency.

In addition, the comet assay is only an indicator test for genotoxicity because the measured effects (DNA damage) might be repaired.

In the following table, studies are summarized that investigated DNA damage in the comet assay in benzene-exposed workers. The studies are ordered with regard to decreasing benzene concentrations.

Table 22: Summary of studies in workers investigating DNA damage (comet assay)

Benzene (ppm)	Result	Parameter	Cohort, characteristics, major shortcomings	Reference
0.46±0.04 **(>1)**	+	comet / PBL	200 fuel filling station attendants, 200 matched controls, India, biomonitoring data (Rekhadevi *et al* 2011) indicate higher endogenous exposure (≥1 ppm), presumably due to dermal absorption; **insufficient working conditions** described in publication	Rekhadevi *et al* 2010
0.330±0.45 0.042±0.12 0.001±0.002	–	comet / blood (stored samples from blood bank)	Archived blod specimens from 139, 38, and 155 workers exposed to JP-8 jet fuel[2)], USA, no external controls, personal exposure measurements	Krieg *et al* 2012
0.27±0.22 (0.005-2.0)	(+)	comet / PBL (haematological parameters not changed)	61 workers from 5 companies (54% SM), no external controls, personal exposure sampling, positive result in carbomer unit with highest BZ exposure	Sul *et al* 2005
0.1-0.25 stationary	(+)	comet / PBL	100 fuel filling station attendants, 100 matched controls (age, body mass index, smoking), India, only air measurements, **insufficient working conditions to be assumed**	Pandey *et al* 2008
0.086±0.077, 0.006±0.005	–	comet / PBL	18 male fuel tank driver (55% SM), 13 male fuel filling station attendants (54% SM), 20 male controls (45% SM), Italy, personal exposure measurements	Lovreglio *et al* 2016

Benzene (ppm)	Result	Parameter	Cohort, characteristics, major shortcomings	Reference
0.042 (0.021-0.680)	+	comet / whole blood	20 non-smoking male fuel filling station attendants, 20 non-smoking male controls, Brazil	Moro *et al* 2017
0.038 (0.020-0.206)	+	comet / whole blood	20 non-smoking female fuel filling station attendants, 20 non-smoking female controls, Brazil	
0.034 (0.003-0.27)	+	comet / whole blood	96 non-smoking petrochemical plant workers, China	Li *et al* 2017
0.023 (0.015-0.396	+	Comet / whole blood	43 non-smoking male fuel filling station attendants, 28 non-smoking male controls, Brazil	Moro *et al* 2013
0.025 (0.002-0.051)	–	comet / PBL	12 non-smoking gasoline pump maintenance workers,	Fracasso *et al* 2010
0.012 (0.003-0.080)	+	comet / PBL	15 non-smoking fuel filling station attendants,	
0.010 (0.001-0.183)	+	comet / PBL	15 non-smoking petrochemical industry operators, Italy	
0.018	+	comet / whole blood	200 fuel filling station workers (49% M; 33 years old; 29% SM), 52 controls (44% M, 34 years old; 31% SM), China, stationary sampling	Xiong *et al* 2016

Abbreviations: Comet: DNA damage detected with the comet assay; PBL: peripheral blood lymphocytes

7.6.2 Animal data

Genotoxicity of benzene

Whysner *et al* (2004) reported that studies of rodents exposed to radiolabelled benzene found a low level of radiolabel in isolated DNA with no preferential binding in target tissues showing neoplasia. Adducts were not identified by ^{32}P-postlabeling (equivalent to a covalent binding index <0.002) under the dosage conditions producing neoplasia in the rodent bioassays, and this method would have detected adducts at 1/10,000th the levels reported in the DNA-binding studies. Adducts were detected by ^{32}P-postlabeling *in vitro* and following high acute benzene doses *in vivo*, but levels were about 100-fold less than those found by DNA binding. These findings suggest that DNA-adduct formation may not be a significant mechanism for benzene-induced neoplasia in rodents.

With respect to gene mutations, two studies with transgenic mice exposed either by inhalation or by oral dosing to benzene provided positive results of less than 2-fold. Whysner *et al* (2004) commented that those low increases contrast with much larger increases of mutagenesis in target tissues of mice exposed to DNA-reactive carcinogens.

The evaluation of other genotoxicity test results revealed that benzene and its metabolites did not produce reverse mutations in Salmonella typhimurium but were clastogenic and aneugenic, producing micronuclei, chromosomal aberrations, sister chromatid exchanges and DNA strand breaks (Whysner *et al* 2004).

Whysner *et al* (2004) compared rodent and human genotoxicity data and concluded that benzene genotoxicity results were (quantitatively) similar for the available tests. Also, the biotransformation of benzene was similar in rodents, humans and non-human primates.

Whysner *et al* (2004) also considered that the genotoxicity test results for benzene and its metabolites were similar to those of topoisomerase II inhibitors and provided less support for proposed mechanisms involving DNA reactivity, mitotic spindle poisoning or oxidative DNA damage as genotoxic mechanisms; all of which have been demonstrated experimentally for benzene or its metabolites.

Erexson *et al* (1986) investigated the induction of sister chromatid exchanges (SCEs) in peripheral blood lymphocytes (PBLs) and micronuclei (MN) in bone marrow polychromatic erythrocytes (PCEs) of mice and rats after single 6 hour inhalation exposure to benzene. Male DBA/2 mice (5 animals per exposuere group, 10 animals in control) were exposed to target concentrations of either 0, 10, 100, or 1,000 ppm benzene. Male Sprague-Dawley rats (5 animals per exposure group, 10 or 20 animals in control groups) were exposed to target concentrations of either 0, 0.1, 0.3, 1, 3, 10, or 30 ppm benzene. Blood was obtained by cardiac puncture 18 hour after exposure, and PBLs were cultured in the presence of lipopolysaccharide (mouse B cells) or concanavalin A (rat T cells) to stimulate blastogenesis for SCE analysis. Femoral bone marrow smears from both species were analysed for MN in PCEs 18 hours after benzene exposure. Mouse PBLs revealed a significant concentration-related increase in the SCE frequency over controls at 10, 100, or 1,000 ppm benzene. Mouse bone marrow showed a significant concentration-dependent increase in MN over controls after exposure to 10, 100, or 1,000 ppm benzene. Rat PBLs showed a significant increase in the SCE frequency after exposure to 3, 10, or 30 ppm benzene. The statistical significance of the 1 ppm benzene result was borderline and dependent on the statistical test chosen. Rat cells revealed a significant concentration-related increase in MN after inhalation of either 1, 3, 10, or 30 ppm benzene. PBLs from treated mice showed significant concentration-dependent decreases in mitotic indices; however, cell cycle kinetics and leucocyte counts remained unaffected. Rat PBLs showed significant decreases in mitotic activity only after exposure to 3 and 30 ppm benzene, whereas cell cycle kinetics and leucocyte counts were unaffected. **This study provides a LOAEC of 1 ppm and a NOAEC of 0.3 ppm for increased micronucleus formation in bone marrow polychromatic erythrocytes of Sprague-Dawley rats after a single 6 hour exposure to benzene.**

Farris *et al* (1996) investigated the frequencies of micronucleated polychromatic erythrocytes (MPCE) in the bone marrow and blood and micronucleated normochromatic erythrocytes (MNCE) in the blood of groups of seven male B6C3F1 mice to benzene in concentration to 0, 1, 10 , 100 and 200 pm for either 1, 2, 4 or 8 weeks. Micronucleus formation was significantly increased at 100 and 200 ppm in polychromatic erythrocytes of the bone marrow and in normochromatic erythrocytes of the blood. A plateau was reached in the bone marrow after 2 weeks but progressively increased in blood for 8 weeks of exposure. At the same concentrations, counts of red blood cells and polychromatic erythrocytes were significantly reduced due to cytotoxicity of replicating and maturing erythrocytes. **This study provides a LOAEC of 100 ppm and a NOAEC of 10 ppm for increased micronucleus frequency in erythrocytes of B6C3F1 mice following up to 8 week benzene exposure.**

In a more recent study, **French *et al* (2015)** exposed male Diversity Outbred (DO) mice to benzene (0, 1, 10, or 100 ppm; 75 mice/exposure group) via inhalation for 28 days (6 hr/day for 5 days/week). DO mice are genetically heterozygous and carry a complex mixture of alleles. Each animal in an outbred population is genetically unique and a level genetic diversity is similar to that of humans. The study was repeated using two independent cohorts of 300 animals each. Micronucleus frequency in reticulocytes from peripheral blood and bone marrow was measured. The authors reported a dose-dependent increase in benzene-induced chromosomal damage (see Table 23) with a statistical significant increase of micronuclei in reticulocytes and erythrocytes of periperal blood at 100 ppm, and in reticulocytes of the bone marrow already at 1 ppm. **The authors estimated a benchmark concentration limit ($BMDC_{10}$) of 0.205 ppm benzene for**

increased micronucleus frequency in reticulocytes of the bone marrow. This estimate is an order of magnitude below the value estimated using B6C3F1 mice. The author conclude that genetically diverse DO mice provided a reproducible response to benzene exposure. The DO mice display interindividual variation in toxicity response and, as such, may more accurately reflect the range of response that is observed in human populations. However, the author also indicate that they did not find any significant association near genes that are traditionally associated with benzene metabolism—such as Cyp2e1, Ephx1, Sult1a1, Mpo, and Nqo1—in the 100 ppm exposure group. The author assumed that this may be due to a lack of functional polymorphisms in these genes in the DO mice, to the relative importance of these genes to benzene metabolism and clearance in the 100 ppm exposure group, or to differences in benzene metabolism between mice and humans. It is also to be noted that exposure duration was only 28 days and the effect levels following long-term exposure are expected to be lower than after 28 day exposure. **This study provides a LOAEC of 1 ppm for increased micronucleus frequency in reticulocytes of the bone marrow of DO mice after inhalation exposure for 28 days (6 hrs/day, 5 days/week), which translates to a $LOAEC_{(worker)}$ of 0.5 ppm (1 x 6/8 x 6.7/10). The $BMDC_{10}$ of 0.205 ppm translates to a $BMDC_{(worker)10}$ of 0.1 ppm for workers**.

Table 23: Micronucleus frequency in reticulocytes from peripheral blood and bone marrow of DO mice (French *et al* 2015)

Benzene (ppm)	n	MN in peripheral blood Mean (C.I.); Student's T-test	n	MN in bone marrow Mean (C.I.); Student's T-test
0	148	2.68 (1.33, 5.39)	142	3.51 (1.27, 9.72)
1	149	2.57 (1.19, 5.54); p 0.93	136	4.31 (1.65, 11.30); p 0.018
10	148	3.14 (1.37, 7.17); p 0.074	146	5.38 (2.07, 14.02); p <0.01
100	145	14.58 (2.29, 92.72); p <0.01	145	14.68 (3.01, 71.70); p <0.01

Genotoxic effects of benzene metabolites in vivo

Whysner *et al* (2004) performed a systematic review of over 1400 genotoxicity test results for benzene and its metabolites. Reactive metabolites are formed during benzene metabolism including phenol, hydroquinones, benzoquinones, catechol, benzenetriol, and muconaldehyde.

As indicated below in Table 24 below, animal studies have connected all of them to one or more different genotoxic effects.

Genotoxic effects of substances with co-exposure to benzene

For toluene (EU RAR 2003), ethylbenzene (DFG 2001) and xylenes (EPA 2003) there are no indications for genotoxicity *in vitro* or *in vivo*. However, co-exposure of mice to benzene (50 ppm) and toluene (100 ppm) resulted in higher frequency of micronuclei in polychromatic erythrocytes compared to exposure to benzene or toluene alone (Bird *et al* 2010; Wetmore *et al* 2008). Bird *et al* (2010) discusses that the increased in clastogenicity upon intermittent co-exposure appears to be associated with induction of hepatic CYP2E1 activity, an increased blood GSH/GSSG ratio, and a 2-fold increase in the level of urinary biomarker s-phenylmercapturic acid (s-PMA) not seen with the same level of benzene exposure alone.

Table 24: Summary of results of rodent genotoxicity tests for benzene and its metabolites *in vivo* (Whysner *et al* 2004).

Substance	MN	CA	Aneuploidy	SCE	DNA strand breaks	Mutation
Benzene	+	+	+	+	+/−	+
Phenol	+/−	+		+	−	
Hydroquinone	+	+	+			
Benzoquinone	+					
Catechol	+/−					
Benzenetriol	+/−					
Muconaldehyde	−			+		

Abbreviations: +: predominantly positive results; +/-: mixed positive and negative results; − : predominantly negative results; CA: chromosomal aberrations; MN: micronuclei;

7.6.3 *In vitro* data

Genotoxicity of benzene metabolites in vitro

Whysner *et al* (2004) performed a systematic review of over 1400 genotoxicity test results for benzene and its metabolites. Table 25 below summarizes the results of *in vitro* genotoxicity tests of benzene and its metabolites. For benzene either negative results or mixed positive and negative results were obtained *in vitro*. In contrast the metabolites showed (in case investigated) either positive or mixed results for micronuclei formation, chromosomal aberrations, aneuploidy, sister-chromatid exchange, DNA strand breaks and forward mutations in mammalian cells. Results for reverse mutagenicity in bacteria and yeast cells for benzene and its metabolites with and without metabolic activation were primarily negative. Whysner *et al* (2004) commented that any agent causing loss of the DNA carrying the gene (or the chromosome) is expected to be positive in the forward mutation assay in mammalian cells, whereas the results of the bacterial reverse mutations are more specific for mutagenicity.

Chen *et al* (2008) showed that benzene, toluene, ethylbenzene, o-, m-, and p-xylene as well as MTBE (Methyl-*tert*-butyl ether) are leading to DNA damage (comet assay) in human lymphocytes *in vitro*.

Table 25: Summary of results of *in vitro* genotoxicity tests for benzene and its metabolites (Whysner *et al* 2004)

Substance	MN	CA	Aneuploidy	SCE	DNA strand breaks	Mammalian gene mutation
Benzene	− (±S9)	+/− (±S9)	+/− (-S9) − (+S9)	− (±S9)	+/− (±S9)	(+)/−
Phenol	+		−	+/−	−	+/−
Hydroquinone	+/−	+/−	+/−	+	+	+
Benzoquinone	+			+		+
Catechol	+/−	+	+	+		+

Substance	MN	CA	Aneuploidy	SCE	DNA strand breaks	Mammalian gene mutation
Benzenetriol	+		+	+		+
Muconaldehyde	+			–		+

Abbreviations: +: predominantly positive results; +/-: mixed positive and negative results; – : predominantly negative results; CA: chromosomal aberrations; DNA damage: either single or double strand breaks; MN: micronuclei; S9: metabolic activation

7.6.4 Epigenetic alterations

The current knowledge on epigenetic mechanisms of chemical carcinogens including benzene has been reviewed by Chappell *et al* (2016). For benzene, the authors identified the following epigenetic alterations:

- DNA Methylation
- Histone modifications
- Non-coding RNA

Chappell *et al* (2016) conclude *that a major challenge in the application of these epigenetic findings in regulatory science is the question of "how" to effectively include them. Epigenetic endpoints are currently being increasingly used in cancer hazard assessments […]. However, while there is extensive information about the fundamental role of epigenetic alterations in cancer development and progression, the understanding of the mechanistic significance and specificity of carcinogen-induced epigenetic abnormalities in the carcinogenic process is insufficient. For example, several studies have demonstrated a mechanistic link between DNA hypomethylation (the most highly reported, and thus assumed best-characterized, epigenetic alteration among the studies included in this review) and genetic changes, and established the role of this epigenetic alteration in carcinogenesis. In contrast, there is not a single study among an extensive list of observational reports on carcinogen-induced DNA hypomethylation that demonstrated a mechanistic link between loss of DNA methylation and cancer development*.

Fenga *et al* (2016) also performed a literature review on epigenetic changes associated with benzene. They conclude that epidemiological and experimental studies have demonstrated the potential epigenetic effects of benzene exposure. Several of the epigenomic changes observed in response to environmental exposures may be mechanistically associated with susceptibility to diseases. However, further elucidation of the mechanisms by which benzene alters gene expression may improve prediction of the toxic potential of novel compounds introduced into the environment, and allow for more targeted and appropriate disease prevention strategies.

7.6.5 Summary

There is evidence that benzene induces micronucleus formation, chromosomal aberrations, aneuploidy, sister chromatid exchange, and DNA strand breaks in humans and in experimental animals (Whysner *et al* 2004).

The induction of gene mutations by benzene seems to be possible *in vitro* and *in vivo*. However, the mutagenic effects observed *in vitro* in mammalian cells might have been secondary to chromosomal damage and the mutagenic effects *in vivo* were of low magnitude (<2-fold) not reflecting the magnitude of DNA-reactive carcinogens (Whysner *et al* 2004).

The leading mechanism for the toxicity of benzene is its clastogenic and aneugenic activity. Investigations in benzene-exposed workers indicate that aneuploidy precedes and may be

a potential mechanism underlying benzene-induced leukemia (Zhang *et al* 2011). Aneugenic effects have been demonstrated to be strongly associated with exposure intensity but not with exposure duration (Qu *et al* 2003a).

In the last two decades, multiple studies investigating benzene-exposed workers were published which are the basis for the following summary.

Several studies have been reviewed that investigate DNA damage using the comet assay with inconsistent results (see Table 22). It is to be noted that the comet assay is only an indicator test for genotoxicity because the measured effects (DNA damage) might be repaired. Furthermore, this test is not specific for benzene-related effects but several substances occurring at the workplace could contribute the effects observed. In addition, several methodological shortcoming (e.g., type, storage, extraction and workup of samples) might have an impact on the result that could potentially lead to either 'false positive' and 'false negative' findings. Hence, results with the comet test are not used to evaluate genotoxicity of benzene, especially considering the availability of studies investigating the clastogenic and aneugenic effects of benzene in workers.

Of relevance in the evaluation of benzene-related effects are studies in workers that investigated the clastogenic and aneugenic effects of benzene.

In the concentration range of 1 ppm benzene and above, clastogenic and aneugenic effects were reported in most studies reviewed.

In 130 Chinese shoe factory workers Qu *et al* (2003a) found a significant exposure-response trend for clastogenic and aneugenic effects with 2.3±1.4 ppm benzene as the lowest investigated concentration. Results from two larger groups of Chinese shoe factory workers (n=385 and 317) showing clastogenic effects at 2.0 ppm (range 0.8-18 ppm) and at 1.6 ppm (range 0.8-12 ppm) contain relevant uncertainties due to missing personal exposure assessment (Zhang et al 2014, 2016). In smaller groups of Chinese shoe factory workers aneugenic effects were found in lymphocytes at benzene concentrations of 7.6±2.3 ppm (Ji *et al* 2012; n=33), 5.0±3.6 ppm (Zhang *et al* 2011; n=47) and >2.6±2.7 ppm (Zhang *et al* 2012; n=28). Aneugenic effects in sperms of Chinese shoe factory workers were also observed at 1.0±2.6 ppm benzene and above (Ji *et al* 2012 and Xing *et al* 2010; n=33; Marchetti *et al* 2012; n=30). Clastogenic effects were also found in Italian car painters exposed to 3.1±5.4 ppm benzene (Testa *et al* 2005) and in Hungarian oil refinery workers at 2.2 ppm benzene (range up to 15 ppm; Major *et al* 1994). No clastogenic and aneugenic effects were reported in 38 Estonian workers (Surrallés *et al* 1997); however, exposure was obviously measured only in the location with higher exposure (1.25±1.46 ppm), but not in the location with much lower benzene exposure.

At concentrations in the range of 0.1 ppm to <1 ppm the results are less consistent and less reliable.

Positive results (aneugenic effects in lymphocytes) were obtained in a study investigating 82 Korean coke oven plant workers at 0.56 ppm (range 0.01-0.74 ppm; Kim *et al* 2004). However, it cannot be excluded that co-exposure to polycyclic aromatic hydrocarbons, differences in smoking habits and unknown previous benzene exposure might have contributed to the clastogenic effect.

Kim *et al* (2008) reported clastogenic effects in 108 petroleum refinery workers and Kim *et al* (2010) aneugenic effects in 30 petroleum refinery workers exposed to 0.51 ppm benzene. However, the positive result may have been due to exposures higher than 0.5 ppm because the measured range was up to 4.3 ppm (Kim *et al* 2008). Furthermore, exposure assessment was not based on personal measurements but on limited number of air measurements and hence, includes some uncertainties.

Clastogenic effects were also reported in 35 shoe factory workers exposed to 0.75±0.73 ppm benzene (Liu *et al* 1996). However, due to insufficient control for confounders, this

result cannot be considered as reliable. Also clastogenic effects reported for 219 workers exposed to <0.17 ppm (Yang *et al* 2012) cannot be considered as reliable due to insufficient exposure assessment and relevant differences in smoking habits. Clastogenic and aneugenic effects reported in 24 Estonian workers at 0.3 to 0.4 ppm benzene (Marcon *et al* 1999) cannot be considered as reliable due to different smoking habits and since exposure ranged up to 8.8 ppm.

Clastogenic effects reported in fuel filling station attendants in India (Pandey *et al* 2008; Rekhadevi *et al* 2010, 2011), Brazil (Moro *et al* 2013, 2017) and China (Xiong *et al* 2016) are not considered in the evaluation because insufficient working conditions have been reported or can be assumed.

Several reliable studies are available with appropriate exposure assessment and control for relevant confounders. However, the number of investigated benzene-exposed workers is limited.

For Italian fuel filling station attendants, Carere *et al* (1995) reported at 0.46±0.14 ppm benzene a borderline positive increase of chromosomal aberrations in 23 male fuel filling station attendants but no increase in the micronucleus frequency. Lovreglio *et al* (2014) found in 19 fuel tank drivers exposed to 0.1±0.1 ppm benzene no increase in the frequency of chromsomal aberration but an increase in the mean frequency of micronuclei. However, since there was no difference for the median micronucleus frequency or the range, the positive results seems to be of questionable relevance.

Negative results have been obtained for 50 male Spanish fuel filling station attendants exposed to 0.28±0.04 ppm benzene (Pitarque *et al* 1996) and for 12 Italian fuel filling station attendants exposed to 0.1±0.1 ppm benzene (Carere *et al* 1998).

At concentrations below 0.1 ppm the results from reliable studies are negative.

No clastogenic effects were reported for 21 Italian fuel filling station attendants exposed to 0.072 ppm benzene (Bukvic *et al* 1998) and for 19 Italian fuel filling station attendants exposed to 0.012 ppm benzene. In a more robust study investigating 79 male Italian petroleum refinery workers exposed to 0.03 ppm benzene (Basso *et al* 2011) also no micronucleus formation was found. In 132 decorators and 129 painters using face maks for which benzene exposure near breathing zone was measured with 0.06 and 0.009 ppm, no clastogenic effects were reported (Sha *et al* 2014).

Results for traffic personnel (Angelini *et al* 2011; Leopardi *et al* 2003; Maffei *et al* 2005; Violante *et al* 2003) cannot be considered as relevant to identify a dose-response for benzene because of the significant contribution of the complex mixtures of traffic/engige exhausts to the total exposure. For example, exposure to polycyclic aromatic hydrocarbons (PAH) was demonstrated to lead to increased micronucleus frequencies (Sram *et al* 2016).

Taking into account all data reviewed, and considering that the positive results obtained in the concentration range below 1 ppm are less reliable, **a LOAEC of 1.0 ppm can be derived for clastogenic and aneugenic effects in peripheral lymphocytes.**

Several reliable studies in workers with exposure below 1 ppm benzene are available. At 0.46 ppm, no effects on micronucleus formation but a borderline increase in chromosomal aberrations was observed (Carere et al 1995). No clastogenic effects were reported at 0.28 ppm (Pitarque *et al* 1996; n=50), 0.1 ppm (Carere *et al* 1998, n=12; Lovreglio *et al* 2014, n=19); 0.07 ppm (Bukvic *et al* 1998, n=21); 0.06 and 0.009 ppm (Sha *et al* 2014, n=132 and 129); 0.03 ppm (Basso *et al* 2011, n=79); 0.01 ppm (Fracasso *et al* 2010). Due to the limited number of workers investigated, those studies might not have sufficient statistical power to detect small benzene-related effect. However, the studies were able to detect age and smoking-related effects.

Taking together those data reviewed, **an overall weight-of-evidence NOAEC in the range of ≤0.1 ppm is indicated for clastogenic and aneugenic effects in peripheral lymphocytes**.

A recent study by French *et al* (2015) with male Diversity Outbred (DO) mice report a dose-dependent increased micronucleus frequency in reticulocytes of the bone marrow with increasing exposure to 1, 10, and 100 ppm benzene for 28 days (6 hours/day, 5 days/week). **This study provides a LOAEC of 1 ppm for bone marrow-derived reticulocytes in DO mice.** The authors modelled the data using two different approaches, leading to a $BMDC_{10}$ of 0.205 or 1.52 ppm (the former would translate to a $BMDC_{(worker)10}$ of 0.1 ppm (0.2*6/8*6.7/10)).

7.7 Carcinogenicity

7.7.1 Human data

LEUKAEMIA AND LYMPHOMA

There is extensive epidemiological literature on benzene carcinogenicity. The studies of greatest relevance concern the haematopoietic and lymphoid system. The association is considered most definitive for acute myeloid leukaemia (AML), which is also called acute non-lymphocytic leukaemia (ANLL). The WHO classification of haematopoietic and lymphoid tumours was revised in 2008 (Swerdlow *et al* 2008). In the revised classification the tumours are no longer classified according to their localization but according to their cells of origin. This may somewhat complicate the comparison of historical and more recent data at least if only aggregate level diagnostic entities are reported. Especially as regards B-cell neoplasms.

In the following chapters abbreviations are used for the following subtypes of these malignancies:

AML acute myeloid leukaemia (or ANLL, acute non-lymphocytic leukaemia)

ALL acute lymphocytic leukaemia

CLL chronic lymphocytic leukaemia

CML chronic myeloid leukaemia

HL Hodgkin lymphoma

MM Multiple myeloma

MPD myeloproliferative disease

MDS myelodysplastic syndrome

NHL non-Hodgkin lymphoma

For MDS it took a long time for it to be recognised as a haematopoietic malignancy and some of the earliest studies may have reported it under AML or aplastic anaemia and furthermore a fraction of MDS cases progress to AML.

A summary of the most relevant cohort studies and nested case-control studies assessing the association between occupational exposure to benzene and risk of leukaemia or its subtypes is given in Table 43.

Leukaemia

As the epidemiological data base contains numerous cohort based studies and meta-analyses, individual case-control studies are not further described.

Cohort studies

Cohort studies that have investigated the risk of leukaemia from exposure to benzene in various industries are summarised in Table 43, which also includes the nested case-control

studies conducted in those cohorts. The focus is on studies that include some quantitative information on the level of exposure.

Historically the most extensively studied cohort is the **'Pliofilm' cohort** consisting of workers exposed to benzene in three rubber hydrochloride manufacturing plants at two locations in Ohio (Rinsky *et al* 1981). Numerous mortality updates and reassessments have been published (see Table 43). Data from this cohort have been central in setting health-based exposure standards for benzene by US EPA, OSHA and ACGIH (Paxton 1996). In addition to extending the follow-up time for cancer occurrence, the later analyses have also proposed alternative exposure estimates. Regardless of which exposure estimates were used, the level of exposure to benzene has consistently shown a relationship to leukaemia mortality (Table 43). Data from this cohort has also provided evidence that exposures in the most recent 10 years were most strongly associated with leukaemia risk The RR was 1.19 (95%CI 1.10-1.29) per 10 ppm-years in the time window less than 10 years since exposure, also a statistically non-significantly increased risk for was observed for the time-window of 10-20 years since exposure (RR=1.05, 95% CI 0.97 – 1.13) while no increase in risk was observed for the time window more than 20 years since exposure (RR=1.00, 95% CI 0.90 – 1.05) (Richardson 2008, see Table 42). The risk of leukaemia has decreased from the earliest reports, but now that more than half of the cohort has already deceased, the risk is still statistically significantly increased both overall (SMR=2.47, 95% CI 1.38 – 4.07) and for white males (SMR=2.56; 95% CI 1.43-4.22) (Rinsky *et al* 2002). However, it is obvious that these risk estimates accumulate both the old and the more recent follow-up periods and consequently reflect the sum of old (higher) and more recent (lower) exposures in the cohort. Most recently Rhomberg *et al* (2016) updated the exposure information and performed analyses specifically for the risk of ANLL or AML. The risk was increased only in the highest exposure category when cohort members were divided into tertiles, quartiles or quintiles based on cumulative exposure. Rhomberg (2016) concluded that "the dose-response relationship at lower benzene exposures remains difficult to resolve with this dataset, owing to a lack of cases in lower exposure categories, Still, the absence of cases in lower exposure categories suggests that benzene may have a threshold effect on induction of certain types of leukaemia". Dose response analyses for other leukaemia types were not reported in this latest follow-up study. A risk specifically linked to ANLL/AML was also reported in some earlier Pliofilm cohort updates (Crump 1996, Wong 1995), while Wong (1995) admitted that for other subtypes the number of cases was not sufficient for any meanningful analysis.

The largest cohort study conducted to date is the **'NCI/CAPM' study** conducted by the US National Cancer Institute and the Chinese Academy of Preventive Medicine (see Table 43). Incidence of lympho-haematopoietic malignancies was followed among about 75 000 exposed and 36 000 unexposed workers employed (from 1972 to 1987) in 672 factories in 12 cities in China. These included a variety of industries and occupations, including painting, printing, and manufacture of footware, paint and other chemicals. There was a statistically significant trend of increased risk across three categories of cumulative exposure to benzene for all leukaemia and ANLL / MDS (Hayes *et al* 1997). The risk of ANLL/MDS was strongly associated with increasing amounts of recent (less than 10 years ago) exposure (p for trend 0.003) but not with distant (at least 10 years ago) exposure (p for trend 0.51). However, in a later follow-up the risk of all leukaemia was highest 2-9 years since first exposure (RR 6.7; 95% CI 1.4-120), the risk was increased at 10-24 years since first exposure (RR 2.1, 95% CI 1.0-5.2), and still increased after more than 25 years since first exposure, although not statistically significantly (RR 2.2; 95% CI 0.7-8.0) (Linet *et al* 2015). However the lag time trend was not statistically significant (p=0.40) . For none of the categories (e.g. >25 years or 10-24 years from start of exposure) there was information on how exposure might have been distributed between more recent and more distant exposures. In this latest follow-up there were also 8 cases of MDS among the exposed indicating a statistically significantly increased risk (lower limit of 95% CI=1.9), but as there were no cases among the unexposed, a quantitative risk estimate could not be calculated. It should be noted that as the study covers various industries, the workers

might have been exposed also to other chemicals. While any effect of such expoures was not assessed in the studies, it is to be noted that there are not many occupatinal factors for which a causal association has been established with leukaemia (see sub-chapter Quality considerations) and which would consequently have confounded the results if not controlled for.

The risk of leukaemia following benzene exposure was investigated in three cohorts of **petroleum industry** and distribution workers : the 'UK Petroleum cohort' (Lewis *et al* 1997, Rushton and Romaniuk 1997), the 'Australian Health Watch' (AHW) cohort (Glass *et al* 2000, 2003 and 2005) and the 'Canadian petroleum marketing and distribution worker' cohort (Schnatter *et al* 1996). In the UK study, although odds ratios[9] were increased in some exposure categories for all leukaemia, CLL and AML, none of the risk estimates was statistically significantly increased. In the Australian study a strong association was found between leukaemia risk and exposure to benzene. Increase in risk by cumulative exposure was reported from 1-2 ppm-years onwards, with statistically significant increases for exposure levels of 2-4, 8-16 and $\geq$16 ppm-years (Table 43). In the Canadian cohort no increase in risk was observed. The authors, however, acknowledged that the power of the study to detect e.g. a two-fold risk was limited (Schnatter *et al* 1996). A pooled analysis of the UK, Australian and Canadian cohorts with an extended follow-up time was conducted (Schnatter *et al* 2012, Rushton *et al* 2014, Glass *et al* 2014). Those studies did no longer assess the risk of leukaemia overall, but focused on specific subtypes. While there was little evidence of dose-response relationship for AML, CLL, CML and myeloproliferative disease, cumulative exposure to benzene increased the risk of MDS (OR=4.3; 95% CI 1.3-14) when the highest exposure category (>2.93 ppm-years) was compared with the lowest ($\leq$0.348 ppm-years). The risk of MDS was also increased among workers with likely frequent peak exposures vs those without (OR=6.32; 95% CI 1.32 – 30.2). The results suggested that MDS may be the most relevant health risk for lower exposures. For CML the risks were increased when exposures were restricted to a time window of 2-20 years before diagnosis, but there was no clear dose-response (p for trend 0.16, see Table 43). For MPD the 2-20 year time window analysis also indicated a significant trend by increasing cumulative exposure as well as a risk from peak exposures. The strength of these more recent follow-ups is that the diagnoses were systematically reviewed by haematopathologists ensuring a higher accruacy of leukaemia subtype assessment than what was possible in the earlier reports.

Two cohort studies have been conducted among **offshore petroleum industry** workers. Kirkeleit *et al* (2008) followed 27 919 Norwegian offshore workers and observed an increased risk of AML (RR=2.9; 95% CI 1.3–6.7). A statistically significant risk of AML was found among workers with their first employment in this industry in 1981-1985, but not for those employed for the first time in 1986-2003. Benzene exposure was not quantified, but based on previous studies for this industry, the authors estimated that exposure ranged from below 0.001 to 0.7 ppm. Stenehjem *et al* (2015) followed 24 917 Norwegian men reporting offshore work between 1965 and 1999. The cumulative exposures were relatively low, <1 ppm-years. There was evidence of a dose-response pattern for AML (p for trend 0.052) when exposure tertiles of <0.001 – 0.0037, >0.0037 – 0.128 and 0.124 – 0.948 ppm-years were compared to the unexposed. For AML there was also indication of a trend by average exposure in ppms (p for trend 0.092) and average number of peak exposures exceeding the Norwegian STEL value of 3 ppm (p for trend 0.056). The trend test did not indicate an association between cumulative exposure and risk of CLL (p for

[9] An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Odds ratios are most commonly used in case-control studies

trend 0.212) or for all myeloid neplasms overall (p for trend 0.188) which included AML, CML and MDS together.

There are two cohorts of chemical industry workers: the **'Dow' cohort** and the **'Chemical Manufacturers Association (CMA)' cohort**. In the Dow cohort a non-significantly increased leukaemia mortality was observed earlier (SMR 1.9; 95% CI 0.5-4.9), but based on only 4 cases (Bond 1986). In the later follow-ups the risk was only slightly above the background (SMR 1.1; 95% CI 0.6-2.0, (Bloemen *et al* 2004 and SMR 1.2; 95% CI 0.7 – 2.0, Collins *et al* 2015). In the CMA cohort there was an indication of a dose-response (p for trend 0.01), but based on only 6 cases and no cases at all in the unexposed (Wong 1987). In a later follow-up of one of the CMA cohort plants Collins *et al* (2003) found indication that for benzene related risk of leukaemia and ANLL the number of days with peak exposures above 100 ppm would be a better predictor than cumulative exposure. Yet the number of deaths for these endpoints were small. Chemical industry workers may obviously be exposed to various chemicals (see chapter Quality considerations for discussion about confounding).

Swaen *et al* (2005) followed cancer mortality in a cohort of 311 men exposed to benzene solvent in caprolactam production during 1952-68 in the Netherlands. The average exposure was estimated as 159 ppm-years. Based on one observed case of leukaemia the mortality was not increased (SMR=0.86; 95% CI 0.01 – 4.3).

Guénel *et al* (2002) conducted a nested case-control study within a cohort of 170 000 male electricity and gas utility workers employed by EDF-GDF. The risk of leukaemia was increased among workers with estimated cumulative benzene exposure of $\geq$16.8 ppm-years (OR=3.6; 95% CI 1.1–12). There was an indication of dose-response relation (OR=1.2, 95% CI 1.0 – 1.5 per 10 ppm-years increase in exposure). It is to be noted that the study assessed exposure originally with exposure scores that were then "converted roughly" to ppms based on publicly available literature data.

Seniori Constantini *et al* (2003) followed 796 women and 891 men employed by an Italian shoe factory between 1939 and 1984. The source of exposure was benzene-based glues and the primary route of exposure was via inhalation, although the potential for skin exposure existed. Leukaemia mortality indicated an increasing trend for cumulative benzene exposure categories of <40, 40-99, 100-199 and >200 ppm-years (p for trend 0.02).

Meta-analyses

Several meta-analyses have recently been conducted to investigate further the relationship between benzene exposure and leukaemia for issues where each individual study had too few cases for a proper analysis or where studies provided heterogeneous results. Especially the dose-response relationship and the risk for subtypes of leukaemia.

Khalade *et al* (2010) analysed the relationship between occupational benzene exposure and the risk of leukaemia overall and the four main subtypes based on 15 studies. A statistically significant increase for the effect estimate (risk estimate like OR, RR, SMR etc) was found for all leukaemia combined, but the study-specific estimates were strongly heterogeneous. For 9 studies effect estimates were available by cumulative exposure (ppm-years). Taking into account the average level of cumulative exposure in each study practically eliminated the heterogeneity, so the variable exposure levels seemed to explain the heterogeneity observed in the overall estimate. The risk was statistically significantly increased in each exposure category and the trend was significant (p 0.015) (Table 26). For AML there were less studies available, but similar results were obtained although without statistical significance (Table 26), p for trend 0.8. For CLL there was some indication of an increased risk overall (1.31; 95% CI 1.09 – 1.57), but the trend was not statistically significant. For CML there were no studies available with cumulative exposure estimates. It is to be noted, however, that when allocating a given study in exposure categories described in Table 21, studies with quite different exposures might be placed in the same category. So some caution is needed in interpeting the results. It is also noted

that for example for the Hayes *et al* (1997) study Khalade seems to have used NHL risk estimates for CLL, which seems quite questionable as CLL is only one type of NHL in the latest WHO classification. Furthermore Khalade *et al* (2010) used cumulative exposures for the study of Constantini *et al* (2008) while no such estimates are reported in the original study.

Table 26: Risk estimate for risk of all leukaemia and AML by cumulative occupational exposure to benzene according to Khalade *et al* (2010)

Cumulative exposure (ppm-years)	Risk estimate (95% CI)	
	All leukaemia	AML
<40	1.64 (1.13 – 2.39)	1.94 (0.95 – 3.95)
40 – 99.9	1.90 (1.26 – 2.89)	2.32 (0.91 – 5.94)
≥100	2.62 (1.57 – 4.39)	3.20 (1.09 – 9.45)

Vlaanderen *et al* (2010) used meta-regression techniques to explore the shape of the exposure-response curve between occupational exposure to benzene and risk of leukaemia. Nine studies had sufficient quality to be included and they provided altogether 30 effect estimates at various exposure levels. The natural spline showed a supra-linear shape at cumulative exposures less than 100 ppm-years,i.e. the risk at those levels would be higher than that predicted by a linear model. However the natural spline fitted the data only marginally better than a linear model (p=0.06). The results suggested that the Pliofilm and the NCI/CAPM cohorts were particularly influential for the high-exposure region of the predicted exposure-response curve. Exclusion of the Pliofilm cohort resulted in a strong reduction of risks predicted for cumulative exposures >100 ppm-years, whereas exclusion of the NCI/CAPM study had the opposite effect. Exclusion of the other studies had little impact on the predicted exposure-response curve. Impacts of individual studies in the lower exposure-range were less pronounced. The natural spline based on all data indicated a significantly increased risk of leukaemia at an exposure level as low as 10 ppm-years (RR=1.14; 95% CI 1.04 – 1.26). Using a linear model (without intercept) resulted in a somewhat lower risk estimate for 10 ppm-years (RR=1.05; 95% CI 1.02 – 1.07). These estimates do not incorporate any time-window as regards which exposures before diagnosis were considered. There was also no assessment of the heterogeneity of the data.

In an earlier report **Vlaanderen *et al* (2008)** developed a tiered quality framework specific for human observational studies and quantitative risk assessment (QRA). Specific focus was on the quality of expsoure assessment. The framework was then applied to rank seven studies that had assessed the association between exposure to benzene and risk of AML. The ranking was 1. UK Petrol, 2. AHW, 3. CAPM-NCI, 4. Pliofilm and 5. Dow. Two studies, EDF-GDF (Guénel *et al* 2002) and Monsanto sub-cohort of CMA (Collins *et al* 2003), were ranked as not suitable for QRA. For UK Petrol, AHW and Pliofilm there have been more recent publications than those ranked by Vlaanderen *et al* 2008. However, it is noteworthy that the study historically most widely used for regulatory quantitative risk assessment (Pliofilm) ranked only second last in quality among the 5 studies that passed the assessment. However, the framework does not illustrate how muh better or worse the quality of a given study was compared to the next or the higehst or lowest ranked one.

Vlaanderen *et al* (2011 and 2012) conducted meta-analyses incorporating a stratification by three study quality indicators. Higher quality was assumed (1) for studies with follow-up starting in 1970 or later than for those with an earlier start of follow-up, (2) for studies that identified a statistically significantly increased risk of AML (5 different quality categories) and (3) for studies with quantitative exposure estimates instead of

semi-quantitative or qualitative ones (4 categories). For ALL, CLL and CML the relative risks increased with increasing study quality for all three stratification approaches, thereby suggesting, according to the authors, an association with the exposure to benzene. It is to be noted that for some of the studies included, later follow-ups have been published that have also incorporated an improved diagnostic review that has influenced the allocation of leukaemia cases to specific subtypes (see detailed description of the petroleum industry cohorts). However, it is not possible to analyse the impact on the meta-analysis results of those later changes as well as the impact of not having included such a diagnostic review in other studies. Furthermore the quality criterion linked to follow-up start may exclude valuable historical cohorts with a long follow-up and the quality criterion linked identifying an increased risk for AML does not consider MDS. Nevertheless the relative risks of ALL, CLL, and CML increased also when considering the quality criterion based on whether quantitative exposure estimates for benzene were available.

Table 27 shows the results for all studies and those with follow-up in 1970 or later. For the latter, the meta relative risk is statistically significantly increased for all leukaemia subtypes studied.

Earlier meta-analyses had analysed studies in the petroleum industry (Raabe and Wong 1996) or compared studies across different industries (Schnatter *et al* 2005). These meta-analyses did not quantitatively analyse dose-response. Raabe and Wong did not find any increased risk of leukaemia and Schnatter found a significantly increased risk for AML and some indication of increased risk for CLL, while for ALL and CML the data was sparse and inconclusive.

Table 27: Relative risk of subtypes of leukaemia based on meta-analysis of Vlaanderen *et al* (2011 and 2012)**.**

Leukaemia subtype	N of studies	N of exposed cases	Meta relative risk (95% CI)	
			All studies	Follow-up 1970 or later
AML	21	217	1.68 (1.35 – 2.10)	2.08 (1.59 – 2.72)
ALL	17	47	1.44 (1.03 – 2.02)	1.92 (1.00 – 3.67)
CML	17	76	1.23 (0.93 – 1.63)	1.67 (1.02 – 2.74)
CLL	18	111	1.14 (0.78 – 1.67)	1.63 (1.09 – 2.44)

Quality considerations

Control for confounding is an important quality consideration in epidemiological studies. The occupational factors (other than benzene exposure) for which a causal association with leukaemia risk has been established are ionizing radiation and handling of cytostatic drugs, especially alkylating agents (Polychronakis *et al* 2013). As regards non-occupational factors a causal link has been established between certain retrovirus infections and specific types of leukaemia (Carrillo-Infante *et al* 2007). Tobacco smoking has shown a weak association with risk of leukaemia which, however, is considered causal for AML (IARC 2004). The latter conclusion was partly influenced by the presence of benzene in tobacco smoke and the established causal relationship between benzene exposure and AML. IARC found no clear association between tobacco smoking and lymphoid leukaemia/lymphoma.

Apart from adjustment for the effect of age and gender the epidemiological studies referred in the previous chapters usually did not adjust for the confounding factors. Nevertheless it would seem unlikely that retrovirus infections or handling of cytostatic drugs would have confounded the studies performed in these industrial cohorts. Some workers in the petroleum offshore cohorts may have been exposed to ionizing radiation, e.g. due to non-

destructive testing of welding seams (Stenehjem *et al* 2015). However, the exposure levels were low. Control for the effect of smoking was performed in the Australian petroleum worker cohort (Glass *et al* 2003) and the Norwegian offshore worker cohort by Stenehjem *et al* (2015). In the latter study it was also reported that the control for this confounding had little effect as the smoking habits of the exposed and unexposed were quite similar. Overall it can be concluded that due to the lack of control for confounding for smoking in most of the studies, it cannot be excluded that some confounding may have occurred. However, due to the fact that smoking is not a potent causative agent for leukaemia (e.g. compared with lung cancer) it looks unlikely that such confounding (if any) would be a major quality problem for these studies at levels of high occupational exposuer to benzene. However, the comparison of biomonitoring data from smokers and workers with low occupational exposures does not exclude the possibility of confounding at those ranges of exposure, i.e. around 0.1 ppm and below (see chapter 6.1.1).

The epidemiological studies described were either cohort studies or nested case-control studies embedded in cohort studies. Such studies are considered less prone for bias than other types of epidemiological studies and in the review of the studies no particular concern for bias emerged.

The **accuracy of assessing exposure** to benzene is an important quality aspect and especially so for any consideration of dose-response. As pointed out in the dose-response shape meta-analysis paper by Vlaanderen *et al* (2010) all the studies included in that meta-analysis assessed exposure retrospectively based on relatively limited sets of exposure measurements. Exposure estimation was based on decision rules to extrapolate these exposure measurements to (older) time periods and exposure circumstances for which no measurements were available. The significant amount of expert judgement that goes into those decision rules makes it conceivable that systematic differences in exposure assessment may exist between studies. This situation is illustrated by the exposure assessment for the Pliofilm cohort where three groups of authors previously published three different sets of exposure estimates (Crump and Allen 1984, Paustenbach *et al* 1992, Rinsky *et al* 1987, 2002, see also Table 43 row for Pliofilm Paxton 1996). These resulted in significant variation in assigning the leukaemia cases to exposure categories and consequently influenced the risk estimates of the exposure categories. This is also illustrated in the unit risk estimates for additional leukaemia cases calculated by SCOEL (1991) which were based on these Pliofilm cohort reports and ranged from 0.5 to 6.6 per1000 workers exposed to 1 ppm for 40 years (see Table 29). A fourth exposure estimation for the Pliofilm cohort was published recently (Williams and Paustenbach) (2003) and used in the latest follow-up (Rhomberg *et al* 2016). Williams and Paustenbach (2003) suggested that the earlier Paustenbach (1992) estimates over-estimated the exposure for the highest exposure categories, Rinsky (1981 and 1987) under-estimated exposure for most jobs and Crump and Allen (1984) both under- and over-estimated exposures depending of the job category and time period. There is no straightforward protocol to assess or rank the accuracy of the exposure estimates used in the various other cohorts.. Finally, while the most commonly used exposure metric in the studies described was cumulative exposure in ppm-years, some studies have found indications that average exposure in ppms or number or level of peak exposures might play a role as well. The same way as cumulative exposure these estimates are based on retrospective assessments and the different exposure metrics (including cumulative exposure) are correlated with each other making it impossible to definitively confirm or exclude their role one over the other with methods of epidemiology.

Other haematopoietic neoplasms

Some of the cohort studies listed for leukaemia in Table 43 have also analysed the benzene related risk of non-Hodgkin lymphoma (NHL) and/or multiple myeloma (MM). The results have been heterogeneous. There was an increased risk of MM in the earlier follow-up of the Pliofilm cohort (SMR=4.1; 95% CI 1.1 – 10.5) based on four cases, which did not

remain statistically significant in a later follow-up (SMR=2.12; 95% CI 0.69 – 5.0) (Rinsky *et al* 1987 and 2002). In the NCI/CAPM cohort there was no increase in the risk of MM, while for NHL there was a statistically significant trend for increased risk by increasing cumulative exposure (p for trend 0.04) (Hayes *et al* 1997) and the overall risk among the exposed was still statistically significant in the latest follow-up (RR=3.9; 95% CI 1.5 – 13) while no data were reported by exposure level (Linet *et al* 2015). The Norwegian offshore worker cohorts found for MM a statistically significantly increased risk (RR=2.89; 95% CI 1.25 – 6.67) (Kirkeleit *et al* 2008) or a significant trend for increased risk by cumulative exposure (p for trend 0.024) (Stenehjem *et al* 2015). Neither study found an increase of risk for NHL. Collins *et al* (2003) found indication of an increased risk of MM in benzene exposed chemical industry workers with some indication that peak exposures would be a better predictor of risk than cumulative exposure.

The results of meta-analyses have also been heterogeneous for NHL while for MM there is more consistent indication of an association.

For MM **Infante (2006)** found an increased meta relative risk (RR=2.1; 95% CI 1.3 – 3.5) based on data from seven well defined benzene cohorts outside petroleum refining. As further described in the leukaemia section Vlaanderen *et al* (2011) conducted a meta-analysis incorporating a stratification by three quality indicators. The meta relative risk increased with increasing study quality for MM, thereby suggesting an association with the exposure to benzene. The meta relative risks were, however, only slightly above 1 (Table 28)

Table 28: Relative risk of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and multiple myeloma (MM) based on meta-analysis of Vlaanderen *et al* 2011

Disease	N of studies	N of exposed cases	Meta relative risk (95% CI)	
			All studies	Follow-up 1970 or later
NHL *	33	647	1.00 (0.89 – 1.13)	1.21 (0.94 – 1.55)
HL	27	146	0.99 (0.83 – 1.19)	0.91 (0.59 – 1.40)
MM	26	284	1.12 (0.98 – 1.27)	1.26 (0.92 – 1.71)

* Includes both NHL and lymphosarcoma/reticulosarcoma (preferred NHL if the study reported both)

Smith *et al* (2007) reviewed 43 case-control studies on NHL that recognised persons with "probable benzene exposure" and concluded that 40 of them indicated an increased risk and 23 found a statistically significantly increased risk. Steinmaus *et al* (2008) performed a meta-analysis of 22 studies analysing the association between benzene exposure and NHL. The meta relative risk was increased (RR=1.22; 95% CI 1.02 – 1.47). The risk was even higher when excluding studies that likely included unexposed among the exposed, or studies based solely on self-reported exposure, or when correcting for healthy worker effect. Swaen *et al* (2010) pointed out methodological inconsistencies of this meta-analysis, e.g. using outdated follow-ups instead of more recent ones, inconsistent application of selection criteria and did not find an increased risk of NHL in that meta-analysis. In the quality incorporated meta-analysis of Vlaanderen *et al* (2011) there was also some increase of meta RR for NHL by increasing quality, but the effect was less pronounced than that observed for leukaemia subtypes or for MM. For NHL it must be noted that it is a heterogeneous group of histological subtypes, and the definition of NHL overall and its subtypes has evolved over the last several decades with the application and discontinuation of several classification schemes, which complicates the assessment of exposure to benzene and risk for NHL (IARC 2012, Health Council of Netherlands 2014, Vlaanderen *et al* 2011). E.g. under the current WHO classification (Swerdlow *et al* 2008), ALL and CLL are subcategories of lymphomas.

For Hodgkin's disease (HL) there was no evidence of an association in the meta-analysis of Vlaanderen *et al* (2011) (see Table 28) or in the studies assessed by IARC (2012).

Some of the data on MDS are described above in the chapter concerning leukaemia and there are recent studies indicating a risk from benzene exposure (e.g. Schnatter *et al* 2012 and Linet *et al* 2015). More recently a case-control study found that the risk is not evenly ditributed over various subtypes of MDS (Copley *et al* 2017). The risk was most evident for refractory cytopenia with multilineage dysplasia which was also the most common type of MDS in the population studied accounting for 70% of all cases.

International and national assessments of of human data on benzene and haematopoietic and lymphoid malignancies

Despite the different classifications in time, the recent international or national assessments have reached conclusions on the relationship between benzene exposure and development of various haematopoietic and lymphoid malignancies.

IARC (2012) concluded: *Benzene causes AML/ANLL. Also a positive association has been observed between exposure to benzene and ALL, chronic lymphocytic leukaemia (CLL), MM and NHL.* The full monograph of the IARC 2017 re-assessment is not yet available but according to the summary report (Loomis *et al* 2017) the Working Group concluded that *in adult humans, benzene causes ANLL, including AML and that the previous observations of limited evidence for CLL, MM and NHL were also confirmed. Small minorities of the Working Group concluded that theevidence of carcinogenicity was inadequate for lung cance and sufficient for NHL*.

Health Council of the Netherlands (2014) concluded: *Epidemiological studies and case studies provide clear evidence of a causal association between exposure to benzene and leukaemia, especially AML/ANLL. More recently 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 associations with other B-cell lymphomas such as follicular lymphoma and diffuse large B-cell lymphoma remain unclear.*

AGS (2012) concluded (translated from German to English): Numerous studies investigated the carcinogenic effects of benzene after occupational exposure. Abnormalities of acute non-lymphatic leukaemia (ANLL), especially AML, have been reported. In addition, possible associations with other forms of leukaemia have been reported like chronic Myeloid Leukaemia CML), chronic lymphocytic leukaemia (CLL), multiple myelomas (MM), acute lymphocytic leukaemia (ALL) and non-Hodgkin's lymphomas (NHL).

OTHER CANCERS

IARC reviewed 24 cohort studies and one case-control study with information on potential or estimated exposure to benzene and risk of lung cancer (IARC 2012). Most cohort studies and the case-control study showed no association but two cohort studies with quantitative exposure assessment showed evidence of a dose-response and two others observed a statistically significant increase in risk with risk estimates around 1.2. One of the studies identifying a dose-response association was the NCI/CAPM cohort study in China. Since the IARC evaluation, the latest follow-up of this study did not analyse dose-response but a statistically significant increased risk was observed for lung cancer mortality (RR 1.5, 95% CI 1.2 -1.9) (Linet *et al* 2015). However, no data on smoking habits is available in this study to allow control for confounding by smoking. Nor was there any control for confounding for workplace exposure to established lung carcinogens.

IARC reviewed 21 cohort studies and two case-control studies with information on potential or estimated exposure to benzene and risk of kidney cancer (IARC 2012). The cohort

studies did not generally indicate any association. In one of the case control studies an association was found while in the other one no association was found.

For cancers other than the above IARC concluded that while associations have occasionally been found in some of the cohort studies conducted, there was no consistence across the cohorts (IARC 2012). See above also the summary conclusions of IARC 2017 assessment.

CONCLUSIONS ON HUMAN DATA

Epidemiological studies provide clear evidence of a causal association between exposure to benzene and ANLL (including AML). There is also recent evidence of an association between benzene exposure and MDS. Positive associations have also been reported for leukaemia subtypes other that ANLL/AML, i.e. for ALL, CML and CLL.There is also some evidence of an association between benzene exposure and risk of MM and NHL.

7.7.2 Animal data

DECOS (2014) summarized the available data as follows: "*Several studies with inhalation and oral exposure provide evidence that benzene is carcinogenic 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 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)*".

7.7.3 Summary

IARC concluded that "*There is sufficient evidence in humans for the carcinogenicity of benzene. Benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia. Also, 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*" (IARC 2012). "*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)*" (DECOS 2014).

Benzene has a harmonised classification for Carcinogenicity Category 1A (H350) (EC 1272/2008; EU Commission 2008)

7.8 Reproductive toxicity

7.8.1 Human data

Katukam *et al* (2012) investigated industrial workers to explore any association between various reproductive malfunctions in terms of infertility and other related factors and benzene exposure. Blood and semen samples were collected from total 160 industrial workers exposed to benzene. Benzene concentration in the blood was 26.92±21.33

µmol/dL. Workers were divided into three groups depending on the length (years) of exposure for 8 hours/day: Group I; low exposed group with 0–5 years exposure (n= 52); Group II; medium exposed group with 5–10 years exposure (n=73); and Group III; high exposed group with 10–15 years exposure (n=35). Two hundred non-occupationally exposed individuals were used as controls. The sperm DNA integrity was determined by the comet assay method and correlated with benzene concentrations in blood and semen. No significant deviation was observed in macroscopic semen parameters between control and exposed groups. In contrast, there was a significant decrease in total sperm count and sperm motility and a significant increase in abnormal sperm morphology among the exposed groups when compared with the controls. A significant increase in comet tail length was also observed in the exposed groups in comparison to controls. In the regression analysis, the data were observed to be significant for Group II industrial workers but not Group I or III. Authors concluded that the mean tail length seen in the benzene-exposed groups, indicative of DNA damage, is an important step from spermatogenesis to malfunctions such as infertility, as sperm integrity is considered one of the major factors in male infertility.

7.8.2 Animal data

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*" (DECOS 2014).

"*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*" (DECOS 2014).

Developmental effects

"*There are numerous inhalation studies available in which rats or mice have been exposed to benzene during pregnancy. 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*" (DECOS 2014).

7.8.3 Summary

"*No effects on fertility were observed in female rats exposed up to 300 ppm benzene for 10 weeks. In mice, 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)*" (DECOS 2014).

"*Several developmental toxicity studies did not 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/m³ (>50 ppm) often associated with maternal toxicity" (DECOS 2014).

7.9 Mode of action (MoA) and Adverse Outcome Pathways (AoP) considerations

IARC developed key characteristics of carcinogen as a basis for organizing data on mechanism of carcinogenesis (Smith *et al* 2016). The key characteristics identified for leukaemia induced by benzene are

- metabolic activation (electrophilic epoxides, aldehydes and quinones),
- genotoxicity (DNA damage, mutations, chromosome aberrations),
- oxidative stress (reactive oxygen species, oxidative DNA damage),
- altered DNA repair (topoisomerase II inhibition, inhibition of DNA repair pathways, metabolites inducing genomic instability),
- epigenetic alterations (altered DNA methylation, miRNA changes, histone modifications),
- immunosuppression (reduces immune surveillance),
- modulation of receptors (AhR dysregulation), and
- altered cell proliferation (stem cell transformation, proliferation, clonal expansion).

Meek and Klauning (2010) are proposing five key events in the mode of action of benzene-induced leukaemia.

1. Benzene metabolism via Cytochrome P450

Benzene oxide appears to be the principal initial metabolite formed through metabolism by Cytochrome P450 2E1 (CYP2E1). This metabolite is further metabolized to a phenol which subsequently results in catechol and/or hydroquinone metabolites, both of which can be additionally metabolized to toxic forms. In addition, benzene oxide may be further metabolized by a peroxide hydrolase to a benzene dihydrodiol. Also, there is the potential for a ring opening of the benzene leading to the formation of aldehyde metabolites. Several of these metabolites have been proposed as possibly producing genetic damage or initiation of leukaemia in the bone marrow (Meek and Klauning 2010).

2. The interaction of benzene metabolites with target cells in the bone marrow cells

Benzene is mainly metabolised in the liver and lung by cytochromes-P450 and some metabolites may be distributed to the bone marrow. However, since CYP2E1, the enzyme mainly involved in the metabolism of benzene, is also expressed in human bone marrow stem cells *in vitro* (Bernauer *et al* 2000), it can be expected that reactive benzene metabolites may also be formed directly in the bone marrow.

In addition, myeloperoxidase, which is most abundanly expressed in neutrophil granulocytes, metabolises the hydroquinones to their respective reactive benzoquinones which can undergo redox cycling with the production of reactive oxygen species.

Moreover, the benzene metabolite *trans,trans*-muconaldehyde was demonstrated to inhibit gap junction intercellular communication (Rivedal *et al* 2010) within the cells of the bone marrow niche.

The bone marrow niche plays an important role for the homeostasis in the bone marrow and for the development of leukaemia. All mature blood cells are derived from a common cellular ancestor, the haematopoietic stem cell (HSC). HSCs are a unique population of somatic stem cells that can both self-renew for long-term reconstitution of HSCs and differentiate into haematopoietic progenitor cells (HPCs), which in turn give rise, in a hierarchical manner, to the entire myeloid and lymphoid lineages. The differentiation and maturation of these lineages occurs in the bone marrow niche, a microenvironment that regulates self-renewal, survival, differentiation, and proliferation, with interactions among

signaling pathways in the HSCs and the niche required to establish and maintain homeostasis. The accumulation of genetic mutations and cytogenetic abnormalities within cells of the partially differentiated myeloid lineage, particularly as a result of exposure to benzene or cytotoxic anticancer drugs, can give rise to malignancies like acute myeloid leukaemia and myelodysplastic syndrome (Greim *et al* 2014).

Hirabayashi and Inoue (2010) performed experiments with AhR-knockout mice. The Ah receptor regulates xenobiotic-metabolising enzymes such as cytochrome P-450. Based on their findings, the authors categorised the mechanisms of benzene-induced haematopoietic toxicities into two: first, a cell-cycle arrest-induced haematopoietic impairment in haematopoietic progenitor cells carrying AhR, and, second, metabolite-induced cytotoxicity related to hepatic AhR, both after benzene exposure. The former involves a low-dose effect, in general, owing to its mechanism linked to receptor-mediated toxicity; whereas the latter involves metabolite-induced xenobiotic chemical toxicity with a possible threshold, although this requires further study.

Benzene affects nearly all blood cells types and also progenitor cells which circulate in the blood stream. Such cells were suggested to be more sensitive to the haematotoxic effects than mature cells (Lan *et al* 2004).

3. Formation of initiated, mutated target cells

Benzene induces the following effects:

- Chromosome aberrations and aneuploidy. There is overwelming evidence from human and animal studies that benzene induces micronucleus formation, chromosomal aberrations and aneuploidy (Whysner *et al* 2004).
- DNA damage. Benzene also induces sister chromatid exchange and DNA strand breaks in humans and in experimental animals (Whysner *et al* 2004)
- Gene mutations. The induction of gene mutations by benzene seems to be possible *in vitro* and *in vivo*. However, the mutagenic effects observed *in vitro* in mammalian cells might have been secondary to chromosomal damage and the mutagenic effects *in vivo* were of low magnitude (<2-fold) not reflecting the magnitude of DNA-reactive carcinogens (Whysner *et al* 2004).
- 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. This mainly involved binding to proteins, for which benzene oxide and *p*-benzoquinone 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 (DECOS 2014; Whysner *et al* 2004).
- 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 (DECOS 2014).
- Inhibition of topoisomerase II. 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 (DECOS 2014). Also the metabolite *trans,trans*-muconaldehyde has been demonstrated to directly inhibit topoisomerase II (Frantz *et al* 1996). Topoisomerase II inhibitors such as the drugs amsacrine, etoposide, etoposide phosphate, teniposide and doxorubicin are used in the anti-cancer therapy. They are indeed also known to produce leukaemia in humans and some share structural and biological similarities with benzene. Furthermore, several genetic pathways that have been implicated in benzene-induced MDS/AML are associated with the inhibition of topoisomerase II. Whysner *et al* (2004) 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 (DECOS 2014).

- 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 (DECOS 2014).
- 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 tumour suppressor genes) (DECOS 2014).

4. Selective proliferation of the mutated cells

McHale *et al* (2012) hypothesize that the level or type of accumulated damage induced by benzene in haematopoetic stem cells (HSCs) usually leads to apoptosis which manifests as haematotoxicity.

However, benzene produces a number of effects on the bone marrow that can contribute to the proliferation of mutated cells (Meek and Klauning 2010).

Common to these effects is the disruption of the normal cell cycle and/or modification of normal apoptotic process resulting in an increase in the growth of the mutated target cell population. Changes in the cytokines TNFα and IL expression have been noted in the stroma of leukaemia patients which supports stromal involvement in the bone marrow in both the precursor cell and the leukemic cell proliferation. Modification of methylation by benzene can result in changes in the differentiation state of the stem and precursor cells. Benzene metabolites may modify cell differentiation through methylation or other processes. Reactive oxygen species and oxidative stress induced by benzene may induce cell proliferation via activation of second messengers and/or through cytotoxicity (a common link with benzene exposure in humans) (Meek and Klauning 2010).

5. Production of leukaemia

The formation of leukaemia is usually resulting from additional mutations and chromosome damage. The production of the leukaemia frequently occurs several year or decades after exposure to benzene suggesting that changes that occur in the target cells may remain dormant until further additional modification to bone marrow microenvironment and/or maturation or differentiation changes to the bone marrow population expresses itself resulting in a neoplasia (Meek and Klauning 2010).

Immune system dysfunction that leads to decreased immunosurveillance has been discussed as a contributing factor within the leukaemogenic process (IARC 2012).

Acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) are closely related diseases of the bone marrow that arise de novo in the general population or follow therapy with alkylating agents, topoisomerase II inhibitors, or ionising agents. Occupational exposure to benzene is widely thought to cause leukaemias that are similar to therapy-related AML (t-AML) and MDS (t-MDS). AML and MDS both arise from genetically altered CD34+ stem or progenitor cells in the bone marrow and are characterised by many different types of recurrent chromosome aberrations (Smith 2010).

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 (DECOS 2014):

- 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. 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. AML/MDS related to therapy and AML/MDS related to benzene exposure have therefore been considered biologically similar diseases (DECOS 2014).

Recent data suggest that the pattern of clonal cytogenetic abnormalities in benzene-exposed cases more closely resemble that of spontaneous AML than therapy-related AML (Irons *et al* 2013).

Another recent study indicates that subtypes with non-erythroid dysplasia (refractory cytopenia with multilineage dysplasia) may be associated with benzene exposure (Copley *et al* 2017).

Considerations on threshold mechanisms

Approaches based on threshold mechanisms

DECOS (2014) concluded that "*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*." Based on those considerations, DECOS derived an OEL for haematological effects observed in humans.

The LOA REACH Consortium (LOA 2017a) has submitted a document on 'Benzene: Importance of Dose Metrics in Assessing Stochastic versus Threshold Mechanisms'. LOA summarizes that "*For benzene and petrochemicals containing benzene it appears the approach best supported by available data is calculation of a DNEL because the key health effect for risk assessment, incidence of benzene-induced hematologic malignancy acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in humans, is likely to result from a threshold mechanism. The rationale for calculating a DNEL based on expectations of a threshold mechanism is based on several observations:*

1. *Benzene is not a direct-acting mutagen.*
2. *Its mechanistic chemistry is consistent with a threshold mechanism via protein cross-linking.*
3. *The dose metric correlating with MDS risk is incompatible with a stochastic mechanism because it is affected by dose-rate.*

Haematological and immunological effects

Many studies in workers have been published showing effects on haematological parameters at benzene concentrations at and above 2 ppm.

Hirabayashi and Inoue (2010) indicated two mechanisms for benzene-induced haematopoietic toxicities: first, a cell-cycle arrest-induced haematopoietic impairment in haematopoietic progenitor cells carrying AhR, and, second, metabolite-induced cytotoxicity related to hepatic AhR, both after benzene exposure. The former involves a low-dose

effect, in general, owing to its mechanism linked to receptor-mediated toxicity; whereas the latter involves metabolite-induced xenobiotic chemical toxicity with a possible threshold, although this requires further investigations.

Clastogenic and aneugenic effects

Benzene is clearly genotoxic. Several modes of action have been identified for benzene (Smith *et al* 2016) like: genotoxicity (DNA damage, mutations, chromosome aberrations), oxidative stress, altered DNA repair (topoisomerase II inhibition, inhibition of DNA repair pathways, metabolites inducing genomic instability), immunosuppression (reduced immune surveillance), modulation of receptors (AhR dysregulation), epigenetic alterations (altered DNA methylation, miRNA changes, histone modifications) altered cell proliferation (stem cell transformation, proliferation, clonal expansion).

The observed induction of gene mutations by benzene *in vitro* in mammalian cells might have been secondary to chromosomal damage and the mutagenic effects *in vivo* were of low magnitude (<2-fold) not reflecting the magnitude of DNA-reactive carcinogens (Whysner *et al* 2004).

DECOS (2014) concluded that "*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*."

Accordingly, DECOS (2014) concluded that "*leukaemia develops from genotoxic effects in the CD34 progenitor cells in the bone marrow, a primary target in benzene-toxicity. Overwhelming evidence exists that benzene causes chromosomal aberrations in haematopoetic cells in humans and experimental animals. The Committee considers this induction of chromosomal aberrations the most plausible explanation for benzene carcinogenicity*".

In a document submitted by the LOA REACH Consortium (LOA 2017a) it is argued that benzene is not a direct-acting mutagen but that its mechanistic chemistry is consistent with a threshold mechanism via protein cross-linking.

Hence, for the main modes of genotoxic action thresholds could be assumed.

Carcinogenicity

Benzene causes tumours in animals and in humans with the haematopoietic system as main target for humans (AGS 2012).

DECOS (2014) noted that persistent cytopenias and other blood disorders frequently precede the onset of leukemia in patients developing AML secondary to exposure to benzene. It is therefore likely that avoiding exposure causing haematological suppression will significantly reduce the risk for leukemia.

In a document submitted by the LOA REACH Consortium (LOA 2017a) it is argued that "*a DNEL can be derived because the key health effect for risk assessment, incidence of benzene-induced hematologic malignancy acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in humans, is likely to result from a threshold mechanism" because* benzene is not a direct-acting mutagen, its mechanistic chemistry is consistent with a threshold mechanism via protein cross-linking and the dose metric correlating with MDS risk is incompatible with a stochastic mechanism.

It is to be noted that the mode of carcinogenic action of benzene is complex and not fully clear. Several modes of action are described to contribute to benzene induced leukemia and there are remaining uncertainties whether all modes of action would have a threshold.

Even if all modes of action would have a threshold, those thresholds would need to be quantified. In the absence of quantitative data on those thresholds, especially a threshold for the aneugenic effects, benzene should be considered as a SCOEL carcinogen group B substance, for which a linear non-threshold model may be used as a default assumption.

7.10 Lack of specific scientific information

Although benzene is one of the most extensively studied chemicals in the world from a toxicological standpoint, the mode of action is not completely understood. It is well-known that the metabolism of benzene is required prior to the development of haematotoxicity and cancer, but the actual metabolite(s) that is/are responsible and how the blood cells are affected have not been completely elucidated (Arnold *et al* 2013).

8. Cancer Risk Assessment and exposure limit values

8.1 Published Approaches for Cancer Risk Assessment

8.1.1 SCOEL

SCOEL (1991) concluded that the different studies and exposure estimations available for the Pliofilm cohort provide an estimated range of dose-response of 0.5 – 6.6 x 10^{-3} per 1 ppm of benzene over a working lifetime of 40 years for leukaemia. SCOEL did not estimate quantitatively the dose-responses from cohorts other than Pliofilm available at that time, but concluded that they "*show a remarkable external consistency of the risk estimates based on independent data sets. The summarised risk estimates for benzene associated excess leukaemia deaths at a 10 ppm-year exposure are within one order of magnitude and vary between 3-15 cases/1000 exposed*". **SCOEL recommended that the limit value should be below 1 ppm.**

SCOEL estimated that a range of 0.5-6.6 additional leukaemia cases per 1000 workers exposed to 1 ppm benzene over a working lifetime of 40 years (40 ppm-years) represents at best the present knowledge on benzene-induced leukaemias. The linearly extrapolated ranges of additional lifetime leukaemia risks at different exposure levels are summarized below in Table 29.

Table 29: Linear extrapolated ranges of additional lifetime leukaemia risks (SCOEL 1991)

Benzene (ppm)	**Exposure** (ppm x years)	**Range of additional leukaemia risk per 1,000 workers**
0.1	4	0.05-0.7
0.5	20	0.25-3.3
1.0	40	0.5-6.6
3.0	120	2.0-19.8

"*On the basis of available information benzene/metabolized benzene has to be regarded as a genotoxic (clastogenic) substance in animals and humans with growing evidence for a mutagenic activity in in vitro systems (Glatt et al 1989, Glatt and Witz, 1990; Henschler, 1991) and the capability to bind covalently to DNA and other cellular macromolecules. Because of the genotoxic properties of benzene, no threshold of action can be identified at the present time* [1991]*, which means that with current scientific knowledge, no level of exposure can be determined below which there is no risk to health. However, by lowering*

the exposure, the risk can be reduced. This is specified by the need for minimization of exposure according to the requirements of Council Directive 90/394/EEC".

An occupational exposure limit value of 0.5 ppm (1.6 mg/m^3) would reduce the range of best estimated lifetime risks down to 0.25-3.3 additional leukaemia cases per 1000 exposed to 0.5 ppm, corresponding to an exposure of 20 ppm-years [see Table 29 above]*. This does not explicitly take into account the possible influence of target cell toxicity and is therefore thought to be a conservative approach.*

The main and sensitive targets of toxicity in animals and humans are the cells of the bone marrow and the haematopoietic system. Non genotoxic effects of the haematopoietic system in animals indicate a LOAEL of 10 ppm (32 mg/m^3) (Baarson et al 1984; Dempster and Snyder, 1989). No effect levels for non-genotoxic effects of the human haematopoietic system cannot be defined at the current time, but epidemiological studies, including that of Van Damme et al (1991), suggest that the dose-response relationship in humans may be similar to that in animals.

The LOAEL for chromosomal damage – induction of SCE and micronuclei – in peripheral blood cells and cells of the bone marrow of rats and mice ranges from 1 to 10 ppm (3.2-32 mg/m^3) (Tice et al 1984; Erexson et al 1986). Similar LOAEL's (1-10 ppm) have been reported for chromosomal aberrations (mainly structural) in peripheral lymphocytes of benzene exposed workers (Killian and Daniel, 1978; Picciano, 1979; Yardley-Jones, 1990). No information is available on genotoxic effects in bone marrow cells of humans at low exposure levels.

If haematotoxic effects play a role in induction of leukaemia, then avoidance of these will minimise the risk of leukaemia. Taking into account the above figures, and the range of LOAELs, the SF.G recommends that the limit value should be below 1.0 ppm (3.25 mg/m^3). This should also avoid the chromosomal effects.

8.1.2 The Netherlands / DECOS

DECOS (2014) concluded that 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.

DECOS (2014) further considered that benzene acts by a non-stochastic genotoxic mechanism and thereby applied a threshold approach. It was concluded that although several dose-response analyses on the benzene-leukaemia association have been reported, their power at low levels of exposure is low and they do not allow determination of a reliable point of departure for derivation of a health based OEL. Instead, DECOS considered that haematotoxicity is considered to be an early indicator of developing AML/MDS after benzene exposure and that persistent cytopenias and other blood disorders frequently precede the onset of leukaemia in patients developing AML secondary to benzene or other alkylating agents. DECOS, however, admitted that it is currently not proven that benzene-induced haematotoxicity forms an initial (required) step to neoplastic disease, or simply represents bone marrow damage.

DECOS noted that several good quality human studies on haematotoxicity are available that have regularly monitored individual benzene exposure levels before blood samples were collected. Consequently it was decided to consider data on haematotoxicity as the most suitable starting point. After review of the literature it was concluded that at exposure level of 0.6 ppm some studies still show haematotoxic effects while others do not. Based on a pragmatic weight-of-evidence approach applying an uncertainty factor of 3 because 0.6 ppm was a LOAEC and not a NOAEC resulted in a recommended OEL of 0.2 ppm.

8.1.3 Germany / AGS

AGS (2012) presented a non-linear and a linear approach.

Linear approach

The linear approach performed by AGS (2012) is based on the leukaemia ED10, which represents the cumulative exposure that would lead to a life-time leukaemia excess incidence of 10%. The epidemiological studies included are listed in Table 30 below. It is to be noted that there is variation between the dose-responses of those studies. These may reflect the inevitable inaccuracies in assessing past exposures to benzene in those studies (see 'Quality considerations' in section 7.7.1). It is concluded that no individual study among those is methodologically convincingly more reliable than any other. Consequently the average based approach chosen by AGS is justified. Based on the average ED10 of 582 ppm-years, an ED10 of 15 ppm (47 mg/m^3) was calculated for 40 years of occupational exposure. The ED10 of 15 ppm corresponds to a tumour risk of 4:10,000 for lifetime exposures to 0.06 ppm (0.2 mg/m^3) (see Table 31).

Table 30: Expected risk estimates for leukaemia after exposure to benzene based on epidemiological data according to Roller *et al* (2006), with amendments (AGS 2012)

Author	ED 10	ED 10 (considering 40 years exposure)	
	(ppm-years)	ppm	mg/m^3
Pliofilm cohort, USA			
Crump (1996)	912	22.8	74.1
Paxton (1996) with exposure assessment according to			
Rinsky	430	10.8	35.1
Crump	604	15.1	49.1
Paustenbach	1436	35.9	116.7
Rinsky *et al* (1987)	416	10.4	33.8
Rinsky *et al* (2002)	574	14.4	46.8
Shoe factory, Italy			
Seniori Constantini *et al* (2003)	641	16.0	52.0
Chemical wokers, China			
Hayes *et al* (1997)	910	16.6	54.0
Chemical workers, USA			
Bloemen *et al* (2004)	910	22.8	74.1
Wong *et al* (1987a)	800	20	65.0
EDF-GDF, France			
Guénel *et al* (2002)	117	2.9	9.4
Oil industry, Australia			
Glass *et al* (2003)	22	0.6	2.0
Glass *et al* (2005)	50.3	1.3	4.1
Average	**582**	**15**	**47**

Table 31: Tumour risk of benzene based on the linear extrapolation as performed by AGS (2012)

Benzene air		Risk
mL/m³ (ppm)	mg/m³	
0.6	1.9	4 : 1,000
0.06	0.2	4 : 10,000
0.006	0.02	4 : 100,000

Non-linear approach

AGS (2012) argues that the epidemiological studies do not allow a threshold for the carcinogenic effects to be derived due to methodological reasons (e.g., low number of cases with leukaemia and hence, low statistical power). Furthermore, several studies (Collins *et al* 2003; Glass *et al* 2003, 2005; Guénel *et al* 2002) may indicate an increased risk at low cumulative exposures.

AGS (2012) argues that based on findings in persons with specific genetic predispositions leading to error prone DNA repair and genetic instability, a causal relation between haematological effects and carcinogenicity is plausible.

AGS (2012) assumes the threshold for haematotoxic effects in the range of 0.5 ppm benzene (Lan *et al* 2004; Qu *et al* 2003a). AGS further assumes that in similar concentrations other pre-carcinogenic effects may have a breakpoint with increased effects at higher concentrations. AGS uses as breakpoint the BMD of 0.42 ppm for haematological effects as calculated by ATSDR (2007). AGS concludes that at this breakpoint the cancer risk would be reduced by a factor of 10.

Figure 5 shows the linear extrapolation based on ED10 for leukaemia as PoD and the sublinear extrapolation.

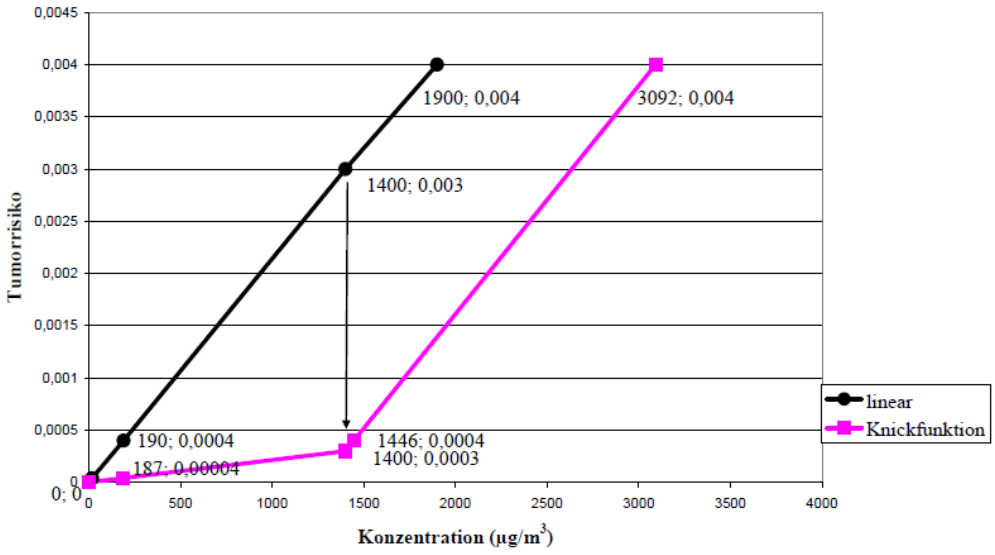

Figure 5: Dose-response relationship for benzene (with and without sublinearity assumption)

Table 32: Cancer risks based on a sub-linear dose-response relationship

Benzene (ppm)	**Benzene** (mg/m^3)	**Risk**
1.00	3.1	4 : 1,000
0.50	1.5	4 : 10,000
0.06	0.2	4 : 100,000

However, based on the following uncertainties, AGS (2012) did not follow the non-linear approach:

- Discussion on mechanism of action not concluded yet;
- Uncertainty in quantification of haematological effects in Lan *et al* 2004;
- Uncertainty of non-carcinogenic effects such as immunotoxicity and oxidative damage at low concentrations;
- Indication that haematological and immunological changes are not initial required steps in carcinogenicity.

8.1.4 France / ANSES

The French Agency for Food, Environmental and Occupational Health and Safety derived a toxicity reference value (TRV) for continuous exposure to benzene, i.e. general population (ANSES 2014). Even though the mechanism of the carcinogenic effect was concluded not be entirely clear, one of the modes of action, i.e. the production of unbalanced chromosomal aberrations, led to the choice of a non-threshold assumption. The critical effects chosen were leukemia. The key studies retained were the studies of Richardson 2008 and Silver *et al* 2002 who reanalyzed the data of the "pliofilm" cohort. Based on the study of Richardson, the RR was 1.19 (95%CI 1.10-1.29) per 10 ppm-years in the time window less than 10 years since exposure. It was converted to RR/ppm year as 1.019 with a 95% CI between 1.01 and 1.029 dividing by 10. The upper confidence interval was then taken as it was considered that the general population may be more sensitive. Conversion for discontinued to continued exposure was performed considering 250 d/365 d, 10 m^3/20 m^3 per 24 h and conversion of 1 ppm to 3200 µg/m^3. ERU was then calculated with the following formula: **ERU**=(RR – 1)/(ppm-year x 1095,9 (µg/m^3/ppm)) leading to **ERU=2,6×10^{-5} (µg.m^{-3})$^{-1}$** (see Table 33). It is to be noted that the above 95% CI of RR reported by Richardson (2008) for 10 ppm-years was restricted to the the time window less than 10 years since exposure. Diving it by 10, as explained above, relies on an assumption that it would have been cumulated during one year within that time-window of 10 years thus resulting in a relatively conservative estimate of what the absolute exposure level behind the cumulative exposure was. Secondly, the calculation does not use the approach of multiplying the excess relative risk (RR-1) with the background incidence or mortality of leukaemia in the population to estimate the excess incidence or mortality per a given dose. Instead, the methodology used by Affset calculates the increase in relative risk (RR-1) linked to a given dose (Affset 2010). As explained in chapter 8.2.2 the cumulative lifetime incidence of leukaemia is about 1%. For this reason it is difficult to compare the unit risk calculated with the other dose-response approaches described in this chapter.

Table 33: Conclusion on TRV (ANSES 2014)

Critical effect and source study	Establishment of method	Toxicity Reference Value (TRV)
Acute leukemia Richardson (2008)	$CI_{95\%}RR_{10\ ppm\text{-}year}=1.29$ Upper limit of the confidence interval for the exposure-risk function calculated by Richardson	**PF=2.6 10^{-5} ($\mu g \cdot m^{-3}$)$^{-1}$** 0.038 $\mu g.m^{-3}$ for a risk of 10^{-6} 0.38 $\mu g.m^{-3}$ for a risk of 10^{-5} 3.8 $\mu g.m^{-3}$ for a risk of 10^{-4} **Confidence level**: HIGH

8.1.5 US EPA

US EPA (2003) derived RfC for chronic inhalation exposure of the general population of benzene with 3 x 10^{-2} mg/m^3 (0.009 ppm). This value is based on benchmark dose modeling of data of decreased lymphocyte count in highly exposed workers (Rothman *et al* 1996). The lower bound 95th percentile benchmark concentration (BMCL) adjusted to continuous exposure (8 to 24 hours) was calculated with 8.2 mg/m^3 (2.6 ppm). An overall uncertainty factor of 300 was applied to the BMCL to derive the RfC.

8.2 Conclusion on Cancer Risk Assessment

8.2.1 Threshold approach

SCOEL (1991) stated that "*because of genotoxic properties of benzene, no threshold of action can be identified at the present time, which means that with the current scientific knowledge, no level of exposure can be determined below which there is no risk to health. However by lowering the exposure, the risk can be reduced*". They further refer to the OSH legislation under which it is mandatory to minimise exposure to carcinogens and mutagens in the workplace. They recommended that the limit value should be below 1.0 ppm, which they considered would also avoid chromosomal effects.

RAC considered, nearly three decades after SCOEL's 1991 recommendation, that there is now sufficient evidence available to set a 'mode of action-based threshold[10]' for benzene, i.e. following the ECHA/RAC –SCOEL Joint Task Force (2017) recommendations on the suitability of such methods for genotoxic carcinogens for which a threshold can be convincingly identified.

Even if benzene was clearly demonstrated to be genotoxic, genotoxic effects on the chromosome level (e.g., chromosome aberration, aneuploidy) and DNA reactivity due to secondary mechanisms (oxygen radical formation and error-prone repair of DNA lesions) could indicate that thresholds exist for the leading genotoxic effects. The observed induction of gene mutations by benzene *in vitro* in mammalian cells might have been secondary to chromosomal damage and the mutagenic effects *in vivo* were of low magnitude (<2-fold) not reflecting the magnitude of DNA-reactive carcinogens (Whysner *et al* 2004).

DECOS (2014) has considered the following: "*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*

[10] SCOEL (2013, p26 extracted form Figure) For weak genotoxins where secondary mechanisms are important, a practical thrshold is likely; a NOAEL would be set and a health based OEL derived.

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."

Furthermore, DECOS (2014) concluded that "*leukaemia develops from genotoxic effects in the CD34 progenitor cells in the bone marrow, a primary target in benzene-toxicity. Overwhelming evidence exists that benzene causes chromosomal aberrations in haematopoetic cells in humans and experimental animals. The Committee considers this induction of chromosomal aberrations the most plausible explanation for benzene carcinogenicity*".

With respect to the reviewed data on genotoxicity, clastogenic and aneugenic effects reported in peripheral blood lymphocytes or sperms of benzene-exposed workers can be considered as relatively specific effects for benzene, whereas for DNA damage (measured by the comet assay) co-exposure to other substances occurring at the workplaces could have contributed to the observed effects. For clastogenic and aneugenic effects, a LOAEC in the range of 1.0 ppm can be derived. Several studies in limited groups of workers do not show clastogenic effects at 0.28 ppm (Pitarque *et al* 1996; n=50), 0.1 ppm (Carere *et al* 1998, n=12; Lovreglio et al 2014, n=19); 0.07 ppm (Bukvic *et al* 1998, n=21); 0.06 and 0.009 ppm (Sha *et al* 2014, n=132 and 129); 0.03 ppm (Basso *et al* 2011, n=79); 0.01 ppm (Fracasso *et al* 2010). Those data indicate a NOAEC for clastogenic effects in the range of ≤0.1 ppm.

8.2.2 Extrapolations

In its evaluation of benzene, RAC also considered non-threshold approaches to estimate the risks and provide comparison. Some of these approaches are outlined below and in Appendix II. Finding that a mode of action-based threshold could be convincingly identified upon which to base an OEL, linear cancer extrapolation in the low dose range is considered overly conservative and therefore only given here for the sake of completeness.

ANSES (2014) performed a linear extrapolation for the general population based on data from the Pliofilm cohort (Rinsky *et al* 1981, 1987).

AGS (2012) included all cohorts that provided quantitative dose-response estimates. For the Pliofilm cohort AGS used also the estimates other than those provided by Rinsky *et al* (1981, 1987). AGS derived a linear non-threshold dose-response between benzene exposure and excess risk of leukaemia based on a mean ED10 value from 13 epidemiological reports with dose-response data coming from 6 cohorts. A 40 year exposure duration and a background leukaemia incidence of 1% were assumed. The ED10 value was 15 ppm which equals to an excess risk of **6.7×10^{-3} per ppm**. A tolerance risk value of 0.6 ppm and two acceptance risk values of 0.06 ppm and 0.006 ppm were derived for excess risk levels of 4:1,000, 4:10,000, and 4:100,000, respectively.

It is noted that Khalade *et al* (2010) identified a meta-analysis RR of 1.64 in the low exposure category of <40 ppm-years for all leukaemia. Assuming that exposure was 40 ppm-years in this category and using the AGS assumptions of background incidence of 1% and 40 years of exposure, results in a dose-response of **6.4×10^{-3} per ppm** (–1.64 – 1.0) x 0.01 / (40 ppm-years/40 years)). Khalade and colleagues used only one report per cohort, either the one with the longest follow-up or the most recent one, while AGS considered multiple studies per cohorts like Pliofilm where varying exposure assessment approaches have been used.

Using the same assumptions and the Vlaanderen *et al* (2010) natural spline meta RR of 1.14 per 10 ppm years results in a dose response of **5.6×10^{-3} per ppm** ((1.14-1) x 0.01 / (10 ppm-years/40 years)). Using the linear model meta risk estimate from Vlaanderen *et al* would result in a dose response of **2.0×10^{-3} per ppm** ((1.05-1) x 0.01 / (10 ppm-years/40 years)). Vlaanderen and colleagues used those 9 epidemiological cohorts that provided a dose-response and m*et al*so all the 6 quality criteria of inclusion (2 studies were excluded due to quality issues).

According to the Globocan 2012 database the cumulative incidence of leukaemia in EU28 in the age category 0-74 years is 0.9% for men, 0.5% for women and 0.7% overall. It is to be noted that leukaemia also occurs in children where occupational exposure wouldn't play a causal role. On the other hand, due to the latency time, occupational factors may play a role in the cases occurring at 75 years of age or later. According to the Globocan 2012 database there were 62 678 incident leukaemia cases in EU28, of which 3777 (6%) in the age category 0-14 years, 35801 (57%) in the age category 15-74 years and 23 100 (37%) in the age category $\geq$75 years. It seems that a cumulative background incidence of 1% overall is a reasonable assumption for estimations concerning the dose-response from occupational exposure.

The dose-response established by AGS (2012) results in the highest risk per a given exposure among all the linear dose-responses cited above. Consequently it is important to review any uncertainties related to that approach and to estimate the potential impact of any uncertainty identified.

The AGS calculations use a 40-year time window to distribute a given cumulative exposure. There is indication from some of the epidemiological studies that the risk is mostly associated with exposure within a time window 10 years since exposure or 20 years since exposure. It is not possible to retrospectively restrict the analyses by AGS to a specific time window. However, if as a very simplistic example, one distributes a given cumulative exposure over 20 years instead of 40 years it would result in two-fold absolute exposure levels. I.e. the dose-responses above should be divided by a factor of two.

It is also to be noted that the AGS approach uses a simple average without any weighting by size or quality of study and furthermore uses 6 follow-ups of Pliofilm and 2 follow-ups of Australian Health Watch thus multiplying the effect of these cohorts. The quality framework developed by Vlaanderen *et al* (2008) indicates that the EDF-GDF study (Guénel *et al* 2002) is of a questionable quality for being used in quantitative risk assessment (see chapter 7.7.1 section on meta-analyses). For Pliofilm it was described earlier that exposure assessment by Crump and Allen, Paustenbach and Rinsky all have deficiencies, so it is difficult to justify selecting only one, but also difficult to justify inclusion of more than one study based on any of these three estimates. If one includes from the AGS average (Table 31) for Pliofilm only the latest Crump (1996), Paustenbach and latest Rinsky (2002), excludes Guénel *et al* 2002, keeps the Italian, Chinese and both Chemical worker cohorts, and includes only the most recent study for AHW (Glass 2005) the ED10 would be 18.7 instead of 14.6. I.e. the dose-response by AGS would need to be divided by a factor of 1.3.

Finally among the leukaemia subtypes, the risk from exposure to benzene is the most established for ANLL including AML. This conclusion was also re-iterated in the summary

report of the most recent international evaluation available, i.e. the IARC evaluation in October 2017 although a full report is not yet available (Loomis *et al* 2017). AGS (2012) uses all leukaemia. AML lifetime cumulative risk is not available in the GLOBOCAN database, but according to US NCI statistics, AML would account for 34% (21 380) of all incident leukaemia (62 130) cases in 2017 (SEER 2017). As a rough estimation, using a background lifetime risk for AML only would result in a dose-response to be divided by a factor of 3. However, this is a simplistic estimate as assuming that risk is increased only for AML, would mean that the risk estimates for all leukaemia that were used to calculate the ED10 estimates would have been "diluted" by the fact that only increases in AML contribute to the increase observed for all leukaemia and one would have to use AML risk estimates. Unfortunately, however, Vlaanderen *et al* (2008) although ranking the AML studies potentially available for quantitative risk assessment, did not calculate a meta-RR for that specific leukaemia type based on the studies that passed the quality assessment.

For uncertainties related to the above issues, it would seem prudent to consider that the dose-response by AGS would represent an upper limit estimate. Nevertheless it is to be noted that the dose-response does not take into account any excess risk from lymphohaematopoietic malignancies other than leukaemia, some of which have shown associations with benzene exposure (see Chapter 7.7.1).

During the Public consulatation of this background document the Lower Olefins and Aromatics REACH Consortium (LOA) provided an alternative dose-response calculation using the lifetable method and based on the leukaemia risk estimates of the Pliofilm cohort published by Richardson 2008 (see Appendix 2). Those estimations are produced separately for leukaemia mortality and incidence and they are based on the assumption that exposures during the time window of 20 most recent years are relevant as no increase in risk (with relatively narrow confidence intervals) was observed in the Pliofilm cohort more than 20 years since exposure; RR=1.00, 95% CI 0.90 – 1.05 (See further Chapter 7.7.1). ECHA considers that incidence calculations are more pertinent for preventing occupational ill-health, which is also in line with the ECHA/RAC-SCOEL task force final report (ECHA 2017c). The excess risk estimates for incidence by LOA (AGS also used incidence) are roughly one order of magnitude lower that the ones by AGS (see Appendix 2).

8.3 Exposure Limit Values

8.3.1 8-hour time weighted average (TWA)

Benzene is a known human carcinogen inducing acute myeloid leukaemia/acute non-lymphocytic leukaemia and is also known to be haematotoxic and genotoxic (clastogenic and aneugenic) in humans.

The metabolism of benzene is inherently complex. The first step in the metabolism of benzene is the oxidation to benzene oxide by cytochrome P-450, mainly CYP2E1, then via several pathways numerous reactive metabolites and also reactive oxygen species (ROS) are formed.

For the non-carcinogenic adverse effects of benzene on the bone marrow and blood system (haematotoxicity and immunotoxicity) and the leading genotoxic effect, i.e. aneugenicity and clastogenicity, thresholds are likely to exist.

Hence, the Dossier Submitter (ECHA) concludes that it is possible to derive a Mode-of-Action-based threshold for benzene.

Multiple studies are available investigating haematological and genotoxic effects in benzene exposed workers. The most significant evidence comes from studies

- investigating larger groups of workers (preferably >100) for which

- appropriate risk management measures have been in place to prevent excessive dermal exposure,
- which used an appropriate control group (industrial workers),
- that considered relevant confounders for the endpoint and method used (e.g., gender, smoking or age),
- which used personal exposure sampling to monitor benzene exposure,
- which excluded workers with previous higher benzene exposure, and
- in which an appropriate regression analysis was performed to control for the effect of confounding factors.

It is to be noted that all available studies have one or more shortcomings. None of the available studies controlled for co-exposure to other substances.

Haematological and immunological effects

The major and most sensitive target organs of benzene are the bone marrow and blood system and benzene has been shown to affect virtually all blood cell types seen as haematological and immunological suppression in workers and experimental animals.

The studies reviewed investigating haematological effects in filling station attendants and studies in traffic personal are not considered in the overall evaluation. More specifically, results from a cohort of Brazilian fuel filling station attendants (Moro *et al* 2015, 2017) are not considered due to assumed additional dermal absorption. The results from one study in traffic personnel (Casale *et al* 2016) were not considered because smoking habits might have influenced the result.

In addition to the criteria cited above for the evaluation of human studies, the strongest evidence comes from those investigating multiple haematological parameters. Considering the individual study shortcomings, the more reliable studies reviewed provide evidence for effects on haematological parameters at benzene concentrations of 1.7 ppm (Pesatori *et al* 2009), above 2 ppm (Lan *et al* 2004), at 2.3 ppm (Qu *et al* 2003a), and at 2.6 ppm (Koh *et al* 2015a, b). Other studies provide some evidence of effects at 1.6 ppm (Zhang *et al* 2016), at 2.0 ppm Ye *et al* (2015), at 7.6 ppm (Rothman *et al* 1996), and at 7.8 ppm (Schnatter *et al* 2010).

In a weight-of-evidence approach and taking into account the studies reviewed showing haematological effects and their reliability, an overall **LOAEC for haematotoxicity in workers in the range of 2 ppm** can be identified as a point of departure to extrapolate to a NOAEC. The following assessment factors are considered appropriate following *ECHA Guidance R. 8, Appendix R.8-15(ECHA 2012)*:

- An assessment factor for intraspecies variability higher than 1 is not required because the number of workers investigated can be considered as sufficiently high to also include sensitive workers with polymorphisms.
- An assessment factor for exposure duration higher than 1 is not required because the studies in workers cover a sufficiently long time span of exposure.
- An assessment factor for dose-response of 3 is applied to extrapolate from the LOAEC to the NOAEC. The minimum value of 3 is used because effects were slight and within the normal range.
- An assessment factor for quality of human data higher than 1 is not required because there are several studies of sufficient quality.

By applying assessment factors in this way, an extrapolated **NOAEC of 0.67 ppm** results.

Based on data from Qu *et al* (2003a) on neutrophils, LOA (2017b) calculated a BMDL of 0.43 ppm for a benchmark response of 5%.

Evidence of lack of relevant haematological effects comes from two health surveillance studies that ingestigated large numbers of workers. No effects were reported among 1200 workers exposed to 0.6 ppm benzene (Tsai *et al* 2004) and in a subgroup among 10,702 workers exposed up to 0.5 ppm benzene (Koh *et al* 2015a).

Considering all reviewed information, a NOAEC for haematological effects of 0.5 ppm is well justifed.

The studies reviewed investigating immunologiocal parameters show effects on the immune system but are not suitable to derive NOAECs or LOAECs for immunological effects of benzene mainly because due to insufficient control groups.

Clastogenic and aneugenic effects

There is evidence that benzene induces micronucleus formation, chromosomal aberrations, aneuploidy, sister chromatid exchange, and DNA strand breaks in humans and experimental animals.

Several studies have been reviewed that investigate DNA damage using the comet assay with inconsistent results. It is to be noted that the comet assay is an indicator test for genotoxicity because the measured effects (DNA damage) might be repaired. Furthermore, this test is not specific for benzene-related effects but several substances occurring at the workplace could contribute the effects observed. In addition, several methodological shortcoming (e.g., type, storage, extraction and workup of samples) might have an impact on the result that could potentially lead to either 'false positive' and 'false negative' findings. Hence, results with the comet test are not used to evaluate genotoxicity of benzene.

Of relevance for the evaluation of benzene-related effects are studies in workers that investigated the clastogenic and aneugenic effects of benzene.

In the concentration range of 1 ppm benzene and above, clastogenic and aneugenic effects were reported in most studies reviewed.

In 130 Chinese shoe factory workers Qu *et al* (2003a) found a significant exposure-response trend for clastogenic and aneugenic effects with 2.3±1.4 ppm benzene as the lowest investigated concentration. Results from two larger groups of Chinese shoe factory workers (n=385 and 317) showing clastogenic effects at 2.0 ppm (range 0.8-18 ppm) and at 1.6 ppm (range 0.8-12 ppm) contain relevant uncertainties due to missing personal exposure assessment (Zhang et al 2014, 2016). In smaller groups of Chinese shoe factory workers aneugenic effects were found in lymphocytes at benzene concentrations of 7.6±2.3 ppm (Ji *et al* 2012; n=33), 5.0±3.6 ppm (Zhang *et al* 2011; n=47) and >2.6±2.7 ppm (Zhang *et al* 2012; n=28). Aneugenic effects in sperms of Chinese shoe factory workers were also observed at 1.0±2.6 ppm benzene and above (Ji *et al* 2012 and Xing *et al* 2010; n=33; Marchetti *et al* 2012; n=30). Clastogenic effects were also found in Italian car painters exposed to 3.1±5.4 ppm benzene (Testa *et al* 2005) and in Hungarian oil refinery workers at 2.2 ppm benzene (range up to 15 ppm; Major *et al* 1994). No clastogenic and aneugenic effects were reported in 38 Estonian workers (Surrallés *et al* 1997); however, exposure was obviously measured only in the location with higher exposure (1.25±1.46 ppm), but not in the location with much lower benzene exposure.

At concentrations in the range of 0.1 ppm to <1 ppm the results are less consistent and less reliable.

Positive results (aneugenic effects in lymphocytes) were obtained in a study investigating 82 Korean coke oven plant workers at 0.56 ppm (range 0.01-0.74 ppm; Kim *et al* 2004). However, it cannot be excluded that co-exposure to polycyclic aromatic hydrocarbons, differences in smoking habits and unknown previous benzene exposure might have contributed to the clastogenic effect.

Kim *et al* (2008) reported clastogenic effects in 108 petroleum refinery workers and Kim *et al* (2010) aneugenic effects in 30 petroleum refinery workers exposed to 0.51 ppm

benzene. However, the positive result may have been due to exposures higher than 0.5 ppm because the measured ange was up to 4.3 ppm (Kim *et al* 2008). Furthermore, exposure assessment was not based on personal measurements but on limited number of air measurements and hence, includes some uncertainties.

Clastogenic effects were also reported in 35 shoe factory workers exposed to 0.75±0.73 ppm benzene (Liu *et al* 1996). However, due to insufficient control for confounders, this result cannot be considered as reliable. Also clastogenic effects reported for 219 workers exposed to <0.17 ppm (Yang *et al* 2012) cannot be considered as reliable due to insufficient exposure assessment and relevant differences in smoking habits. Clastogenic and aneugenic effects reported in 24 Estonian workers at 0.3 to 0.4 ppm benzene (Marcon *et al* 1999) cannot be considered as reliable due to different smoking habits and since exposure ranged up to 8.8 ppm.

Clastogenic effects reported in fuel filling station attendants in India (Pandey *et al* 2008; Rekhadevi *et al* 2010, 2011), Brazil (Moro *et al* 2013, 2017) and China (Xiong *et al* 2016) are not considered in the evaluation because insufficient working conditions have been reported or can be assumed.

However, several studies are available with appropriate exposure assessment and control for relevant confounders but with a limited number of investigated benzene-exposed workers.

Carere *et al* (1995) reported at 0.46±0.14 ppm benzene a borderline positive increase of chromosomal aberrations in 23 male fuel filling station attendants but no increase in the micronucleus frequency. Lovreglio *et al* (2014) found in 19 fuel tank drivers exposed to 0.1±0.1 ppm benzene no increase in the frequency of chromsomal aberration but an increase in the mean frequency of micronuclei. However, since there was no difference for the median micronucleus frequency or the range, the positive results seems to be of questionable relevance.

Negative results have been obtained for 50 male Spanish fuel filling station attendants exposed to 0.28±0.04 ppm benzene (Pitarque *et al* 1996) and for 12 Italian fuel filling station attendants exposed to 0.1±0.1 ppm benzene (Carere *et al* 1998).

At concentrations below 0.1 ppm the results from more reliable studies are negative.

No clastogenic effects were reported for 21 Italian fuel filling station attendants exposed to 0.072 ppm benzene (Bukvic *et al* 1998) and for 19 Italian fuel filling station attendants exposed to 0.012 ppm benzene (Fracasso et al 2010). In a more robust study investigating 79 male Italian petroleum refinery workers exposed to 0.03 ppm benzene (Basso *et al* 2011) also no micronucleus formation was found. In 132 decorators and 129 painters using face maks for which benzene exposure near breathing zone was measured with 0.06 and 0.009 ppm, no clastogenic effects were reported (Sha *et al* 2014).

Results for traffic personnel (Angelini *et al* 2011; Leopardi *et al* 2003; Maffei *et al* 2005; Violante *et al* 2003) cannot be considered as relevant to identify a dose-response for benzene because of the significant contribution of the complex mixtures of traffic/engige exhausts to the total exposure. For example, exposure to polycyclic aromatic hydrocarbons (PAH) was demonstrated to lead to increased micronucleus frequencies (Sram *et al* 2016).

Taking into account all data reviewed, and considering that the positive results obtained in the concentration range below 1 ppm are less reliable, an overall weight of evidence **LOAEC in the range of 1.0 ppm** can be derived for clastogenic and aneugenic effects in peripheral blood lymphocytes and sperms.

To extrapolate the LOAEC of 1 ppm derived from workers to a NOAEC the following assessment factors are considered following *ECHA guidance R. 8, Appendix R.8-15 (ECHA 2012)*:

- An assessment factor for intraspecies variability of 2 may be considered due to the relative small number of workers investigated in the low concentration range which limits the statistical power of such studies.
- An assessment factor for exposure duration higher than 1 is not required because the studies in workers cover a sufficiently long time span of exposure.
- An assessment factor for dose-response and severity of 10 is proposed considering the extrapolation from LOAEC to NOAEC, the severity of the type of effect (clastogenicity and aneugenicity) and since the bone marrow might be a more sensitive target than peripheral blood lymphocytes.
- An assessment factor for quality of human data higher than 1 is not required because there are several studies of sufficient quality.

By applying assessment factors in such a way, an extrapolated NOAEC of 0.05 ppm for chromosomal damage in bone marrow results.

In addition, a **LOAEC of 1.0 ppm is supported by animal data** (Erexson *et al* 1986, French *et al* 2015). French *et al* (2015) identified a LOAEC of 1 ppm for bone marrow derived reticulocytes in male DO mice which would translate to a human $LOAEC_{(worker)}$ of 0.5 ppm (1*6/8*6.7/10). By applying the usual dose-response extrapolation, a NOAEC for bone marrow damage in these animals would be in the range of 0.1 ppm. The above authors modelled a $BMDC_{10}$ of 0.2 ppm, which would also translate to a $BMDC_{(worker)10}$ of 0.1 ppm. Then, considering interspecies variability in toxicokinetics and toxicodynamics, an animal-derived **extrapolated NOAEC** starting from effects in rodent bone marrow cells would again be **well below 0.1 ppm**.

Furthermore, several studies in workers are available that could be used to give a NOAEC of around 0.1 ppm. Considering the insufficient statistical power of such studies to detect small benzene-related effect, and hence the uncertainty that small benzene-related effect could have been missed, it seems to be appropriate to conclude on a **NOAEC of 0.05 ppm based on studies in workers in the low concentration range**.

To conclude, based on the available scientific data on adverse effects of benzene in workers with an extrapolated NOAEC of 0.5 ppm for haematological effects and an extrapolated NOAEC of 0.05 ppm for clastogenic and aneugenic effects, **the Dossier Submitter (ECHA) proposes an 8-hour Time Weighted Average of 0.05 ppm.**

Benzene exposure

At workplaces in Europe, the long-term average exposures to benzene are mainly below 0.1 ppm (0.3 mg/m^3) and even below 0.05 ppm (0.16 mg/m^3). However, higher exposures have been reported for several diverse groups.

Benzene exposures in the range between 0.05 and 0.1 ppm have been reported for example

- for landscaping work (GM 0.003 ppm, maximum 0.06 ppm; Breuer *et al* 2015);
- for service station workers in Italy, (AM 0.02 ppm, maximum 0.09 ppm; Campo *et al* 2016);
- for petrochemical industry (average <0.05 ppm, maximum 0.28 ppm; Carrieri *et al* 2012; Breuer *et al* 2013);
- in research and development laboratories (AM 0.05 ppm, maximum 0.2 ppm; Concawe 2009);
- for service station workers in Spain (AM 0.05 ppm, GM 0.04 ppm, maximum 0.17 ppm; Periago and Prado 2005);
- for fuel tank drivers (AM 0.09 ppm, maximum 0.3 ppm; Lovreglio *et al* 2014, 2016);
- in repair workshops (GM 0.10 ppm, maximum 0.4 ppm; Breuer *et al* 2013).

Benzene exposures above 0.1 ppm have been reported for example

- in gasoline pump calibration (AM 0.13 ppm, maximum 0.28 ppm; Concawe 2009);
- in gasoline pump repair and maintenance (AM 0.25 ppm, maximum 0.89 ppm; Concawe 2009);

- during maintenance work and shut down operations in refineries (mean 0.06-0.3 ppm, maximum 1.4 ppm; Akerstrom *et al* 2016);
- in a repair shop for gasoline-powered gardening tools (AM 0.4 ppm; Breuer *et al* 2013)
- in a simulation experiment with short term use (30 min) of a lacquer spiked with 0.05% benzene (0.91 ppm; HVBG 2001);
- during tank cleaning work on upstream petroleum industry (AM 1.4 ppm, GM 0.3 ppm, maximum 16.8 ppm; Kirkeleit *et al* 2006).

8.3.2 Short-term Exposure Limits (STEL)

In 1991, SCOEL considered that no STEL was necessary.

Benzene is leading to effects in the central nervous system at high concentrations of 975-9,750 mg/m^3 (300-3000 ppm). Considering an OEL of 0.1 ppm to prevent from benzene-related haematological effects, it is not expected that a concentration of 300 ppm will be reached under normal workplace conditions.

Hence, no STEL is recommended by the Dossier Submitter ECHA.

8.3.3 Biological limit values (BLV)

Considering the correlation as published by DFG (2017a, b) and as summarised in Table 8 and Table 9, an OEL of 0.05 ppm corresponds to biological limit values (BLV) of about:

- 0.7 µg benzene /L urine and
- 2 µg S-phenylmercapturic acid (SPMA)/g creatinine

Sampling time is at the end of exposure or the end of the working shift.

The Dossier Submitter ECHA is proposing those values as BLVs.

8.3.4 Biological Guidance Values (BGV)

DFG (2017a, b) published 95 percentils for benzene metabolites for the general non-smoking population. Based on those, **the following Biological Guidance Values(BGV) are recommended by the Dossier Submitter ECHA:**

- 0.3 µg benzene/L urine
- 0.5 µg S-phenylmercapturic acid (SPMA)/g creatinine
- 150 µg *trans,trans*-muconic acid (ttMA)/g creatinine

8.4 Notations

SCOEL (1991) recommended a skin notation because absorption of liquid benzene through the skin may contribute substantially to the amount absorbed at exposure levels below 1.0 ppm (3.25 mg/m^3).

Annex III of Directive 2004/37/EC (EU Parliament and Council Directive 2004) lists a 'skin notation' for benzene indicating that there is a substantial contribution to the total body burden possible via dermal exposure.

DECOS (2014) confirmed the need for a 'skin' notation.

Williams *et al* (2011) analysed the experimental skin absorption data of benzene (both human and animal; *in vitro* and *in vivo*), and concluded that the steady state absorption rate of benzene ranges from 200-400 µg/cm^2*h (DECOS 2014). Considering an OEL of 0.05 ppm (0.16 mg/m^3) this value exceeds by far the critical absorption value (CAV) calculated according to the ECETOC methodology (1998) of 0.08 µg/cm^2*h (with (10 [m^3] x OEL [mg/m^3] x f x 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).

Kalnas and Teitelbaum (2000) found that for solvents used for cleaning that contained benzene at concentrations of less than 0.1%, the amount of benzene absorbed through the skin over a long period was found to be significant, depending on exposure time and exposed skin surface areas.

Hence, the Dossier Submitter ECHA proposes to maintain the 'skin' notation.

9. 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, co-exposures smoking and dietary habits (DECOS 2014).

In addition, polymorphisms in the genes encoding for enzymes involved in the metabolism of benzene can lead to higher risk for benzene toxicity. Current studies indicate that workers with GSTM1 and GSTT1 null genotypes have a higher risk for benzene-induced toxicity. Also other polymorphisms may have an impact on benzene toxicity like cytochrome P450 2E1 (CYP2E1), epoxid hydrolase (EH), NAD(P)H:quinone oxidoreductase (NQO1), Myeloperoxidase (MPO), and polymorphisms in DNA repair genes (see 7.1.1).

Ethanol can increase the severity of benzene-induced anaemia, lymphocytopenia, and reduction in bone marrow cellularity, and produce transient increases in normoblasts in the peripheral blood and atypical cellular morphology. The enhancement of the hepatotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol. Accordingly, increased central nervous system disturbances (e.g., depression) may be expected following concurrent exposure to benzene and ethanol (ATSDR 2007).

REFERENCES

AFFSET [French Agency for Environmental and Occupational Health Safety] (2010) Valeurs toxicologiques de référence (VTR). Méthode de construction de valeurs toxicologiques de référence (VTR) pour les substances chimiques cancérogènes. Edition scientifique-Air et agents chimiques. Mars 2010 (In French). https://www.anses.fr/documents/CHIM2004etAS16Ra.pdf

AGS [Ausschuss für Gefahrstoffe] (2012) Begründung zu Benzol in BekGS 910, Ausgabe: November 2012, Stand: Mai 2012. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/910/910-benzol.pdf?__blob=publicationFile&v=2

AGS [Committee on Hazardous Substances] (2016) Technical Rules for Hazardous Substances Risk-related concept of measures for activities involving carcinogenic hazardous substances TRGS 910. 22.11.2016. https://www.baua.de/EN/Service/Legislative-texts-and-technical-rules/Rules/TRGS/pdf/TRGS-910.pdf?__blob=publicationFile&v=2

Akerstrom M, Almerud P, Andersson EM, Stranberg B and Sallsten G (2016) Personal exposure to benzene and 1,3-butadiene during petroleum refinery turnarounds and work in the oil harbour. Int Arch Occup Environ Health 89: 1289-1297.

Al-Salmani K, Abbas HH, Schulpen S, Karbaschi M, Abdalla I, Bowman KJ, So KK, Evans MD, Jones GD, Godschalk RW, Cooke MS (2011) Simplified method for the collection, storage, and comet assay analysis of DNA damage in whole blood. Free Radic Biol Med 51(3): 719-725.

Almerud P, Akerstrom M, Andersson EM, Stranberg B and Sallsten G (2017) Low personal exposure to benzene and 1,3-butadiene in the Swedish petroleum refinery industry. Int Arch Occup Environ Health: DOI 10.1007/s00420-017-1234-y.

Amin RP, Witz G (2001) DNA-protein crosslink and DNA strand break formation in HL-60 cells treated with trans,trans-muconaldehyde, hydroquinone and their mixtures. Int J Toxicol 20: 69-80.

Andreoli R, Manini P, Bergamaschi E, Brustolin A, Mutti A (1999) Solid-phase microextraction and gas chromatography–mass spectrometry for the determination of monoaromatic hydrocarbons in blood and urine: application to people exposed to air pollutants. Chromatographia 50: 167–172.

Angelini S, Kumar R, Bermejo JL, Maffei F, Barbieri A, Graziosi F, Carbone F, Cantelli-Forti G, Violante FS, Hemminki K, Hrelia P (2011) Exposure to low environmental levels of benzene: evaluation of micronucleus frequencies and S-phenylmercapturic acid excretion in relation to polymorphisms in genes encoding metabolic enzymes. Mutat Res 719: 7-13.

Angelini S, Maffei F, Bermejo JL, Ravegnini G, L'Insalata D, Cantelli-Forti G, Violante FS, Hrelia P (2012) Environmental exposure to benzene, micronucleus formation and polymorphisms in DNA-repair genes: A pilot study. Mutation Research-Genetic Toxicology and Environmental Mutagenesis 743: 99-104.

Angelini S, Bermejo JL, Ravegnini G, Sammarini G, Hrelia P (2016) Application of the lymphocyte Cytokinesis-Block Micronucleus Assay to populations exposed to petroleum and its derivatives: Results from a systematic review and meta-analysis. Mutat Res 770(Pt A): 58-72.

ANSES (2014) Opinion of the French Agency for Food, Environmental and Occupational Health & Safety regarding the establishment of a carcinogenic TRV by inhalation for benzene CAS No 71-43-2.https://www.anses.fr/en/system/files/SUBCHIM2009sa0346EN.pdf. Accessed 18 May 2017.

Aprea C, Sciarra G, Bozzi N, Pagliantini M, Perico A, Bavazzano P, Leandri A, Carrieri M, Scapellato ML, Bettinelli M, Bartolucci GB (2008) Reference values of urinary trans,trans-muconic acid: Italian multicentric study. Arch Environ Contam 55: 329-340.

Arnold SM, Angerer J, Boogaard PJ, Hughes MF, O'Lone RB, Robison SH, Schnatter AR (2013) The use of biomonitoring data in exposure and human health risk assessment: benzene case study. Crit Rev Toxicol 43: 119-153.

ATSDR (2007) Toxicological profile for benzene. Toxicological profile. ATDSR, https://www.atsdr.cdc.gov/toxprofiles/tp3.pdf, accessed 05.07.2017

ATSDR (2015) Addendum to theToxicological profile for benzene. Toxicological profile. ATDSR, https://www.atsdr.cdc.gov/toxprofiles/Benzene_Addendum.pdf, accessed 05.07.2017

Basso E, Cevoli C, Papacchini M, Tranfo G, Mansi A, Testa A (2011) Cytogenetic biomonitoring on a group of petroleum refinery workers. Environ Mol Mutagen 52: 440-447.

Bausinger J, Speit G (2016) The impact of lymphocyte isolation on induced DNA damage in human blood samples measured by the comet assay. Mutagenesis 31(5): 567-572.

Bernauer U, Vieth B, Ellrich R, Heinrich-Hirsch B, Jänig GR, Gundert-Remy U (1999) CYP2E1-dependent benzene toxicity: the role of extrahepatic benzene metabolism. Arch Toxicol 73: 189-196.

Bernauer U, Vieth B, Ellrich R, Heinrich-Hirsch B, Jänig GR, Gundert-Remy U (2000) CYP2E1 expression in bone marrow and its intra- and interspecies variability: approaches for a more reliable extrapolation from one species to another in the risk assessment of chemicals. Arch Toxicol 73: 618-624.

Biotox database. (INSR). http://www.inrs.fr/publications/bdd/biotox.html.

Bird MG, Wetmore BA, Letinski DJ, Nicolich M, Chen M, Schnatter AR, Whitman FT (2010) Influence of toluene co-exposure on the metabolism and genotoxicity of benzene in mice using continuous and intermittent exposures. Chem Biol Interact 184: 233-239.

Bolte G, Heitmann D, Kiranoglu M, Schierl R, Diemer J, Koerner W, Fromme H (2008) Exposure to environmental tobacco smoke in German restaurants, pubs and discotheques. J Expo Sci Environ Epidemiol 18(3): 262-271.

Bloemen LJ, Youk A, Bradley TD, Bodner KM, Marsh G (2004) Lymphohaematopoietic cancer risk among chemical workers exposed to benzene. Occup Environ Med 61: 270-274.

BMAS [Bundesministerium für Arbeit und Soziales] (2017) Bundesantalt für Arbeitsschutz und Arbeitsmedizin, Bekanntmachung von Technischen Regeln hier TRGS 910 „Risikobezogenes Maßnahmenkonzept für Tätigkeiten mit krebserzeugenden Gefahrstoffen“, 08.06.2017. https://www.baua.de/DE/Angebote/Rechtstexte-und-

Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-910-Aenderungen.pdf?__blob=publicationFile&v=3

Bonassi S, Fenech M, Lando C, Lin YP, Ceppi M, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Jia C, Di Giorgio M, Ferguson LR, Fucic A, Lima OG, Hrelia P, Krishnaja AP, Lee TK, Migliore L, Mikhalevich L, Mirkova E, Mosesso P, Müller WU, Odagiri Y, Scarffi MR, Szabova E, Vorobtsova I, Vral A, Zijno A (2001) HUman MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen 37: 31-45.

Bond GG, McLaren EA, Baldwin CL, Cook RR (1986) An update of mortality among chemical workers exposed to benzene. Br J Ind Med 43: 685-691.

Boogaard PJ (2010) The low-dose benzene debate needs a sharp blade. Chem Biol Interact pii: S0009-2797(17)30510-0.

Boogaard PJ, van Sittert NJ (1995) Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans,trans-muconic acid, and phenol. Occup Environ Med 52(9): 611-620.

Boogaard PJ, van Sittert NJ (1996) Suitability of S-phenyl mercapturic acid and trans-trans-muconic acid as biomarkers for exposure to low concentrations of benzene. Environ Health Perspect 104 Suppl 6: 1151-1157.

Bråtveit M, Kirkeleit J, Hollund BE, Moen BE (2007) Biological monitoring of benzene exposure for process operators during ordinary activity in the upstream petroleum industry. Ann Occup Hyg 51(5): 487-494.

Breuer D, Ngazi R, Van Gelder R, Gabriel S, Pflaumbaum W, Meyer G, Schmidt I, Fröhlich H-P, Fendler D, Tigler A, Lewin-Keretzschmar U, Hohenberger L, und Riethmuller A (2015) Benzol – Messungen in verschiedenen Arbeitsbereichen mit Bezug zur Toleranz- und Akzeptanzkonzentration nach TRGS 910. Gefahrstoffe – Reinhaltung der Luft 75 (7/8): 259-263.

Breuer D, Ngazi R, Herrmann S, Schneider W, Moritz A und Lewin-Keretzschmar U (2013) Benzol –Erarbeitung und Praxiserprobung eines Messverfahrens mit Bezug zu dem neuen Risikowert und der Exposition-Risiko-Beziehung fur Tätigkeiten mit krebserzeugenden Gefahrstoffen. Gefahrstoffe – Reinhaltung der Luft 73 (10): 415-421.

Brugnone F, Perbellini L, Maranelli G, Romeo L, Guglielmi G, Lombardini F (1992) Reference values for blood benzene in the occupationally unexposed general population. Int Arch Occup Environ Health 64: 179-184.

Brugnone F, Perbellini L, Romeo L, Cerpelloni M, Bianchin M, Tonello A (1999) Benzene in blood as a biomarker of low level occupational exposure. Sci Total Environ 235: 247-252.

Bruinen de Bruin Y, Koistinen K, Kephalopoulos S, Geiss O, Tirendi S and Kotzias D (2008) Characterisation of urban inhalation exposures to benzene, formaldehyde and acetaldehyde in the European Union. Environmental Science and Pollution Research 15(5): 417-30.

Bukvic N, Bavaro P, Elia G, Cassano F, Fanelli M, Guanti G (1998) Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of gasoline station attendants. Mutat Res 415: 25-33.

Burns A, Shin J *et al* (2017) Combined analysis of job and task benzene air exposures among workers at four US refinery operations. Toxicol Ind Health 33: 193-210.

Campagna M, Satta G, Campo L, Flore V, Ibba A, Meloni M, Tocco MG, Avataneo G,Flore C, Fustinoni S, Cocco P (2012) Biological monitoring of low-level exposure to benzene. Med Lav 103: 338-346.

Campagna M, Satta G, Campo L, Flore V, Ibba A, Meloni M, Tocco MG, Avataneo G, Flore C, Fustinoni S, Cocco P (2014) Analysis of potential influence factors on background urinary benzene concentration among a non-smoking, non-occupationally exposed general population sample. Int Arch Occup Environ Health 87: 793-799.

Campo L, Mercadante R, Rossella F, Fustinoni S (2009) Quantification of 13 priority polycyclic aromatic hydrocarbons in human urine by headspace solid-phase microextraction gas chromatography-isotope dilution mass spectrometry. Anal Chim Acta 631: 196–205.

Campo L, Rossella F, Mercadante R, Fustinoni S (2016) Exposure to BTEX and Ethers in Petrol Station Attendants and Proposal of Biological Exposure Equivalents for Urinary Benzene and MTBE. Ann Occup Hyg 60: 318-333.

Capleton AC, Levy LS (2005) An overview of occupational benzene exposures and occupational exposure limits in Europe and North America. Chem Biol Interact 153-154: 43-53.

Carbonari D, Chiarella P, Mansi A, Pigini D, Iavicoli S, Tranfo G (2016) Biomarkers of susceptibility following benzene exposure: influence of genetic polymorphisms on benzene metabolism and health effects. Biomark Med 10(2):145-163.

Carere A, Antoccia A, Crebelli R, Degrassi F, Fiore M, Iavarone I, Isacchi G, Lagorio S, Leopardi P, Marcon F, et al (1995) Genetic effects of petroleum fuels: cytogenetic monitoring of gasoline station attendants. Mutat Res 332: 17-26.

Carere A, Antoccia A, Cimini D, Crebelli R, Degrassi F, Leopardi P, Marcon F, Sgura A, Tanzarella C, Zijno A (1998) Genetic effects of petroleum fuels: II. Analysis of chromosome loss and hyperploidy in peripheral lymphocytes of gasoline station attendants. Environ Mol Mutagen 32: 130-138.

Carrieri M, Bartolucci GB, Scapellato ML, Spatari G, Sapienza D, Soleo L,Lovreglio P, Tranfo G, Manno M, Trevisan A (2012) Influence of glutathione S-transferases polymorphisms on biological monitoring of exposure to low doses of benzene. Toxicol Lett 213: 63-68.

Carrieri M, Tranfo G, Pigini D, Paci E, Salamon F, Scapellato ML, Fracasso ME,Manno M, Bartolucci GB (2010) Correlation between environmental and biological monitoring of exposure to benzene in petrochemical industry operators. Toxicol Lett 192: 17-21.

Carrillo-Infante C, Abbadessa G, Bagella L, Giordano A (2007) Viral infections as a cause of cancer (Review). Int J Oncol 30: 1521-1528.

Casale T, Sacco C, Ricci S, Loreti B, Pacchiarotti A, Cupelli V, Arcangeli G, Mucci N, Antuono V, De Marco F, Tomei G, Tomei F, Rosati MV (2016) Workers exposed to low levels of benzene present in urban air: Assessment of peripheral blood count variations. Chemosphere 152: 392-398.

Chaney AM, Carlson GP (1995) Comparison of rat hepatic and pulmonary microsomal metabolism of benzene and the lack of benzene-induced pneumotoxicity and hepatotoxicity. Toxicology 104: 53-62.

Chappell G, Pogribny IP, Guyton KZ, Rusyn I (2016) Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: A systematic literature review. Mutat Res Rev Mutat Res 768: 27-45.

Chen CS, Hseu YC, Liang SH, Kuo JY, Chen SC (2008) Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay. J Hazard Mater 153(1-2): 351-356.

Ciarrocca M, Tomei F, Caciari T, Capozzella A, Scimitto L, Nardone N, Andreozzi G, Scala B, Fiaschetti M, Cetica C, Di Giorgio V, Schifano MP, Tomei G, Sancini A (2012a) Environmental and biological monitoring of benzene in traffic policemen, police drivers and rural outdoor male workers. J Environ Monit 14: 1542-1550.

Ciarrocca M, Tomei G, Fiaschetti M, Caciari T, Cetica C, Andreozzi G, Capozella A, Schifano MP, Andre J-C, Tomei F, Sancini A (2012b) Assessment of occupational exposure to benzene, toluene and xylenes in urban and rural female workers. Chemosphere 87: 813-819.

Collins JJ, Conner P, Friedlander BR, Easterday PA, Nair RS, Braun J (1991) A study of the hematologic effects of chronic low-level exposure to benzene. J Occup Med 33: 619-626.

Collins JJ, Ireland BK, Easterday PA, Nair RS, Braun J (1997) Evaluation of lymphopenia among workers with low-level benzene exposure and the utility of routine data collection. J Occup Environ Med 39: 232-237.

Collins JJ, Anteau SE, Swaen GMH, Bodner KM, Bodnar CM (2015) Lymphatic and hematopoietic cancers among benzene-exposed workers. J Occup Environ Med 57: 159-163.

Collins JJ, Ireland B, Buckley CF, Shepperly D (2003) Lymphohaematopoietic cancer mortality among workers with benzene exposure. Occup Environ Med 60: 676-679.

Concawe (1999) Environmental exposure to benzene. Prepared by: M Claydon, M Evans, J-P Gennart, C Roythorne, B Simpson, J Urbanus (Technical Co-ordinator), Brussels October 1999. https://www.concawe.eu/wp-content/uploads/2017/01/2002-00206-01-e.pdf, accessed 05.07.2017

Concawe (2002) A survey of europeans gasoline exposures for the period 1999-2001. Concawe Report no 9/02. https://www.concawe.eu/wp-content/uploads/2017/01/rpt_02-9-2003-01128-01-e.pdf, accessed 05.07.2017

Concawe (2006) French service station study of ambient benzene levels (2005) https://www.concawe.eu/wp-content/uploads/2017/01/cr152ambientbenzeneatservicestations-2006-02007-01-e.pdf, accessed 05.07.2017

Concawe (2009) Report no 5/09. Additional human exposure information for gasoline substance risk assessment (period 2002-2007).

Concawe (2012) A new 'pooled' analysis of benzene effects on human health, https://www.concawe.eu/wp-content/uploads/2017/01/cr212-bz_pooled_analysis-2013-00214-01-e.pdf, accessed 05.07.2017

Concawe (2013) Comments on the DECOS Draft: "Benzene Health-based recommended occupational exposure limit", dated 26 April 2013. https://www.gezondheidsraad.nl/sites/default/files/commentaar_concawe-cefic_benzeen_0_1.pdf

Concawe (2014) Report no 14/14. Dermal exposures associated with service station refuelling activities: preliminary evaluation. Copley GB, Schnatter AR, Armstrong TW, Irons RD, Chen M, Wang XQ, Kerzic P (2017) Hospital-based case-control study of MDS suptypes and benzene exposure in Shanghai. J Ocuup Environ Med 59: 349-355.

Constantini A, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazotti V, Rodella A, Stagnaro E, Crosignani P, Amadori D, Mirabelli D, Sommani L, Belletti I, Troschel L, Romea L, Miceli G, Tozzi G, Mendico I, Maltoni S, Miligi L (2008) Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian multicentre case-control study. Am J Ind Med 51:803-811.

Copley GB, Schnatter AR, Armstrong TW, Irons RD, Chen M, Wang XQ, Kerzic P (2017) Hospital-Based Case-Control Study of MDS Subtypes and Benzene Exposure in Shanghai. J Occup Environ Med 59: 349–355.

Cox LA, Schnatter AR, Boogaard PJ, Banton M, Ketelslegers HB (2017) Non-parametric estimation of low-concentration benzene metabolism. Chem Biol Interact pii: S0009-2797(17)30495-7.

Crebelli R, Tomei F, Zijno A, Ghittori S, Imbriani M, Gamberale D, Martini A, Carere A (2001) Exposure to benzene in urban workers: environmental and biological monitoring of traffic police in Rome. Occup Environ Med 58: 165-171.

Crump KS (1996) Risk of benzene-induced leukaemia predicted from the Pliofilm cohort. Environ Health Perspect 104 (Suppl 6): 1437-1441.

Crump K, Allen B (1984). Quantitative estimates of risk of leukaemia from occupational exposure to benzene. Unpublished report prepared fro the Occupational Safety and Health Administration, 1984.

DECOS [Dutch Expert Committee an Occupational Safety of the Health Council of the Netherlands] (2014) Benzene, Health-based recommended occupational exposure limit, No. 2014/03, The Hague: the Health Council of the Netherlands, February 21, 2014. https://www.gezondheidsraad.nl/en/task-and-procedure/areas-of-activity/healthy-working-conditions/benzene-health-based-recommended

Deng Q, Huang S, Zhang X, Zhang W, Feng J, Wang T, Hu D, Guan L, Li J, Dai X, Deng H, Zhang X, Wu T (2014) Plasma microRNA expression and micronuclei frequency in workers exposed to polycyclic aromatic hydrocarbons. Environ Health Perspect 122: 719-72DEFRA[Department for Environment Food & Rural Affairs](2015). Air Pollution in the UK 2015. https://uk-air.defra.gov.uk/library/annualreport/index

DFG [Deutsche Forschungsgemeinschaft] (1993) Biomonitoring Methods for Benzene and alkylbenzenes (BTX aromatics). The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.bi7143e0004/pdf, accessed 05.07.2017

DFG [Deutsche Forschungsgemeinschaft] (1995a) Air Monitoring Methods for Benzene. Method No 1. The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.am7143e0005/pdf, accessed 05.07.2017

DFG [Deutsche Forschungsgemeinschaft] (1995b) Biomonitoring Methods for t,t-Muconic acid. The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.bi358817e0005/pdf, accessed 05.07.2017

DFG [Deutsche Forschungsgemeinschaft] (1995c) Biomonitoring Methods for S-Phenylmercapturic acid. The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.bi477580e0005/pdf, accessed 05.07.2017

DFG [Deutsche Forschungsgemeinschaft] (2001) MAK Value documentation for Ethylbenzene. The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb10041e3316/pdf, accsessed 04.08.32017

DFG [Deutsche Forschungsgemeinschaft] (2014) Air Monitoring Methods for Benzene, Sovent Mixtures. The MAK Collection for Occupational Health and Safety, http://onlinelibrary.wiley.com/doi/10.1002/3527600418.am0lmix001e1816/pdf, accessed 05.07.2017

DFG [Deutsche Forschungsgemeinschaft] (2017a) List of MAK and BAT Values, Assessment values in biological material, XII List of substances (in German). http://onlinelibrary.wiley.com/doi/10.1002/9783527812110.ch12/pdf

DFG [Deutsche Forschungsgemeinschaft] (2017b) List of MAK and BAT Values, Assessment values in biological material, XIII Carcinogenic substances (in German). http://onlinelibrary.wiley.com/doi/10.1002/9783527812110.ch13/pdf

DGUV [Deutsche Gesetzliche Unfallversicherung] (2007) Occupational Disease Report 2/2007e. Occupational Disease No. 1317.

Dougherty D, Garte S, Barchowsky A, Zmuda J, Taioli E (2008) NQO1, MPO, CYP2E1,GSTT1 and GSTM1 polymorphisms and biological effects of benzene exposure—a literature review. Toxicol Lett 182: 7-17.

Eastmond DA, Schuler M, Frantz C, Chen H, Parks R, Wang L, Hasegawa L (2001) Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans. Res Rep Health Eff Inst 103: 1-68; discussion 69-80.

ECETOC [European Centre for Ecotoxicology and Toxicology of Chemicals] (1998). Strategy for Assigning a "Skin Notation". Brussels, Belgium: ECETOC Copyright.

ECHA [European Chemicals Agency] (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. Version 2.1, November 2012. https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

ECHA [European Chemicals Agency] (2017) Registered substances: benzene. https://echa.europa.eu/information-on-chemicals/registered-substances/-/disreg/substance/100.000.685; Accessed 23.07.2017

ECHA [European Chemicals Agency] (2017b) Substance description: benzene. https://echa.europa.eu/brief-profile/-/briefprofile/100.000.685.

ECHA [European Chemicals Agency] (2017c). Joint Task Force ECHA Committee for Risk Assessment (RAC) and Scientific Committee on Occupational Exposure Limits (SCOEL) on Scientific aspects and methodologies related to the exposure of chemicals at the workplace. Task 2. Final Report 6 December 2017. https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf/db8a9a3a-4aa7-601b-bb53-81a5eef93145

Edokpolo B, Yu JQ, Connell D (2014) Health Risk Assessment of Ambient Air Concentrations of Benzene, Toluene and Xylene (BTX) in Service Station Environments. Int J Environ Res Public Health 11: 6354-6374.

EPA [US Environmental Protection Agency] (1998) Carcinogenic effects of benzene: An Update (draft report). https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=2806

EPA [US Environmental Protection Agency] (2003) Integrated Risk Information System (IRIS), Chemical Assessment Summary Xylenes; CAS No. 1330-20-7, https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0270_summary.pdf

Erexson GL, Wilmer JL, Steinhagen WH, Kligerman AD (1986) Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Environ Mutagen 8: 29-40.

EU Commission (2008) COMMISSION REGULATION (EC) No 1271/2008 of 16 December 2008 amending Regulation (EC) No 1255/2008 fixing the import duties in the cereals sector applicable from 16 December 2008. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R1271&from=EN

EU Council Recommendation (2009) Council recommendation of 30 November 2009 on smoke-free environments. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009H1205(01)&from=EN

EU OSHA [European Agency for Safety and Health at Work] (2009) European Risk Observatory report. https://osha.europa.eu/en/tools-and-publications/publications/reports/548OELs, accessed 04.07.2017

EU Parliament and Council Directive (1998) DIRECTIVE 98/70/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 13 October 1998 relating to the quality of petrol and diesel fuels and amending Council Directive 93/12/EEC. http://eur-lex.europa.eu/resource.html?uri=cellar:9cdbfc9b-d814-4e9e-b05d-49dbb7c97ba1.0008.02/DOC_1&format=PDF

EU Parliament and Council Directive (2004) Corrigendum to 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 (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC) (codified version); http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:229:0023:0034:EN:PDFEU RAR [Risk Assessment Report] (2003) Toluene. https://echa.europa.eu/documents/10162/24a34bd6-55cd-4e28-ae24-5bae281bf3c2, accessed 04.08.2017

EU Parliament and Council Directive (2008) DIRECTIVE 2008/50/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 May 2008 on ambient air quality and cleaner air for Europe. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0050&from=en

EU Parliament and Council Directive (2010) DIRECTIVE 2010/75/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 24 November 2010. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0075&from=EN.

EU RAR [Risk Assessment Report] (2008) Benzene. https://echa.europa.eu/documents/10162/be2a96a7-40f6-40d7-81e5-b8c3f948efc2, accessed 10.07.2017

Farmer PB, Kaur B, Roach J, Levy L, Consonni D, Bertazzi PA, Pesatori A, Fustinoni S, Buratti M, Bonzini M, Colombi A, Popov T, Cavallo D, Desideri A, Valerio F, Pala M, Bolognesi C, Merlo F (2005) The use of S-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. Chem Biol Interact 153-154: 97-102.

Fenga C, Gangemi S, Costa C (2016) Benzene exposure is associated with epigenetic changes (Review). Mol Med Rep 13: 3401-3405.

Finland Ministry of Social Affairs and Health (2016) HTP-arvot 2016 (in Finnish). http://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/79109/08_2016_HTP-arvot_suomi_22122016_netti_kansilla.pdf?sequence=1

FIOH (Finnish Institute of Occupational Health/ Työterveyslaitos) (2010) Työympäristön kemikaalien altistumismittaukset 2004-2007. Työympäristötutkimuksen raporttisarja 47 (in finnish).

Fracasso ME, Doria D, Bartolucci GB, Carrieri M, Lovreglio P, Ballini A, Soleo L, Tranfo G, Manno M (2010) Low air levels of benzene: correlation between biomarkers of exposure and genotoxic effects. Toxicol Lett 192: 22-28.

Fustinoni S, Gianpiccolo L, Pulvirenti S, Buratti M, Colombi A (1999) Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. J Chromat B Biomed Sci Appl 723: 105–115.

Fustinoni S, Buratti M, Campo L, Colombi A, Consonni D, Pesatori AC, Bonzini M, Farmer P, Garte S, Valerio F, Merlo DF, Bertazzi PA (2005) Urinary t,t-muconic acid, S-phenylmercapturic acid and benzene as biomarkers of low benzene exposure. Chem Biol Interact 153-154: 253-256.

Fustinoni S, Rossella F, Campo L, Mercadante R, Bertazzi PA (2010a) Urinary BTEX, MTBE and naphthalene as biomarkers to gain environmental exposure profiles of the general population. Sci Total Environ 408: 2840-2849.

Fustinoni S, Campo L, Mercadante R, Manini P (2010b) Methodological issues in the biological monitoring of urinary benzene and S-phenylmercapturic acid at low exposure levels. J Chromatogr B Analyt Technol Biomed Life Sci 878: 2534-2540.

Fustinoni S, Campo L, Mercadante R, Consonni D, Mielzynska D, Bertazzi PA (2011) A quantitative approach to evaluate urinary benzene and S-phenylmercapturic acid as biomarkers of low benzene exposure. Biomarkers 16: 334-345.

Gad SC, Dunn BJ, Dobbs DW, Reilly C, Walsh RD (1986) Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). Toxicol Appl Pharmacol 84: 93-114.

Glass DB, Gray CN, Jolley DJ, Gibbons C, Sim MR (2005) Health Watch exposure estimates: do they underestimate benzene exposure? Chem-Biol Interact 153-154: 23-32.

Glass DB, Gray CN, Jolley DJ, Gibbons C, Sim MR, Fritschi L, Adams GA, Bisby JA, Manuell R (2003) Leukaemia risk associated with low-level benzene exposure. Epidemiology 14: 569-577.

Glass DC, Adams GG, Manuell RW, Bisby JA (2000) Retrospective exposure assessment for benzene in the Australian petroleum industry. Ann Occup Hyg 44: 301-320.

Glass DC, Schnatter AR, Tang G, Irons RD, Rushton L (2014) Risk of myeloproliferative disease and chronic myeloid leukaemia following exposure to low-level benzene in a nested case-control study of petroleum workers. Occu Environ Med 71: 266-274.

Globocan (2012) Estimated cancer incidence, mortality and prevalence worldwide in 2012. IARC, WHO. http://globocan.iarc.fr/Default.aspx. Accessed 18 May 2017.

Göethel G, Brucker N, Moro AM, Charão MF, Fracasso R, Barth A, Bubols G, Durgante J, Nascimento S, Baierle M, Saldiva PH, Garcia SC (2014) Evaluation of genotoxicity in workers exposed to benzene and atmospheric pollutants. Mutat Res Genet Toxicol Environ Mutagen 770: 61-65.

Greim H, DA, Larson RA, Palermo CM, Rice JM, Ross D, Snyder R (2014) The bone marrow niche, stem cells, and leukaemia: impact of drugs, chemicals, and the environment. Ann N Y Acad Sci 1310: 7-31.

Guénel P, Imbernon E, Chevalier A, Grinquard-Calastreng A, Goldberg M (2002) Leukaemia in relation to occupational exposures to benzene and other agents: a case-control study nested in a cohort of gas and electricity utility workers. Am J Ind Med 42: 87-97.

Hartwig A (2010) The role of DNA repair in benzene-induced carcinogenesis. Chem Biol Interact 184: 269-272.

Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Travis LB, Li CY, Rothman N, Hoover RN, Linet MS (1997) Benzene and dose-related incidence of haematologic neoplasms in China. J Nat Cancer Inst 89: 1065-1071.

Hirabayashi Y, Inoue T (2010) Benzene-induced bone-marrow toxicity: a hematopoietic stem-cell-specific, aryl hydrocarbon receptor-mediated adverse effect. Chem Biol Interact. 184(1-2): 252-258.

Hollins D, Kerger B, Unice K, Knutsen J, Madl A, Sahmel J and Paustenbach D (2013) Airborne benzene exposures from cleaning metal surfaces with small volumes of petroleum solvents. International Journal of Hygiene and Environmental Health 216: 324-332.

Hopf NB, Kirkeleit J, Bråtveit M, Succop P, Talaska G, Moen BE (2012) Evaluation of exposure biomarkers in offshore workers exposed to low benzene and toluene concentrations. Int Arch Occup Environ Health 85: 261-271

Hosny G, Elghayish M, Noweir N (2017). Health Risk Assessment for benzene-exposure in oil refineries. Int. J. Environ. Sci. Toxic. Res. Vol. 5(1): 23-30.

HSE (Health & Safety Executive)(2000) Occupational Exposure to Benzene, Toluene, Xylene and Ethylbenzene during Routine Offshore Oil&Gas Production Operations. February 2000. Offshore Technology report – OTO 1999 088

Huang J, Zhao M, Wang P, Li X, Ma L, Zhang J, Zhou Y (2014) Effects of low concentrations of benzene exposure on levels of platelet-associated antibodies and platelet parameters. J Occup Environ Med 56: e92-97.

HVBG [Hauptverband der gewerblichen Berufsgenossenschaften] (2001) BIA-Report 3/2001: Berechnungsverfahren und Modellbildung in der Arbeitsbereichsanalyse. http://www.dguv.de/medien/ifa/de/pub/rep/pdf/rep01/biar0301/rep3_01.pdf

IAEA [International Atomic Energy Agency] (2011). Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. Vienna; International Atomic Energy Agency. http://www-pub.iaea.org/MTCD/Publications/PDF/EPR-Biodosimetry%202011_web.pdf

IARC [International Agency for Research on Cancer] (1982) Some industrial chemicals and dyestuffs. IARC Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC.

IARC [International Agency for Research on Cancer] (2004). Tobacco smoke and involuntary smoking. In: Chemical agents and related occupations IARC monographs on the evaluation of carcinogenic risks of chemicals to humans, vol. 83. http://monographs.iarc.fr/ENG/Monographs/vol83/

IARC [International Agency for Research on Cancer] (2012) Benzene. In: Chemical agents and related occupations IARC monographs on the evaluation of carcinogenic risks of chemicals to humans, vol. 100F. 2012. http://monographs.iarc.fr/ENG/Monographs/vol100F/index.php

Infante PF (2001) Benzene: an historical perspective on the American and European occupational setting. In : Gee D, Harremoes P, Keys J, MacGarvin M, Stirling A Vaz S, Wynne B (editors). Late lessons from early warnings: the precautionary princuiple 1896-2000. Copenhagen: European Environment Agency; 38-51. https://www.google.de/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0ahUKEwivq7b9j7DUAhWQI1AKHWb0CzwQFggpMAE&url=https%3A%2F%2Fwww.eea.europa.eu%2Fpublications%2Fenvironmental_issue_report_2001_22%2Fissue-22-part-04.pdf&usg=AFQjCNHrBgAnDeUtEiFHE5QZbHpo4j5EiQ, Accessed 09 June 2017

Infante PF (2006) Benzene exposure and multiple myeloma – A detailed meta-analysis of benzene cohort studies. Ann N Y Acad Sci 1076: 90-109.

INRS [L'Institut national de la recherche scientifique] (2016) Valeurs limites d'exposition professionnelle aux agents chimiques en France. http://www.inrs.fr/media.html?refINRS=ED%20984

INRS [L'Institut national de la recherche scientifique] (2017) MétroPol Benzène M-40. http://www.inrs.fr/publications/bdd/metropol/fiche.html?refINRS=METROPOL_40

IFA [Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung] (2013) Benzol. Kennzahl 6265. Messung von Gefahrstoffen. IFA-Arbeitsmappe. https://www.ifa-arbeitsmappedigital.de/ce/benzol/detail.html

INSHT [Instituto Nacional de Seguridad e Higiene en el Trabajo] (2015) Determinación de ácido trans,trans-mucónico en orina – Método de extracción en fase sólida y detección ultravioleta/cromatografía líquida de alta resolución. MTA/MB-026/A06. http://www.insht.es/InshtWeb/Contenidos/Documentacion/FichasTecnicas/MetodosAnalisis/Ficheros/MB/MB_026_A06.pdf

INSHT [Instituto Nacional de Seguridad e Higiene en el Trabajo] (2017) Límites de Exposición Profesional para Agentes Químicos en España. Instituti Nacional de Seguridad e Higiene en el Trabajo. http://www.insht.es/InshtWeb/Contenidos/Documentacion/LEP%20_VALORES%20LIMITE/Valores%20limite/LEP%202017.pdf.

Jakasa I, Kezic S, Boogaard P (2015) Dermal uptake of petroleum substances. Toxicology Letters 235: 123-139.

Jamebozorgi I, Mahjoubi F, Pouryaghoub G, Mehrdad R, Majidzadeh T, Saltanatpour Z, Nasiri F (2016) Micronucleus, Nucleoplasmic Bridge, and Nuclear Budding in Peripheral Blood Cells of Workers Exposed to Low Level Benzene. Int J Occup Environ Med 7(4): 227-233.

Ji Z, Weldon RH, Marchetti F, Chen H, Li G, Xing C, Kurtovich E, Young S, Schmid TE, Waidyanatha S, Rappaport S, Zhang L, Eskenazi B (2012) Comparison of aneuploidies of chromosomes 21, X, and Y in the blood lymphocytes and sperm of workers exposed to benzene. Environ Mol Mutagen 53: 218-226.

Jiřík V, Machaczka O, Miturová H, Tomášek I, Šlachtová H, Janoutová J, Velická H and Janout V (2016) Air Pollution and Potential Health Risk in Ostrava Region-A Review. Cent Eur J Public Health 24 Suppl, S4-S17.

Irons RD, Chen Y, Wang X-Q, Ryder J, Kerzic PJ (2013) Acute myeloid leukaemia following benzene exposure more closely resembles de novo than therapy-related disease. Genes Chromosomes Cancer 52: 887-894.

Kang SK, Lee MY, Kim TK, Lee JO, Ahn YS (2005) Occupational exposure to benzene in South Korea. Chem Biol Interact 153-154: 65-74.

Kalnas J, Teitelbaum DT (2013) Dermal absorption of benzene: implications for work practices and regulations. Int J Occup Environ Health 6: 114-121.

Katukam V, Kulakarni M, Syed R, Alharbi K, Naik J (2012) Effect of benzene exposure on fertility of male workers employed in bulk drug industries. Genetic Testing and Molecular Biomarkers 16: 592-597.

Khalade A, Jaakkola MS, Pukkala E, Jaakkola JK (2010) Exposure to benzene at work and the risk of leukaemia: a systematic review and meta-analysis. Environ Health 9: 31. Doi: 10.1186/1476-069X-9-31.

Kim SY, Choi JK, Cho YH, Chung EJ, Paek D, Chung HW (2004) Chromosomal aberrations in workers exposed to low levels of benzene: association with genetic polymorphisms. Pharmacogenetics 14: 453-463.

Kim S, Vermeulen R, Waidyanatha S, Johnson BA, Lan Q, Smith MT, Zhang L, Li G,Shen M, Yin S, Rothman N, Rappaport SM (2006) Modeling human metabolism of benzene following occupational and environmental exposures. Cancer Epidemiol Biomarkers Prev 15: 2246-2252.

Kim YJ, Choi JY, Paek D, Chung HW (2008) Association of the NQO1, MPO, and XRCC1 polymorphisms and chromosome damage among workers at a petroleum refinery. J Toxicol Environ Health A 71: 333-341.

Kim YJ, Choi JY, Cho YH, Woo HD, Chung HW (2010) Micronucleus-centromere assay in workers occupationally exposed to low level of benzene. Hum Exp Toxicol 29: 343-350.

Kirkeleit J, Riise T, Bråtveit M, Moen BE (2006) Benzene exposure on a crude oil production vessel. Ann Occup Hyg 50: 123-129.

Kirkeleit J, Riise T, Bratveit M, Moen BE (2008) Increased risk of acute myelogenous leukaemia and multiple myeloma in a historical cohort of upstream petroleum workers exposed to crude oil. Cancer Causes Control 19: 13-23.

Knutsen JS, Kerger BD, Finley B, Paustenbach DJ (2013) A calibrated human PBPK model for benzene inhalation with urinary bladder and bone marrow compartments. Risk Anal 33:1237-1251.

Koh DH, Jeon HK, Lee SG, Ryu HW (2015a) The relationship between low-level benzene exposure and blood cell counts in Korean workers. Occup Environ Med 72: 421-427.

Koh DH (2015b) Personal communication between AR Schnatter and DH Koh. 12.11.2015

Krieg EF Jr, Mathias PI, Toennis CA, Clark JC, Marlow KL, B'hymer C, Singh NP, Gibson RL, Butler MA (2012) Detection of DNA damage in workers exposed to JP-8 jet fuel. Mutat Res 747: 218-227.

Lagorio S, Crebelli R, Ricciarello R, Conti L, Iavarone I, Zona A, Ghittori S, Carere A (1998) Methodological issues in biomonitoring of low level exposure to benzene. Occup Med 48: 497-504.

Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N, Smith MT (2004) Haematotoxicity in workers exposed to low levels of benzene. Science 306: 1774-1776.

Leopardi P, Zijno A, Marcon F, Conti L, Carere A, Verdina A, Galati R, Tomei F, Baccolo TP, Crebelli R (2003) Analysis of micronuclei in peripheral blood lymphocytes of traffic wardens: effects of exposure, metabolic genotypes, and inhibition of excision repair *in vitro* by ARA-C. Environ Mol Mutagen 41: 126-130.

Lewis SJ, Bell GM, Cordingly N, Pearlman ED, Ruhston L (1997) Retrospective estimation of exposure to benzene in a leukaemia case-control study of petroleum marketing and distribution workers in the United Kingdom. Occup Environ Med 54: 167-175.

Li K, Jing Y, Yang C, Liu S, Zhao Y, He X, Li F, Han J, Li G (2014) Increased leukaemia-associated gene expression in benzene-exposed workers. Scientific Reports 4: 5369. Doi: 10.1038/srep05369.

Li J, Zhang X, He Z, Sun Q, Qin F, Huang Z, Zhang X, Sun X, Liu L, Chen L, Gao C, Wang S, Wang F, Li D, Zeng X, Deng Q, Wang Q, Zhang B, Tang H, Chen W, Xiao Y (2017) MGMT hypomethylation is associated with DNA damage in workers exposed to low-dose benzene. Biomarkers 12: 1-6.

Liang Y, Wong O, Yang L, Li T, Su Z (2006) The development and regulation of occupational exposure limits in China. Regul Toxicol Pharmacol 46: 107-113.

Linet MS, Yin SN, Gilbert ES, Dores GM, Hayes RB, Vermeulen R, Tian HY, Lan Q, Portengen L, Ji BT, Li GL, Rothman N (2015) A retrospective cohort study of cause-specific mortality and incidence of hematopoietic malignancies in Chinese benzene exposed workers. In J Cancer 137: 2184-2197.

Liu L, Zhang Q, Feng J, Deng L, Zeng N, Yang A, Zhang W (1996) The study of DNA oxidative damage in benzene-exposed workers. Mutat Res 370: 145-150.

LOA [Lower Olefins and Aromatics REACH Consortium vzw] (2017a) Benzene: Importance of Dose Metrics in Assessing Stochastic versus Threshold Mechanisms. Technical Steering Committee, benzene Working Group. Unpublished; submitted to ECHA on 09.08.2017.

LOA [Lower Olefins and Aromatics REACH Consortium vzw] (2017b) Benzene: BMD analysis for neutrophil count and white blood cell count based on tha data from Qu *et al* 2003a, Table 9 of Appendix A. Unpublished; submitted to ECHA on 13. and 15.12.2017.

Loomis D, Guyton K, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Vilahur N, Mattock H, Straif K (2017) Carcinogenicity of benzene. The Lancet Oncology. Available online 26 Oct 2017. Http://dx.doi.org/10.1016/S1470-2045(17)30832-X.

Lovreglio P, Doria D, Fracasso ME, Barbieri A, Sabatini L, Drago I, Violante FS, Soleo L (2016) DNA damage and repair capacity in workers exposed to low concentrations of benzene. Environ Mol Mutagen 57: 151-158.

Lovreglio P, De Palma G, Barbieri A, Andreoli R, Drago I, Greco L, Gallo E, Diomede L, Scaramuzzo P, Ricossa MC, Fostinelli J, Apostoli P, Soleo L (2017) Biological monitoring of exposure to low concentrations of benzene in workers at a metallurgical coke production plant: new insights into S-phenylmercapturic acid and urinary benzene. Biomarkers 16: 1-8.

Lovreglio P, Maffei F, Carrieri M, D'Errico MN, Drago I, Hrelia P, Bartolucci GB, Soleo L (2014) Evaluation of chromosome aberration and micronucleus frequencies in blood lymphocytes of workers exposed to low concentrations of benzene. Mutat Res Genet Toxicol Environ Mutagen 770: 55-60.

Maestri L, Negri S, Ferrari M, Ghittori S, Imbriani M (2005) Determination of urinary S-phenylmercapturic acid, a specific metabolite of benzene, by liquid chromatography/single quadrupole mass spectrometry. Rapid Commun Mass Spectrom 19: 1139-1144.

Maffei F, Hrelia P, Angelini S, Carbone F, Cantelli Forti G, Barbieri A, Sanguinetti G, Mattioli S, Violante FS (2005) Effects of environmental benzene: micronucleus frequencies and haematological values in traffic police working in an urban area. Mutat Res 583: 1-11.

Major J, Jakab M, Kiss G, Tompa A (1994) Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. Environ Mol Mutagen 23: 137-142.

Manini P, De Palma G, Andreoli R, Poli D, Mozzoni P, Folesani G, Mutti A, Apostoli P (2006) Environmental and biological monitoring of benzene exposure in a cohort of Italian taxi drivers. Toxicol Lett 167: 142-151.

Manini P, De Palma G, Andreoli R, Poli D, Petyx M, Corradi M, Mutti A, Apostoli P (2008) Biological monitoring of low benzene exposure in Italian traffic policemen. Toxicol Lett 181: 25-30.

Manini P, De Palma G, Andreoli R, Mozzoni P, Poli D, Goldoni M, Petyx M, Apostoli P, Mutti A (2010) Occupational exposure to low levels of benzene: Biomarkers of exposure and nucleic acid oxidation and their modulation by polymorphic xenobiotic metabolizing enzymes. Toxicol Lett 193(3): 229-235.

Mansi A, Bruni R, Capone P, Paci E, Pigini D, Simeoni C, Gnerre R, Papacchini M, Tranfo G (2012) Low occupational exposure to benzene in a petrochemical plant: modulating effect of genetic polymorphisms and smoking habit on the urinary t,t-MA/SPMA ratio. Toxicol Lett 213: 57-62.

Marchetti F, Eskenazi B, Weldon RH, Li G, Zhang L, Rappaport SM, Schmid TE, Xing C, Kurtovich E, Wyrobek AJ (2012) Occupational exposure to benzene and chromosomal structural aberrations in the sperm of Chinese men. Environ Health Perspect 120: 229-234.

Marcon F, Zijno A, Crebelli R, Carere A, Veidebaum T, Peltonen K, Parks R, Schuler M, Eastmond D (1999). Chromosome damage and aneuploidy detected by interphase multicolour FISH in benzene-exposed shale oil workers. Mutat Res 445: 155-166.

Mascelloni M, Delgado-Saborit JM, Hodges NJ, Harrison RM (2015) Study of gaseous benzene effects upon A549 lung epithelial cells using a novel exposure system.Toxicol Lett 237: 38-45.

McHale CM, Zhang L, Lan Q, Vermeulen R, Li G, Hubbard AE, Porter KE, Thomas R, Portier CJ, Shen M, Rappaport SM, Yin S, Smith MT, Rothman N (2011) Global gene expression profiling of a population exposed to a range of benzene levels. Environ Health Perspect 119: 628-634.

McHale CM, Zhang L, Smith MT (2012) Current understanding of the mechanism of benzene-induced leukaemia in humans: implications for risk assessment. Carcinogenesis 33: 240-252.

McNally K, Sams C, Loizou GD, Jones K (2017) Evidence for non-linear metabolism at low benzene exposures? A reanalysis of data. Chem Biol Interact. Pii: S0009-2797(17)30493-3.

Meek ME, Klaunig JE (2010) Proposed mode of action of benzene-induced leukaemia: Interpreting available data and identifying critical data gaps for risk assessment. Chem Biol Interact 184: 279-285.

Melikian AA, O'Connor R, Prahalad AK, Hu P, Li H, Kagan M, Thompson S (1999) Determination of the urinary benzene metabolites S-phenylmercapturic acid and trans,trans-muconic acid by liquid chromatography-tandem mass spectrometry. Carcinogenesis 20: 719-726.

Mendes MPR, Silveira JN, Andre LC (2017) An efficient analytical method for determination of S-phenylmercapturic acid in urine by HPLC fluorimetric detector to assessing benzene exposure. J Chromatogr B Analyt Technol Biomed Life Sci 1063: 136-140.

Monks TJ, Butterworth M, Lau SS (2010) The fate of benzene-oxide. Chem Biol 184: 201-206.

Moolla R, Curtis CJ and Knight J (2015) Occupational Exposure of Diesel Station Workers to BTEX Compounds at a Bus Depot. Int J Environ Res Public Health 12: 4101-4115.

Moro AM, Charão MF, Brucker N, Durgante J, Baierle M, Bubols G, Goethel G, Fracasso R, Nascimento S, Bulcão R, Gauer B, Barth A, Bochi G, Moresco R, Gioda A, Salvador M, Farsky S, Garcia SC (2013) Genotoxicity and oxidative stress in gasoline station attendants. Mutat Res 754: 63-70.

Moro AM, Brucker N, Charão MF, Sauer E, Freitas F, Durgante J, Bubols G,Campanharo S, Linden R, Souza AP, Bonorino C, Moresco R, Pilger D, Gioda A, Farsky S, Duschl A, Garcia SC (2015) Early haematological and immunological alterations in gasoline station attendants exposed to benzene. Environ Res 137: 349-356.

Moro AM, Brucker N, Charão MF, Baierle M, Sauer E, Goethel G, Barth A, Nascimento SN, Gauer B, Durgante J, Amaral BS, Neto FR, Gioda A, Garcia SC (2017) Biomonitoring of gasoline station attendants exposed to benzene: Effect of gender. Mutat Res 813: 1-9.

Nakayama A, Noguchi Y, Mori T, Morisawa S, Yagi T (2004) Comparison of mutagenic potentials and mutation spectra of benzene metabolites using supF shuttle vectors in human cells. Mutagenesis 19: 91-97.

Neri F, Foderi C, Laschi A, Fabiano F, Cambi M, Sciarra G, Aprea MC, Cenni A and Marchi E (2016) Determining exhaust fumes exposure in chainsaw operations. Environmental Pollution 218: 1162-1169.

NL (2017) Regeling van de Miniter van Social Zaken …7 Feb 2017 nr 2017-0000005838 and applies as of 1 Oct 2017. https://zoek.officielebekendmakingen.nl/stcrt-2017-8098.pdf

Ong CN, Kok PW, Ong HY, Shi CY, Lee BL, Phoon WH, Tan KT (1996) Biomarkers of exposure to low concentrations of benzene: a field assessment. Occup Environ Med 53(5): 328-333.

Ott MG (1978) Mortality among individuals occupationally exposed to benzene. Arch Occup Env Health 33: 3-10.

OSHA (2002) Sampling and Analytical Methods. Method 1005. Benzene. https://www.osha.gov/dts/sltc/methods/validated/1005/1005.html, accessed 05.07.2017

Paci E, Pigini D, Cialdella AM, Faranda P, Tranfo G (2007) Determination of free and total S-phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure. Biomarkers 12: 111-122.

Pandey AK, Bajpayee M, Parmar D, Kumar R, Rastogi SK, Mathur N, Thorning P, de Matas M, Shao Q, Anderson D, Dhawan A (2008) Multipronged evaluation of genotoxicity in Indian petrol-pump workers. Environ Mol Mutagen 49: 695-707.

Paustenbach DJ, Price PS, Ollison W, Blank C, Jernigan JD, Bass RD, Peterson HD (1992) Reevaluation of benzene exposure for the Pliofilm (rubberworker) cohort (1936-1976). Toxicol Environ Health 36: 177-231.

Pavanello S, Kapka L, Siwinska E, Mielzyñska D, Bolognesi C, Clonfero E (2008) Micronuclei related to anti-B[a]PDE-DNA adduct in peripheral blood lymphocytes of heavily polycyclic aromatic hydrocarbon-exposed nonsmoking coke-oven workers and controls. Cancer Epidemiol Biomarkers Prev 17(10): 2795-2799.

Paxton MB (1996) Leukaemia risk associated with benzene exposure in the Pliofilm cohort. Environ Health Perspect 104(Suppl 6): 1431-1436.

Periago JF and Prado C (2005) Evolution of Occupational Exposure to Environmental Levels of Aromatic Hydrocarbons in Service Stations. Ann Occup Hyg 49(3): 233-240.

Pesatori AC, Garte S, Popov T, Georgieva T, Panev T, Bonzini M, Consonni D, Carugno M, Goldstein BD, Taioli E, Fontana V, Stagi E, Bertazzi PA, Merlo DF (2009) Early effects of low benzene exposure on blood cell counts in Bulgarian petrochemical workers. Med Lav 100(2):83-90.

Pitarque M, Carbonell E, Lapeña N, Marsá M, Torres M, Creus A, Xamena N, Marcos R (1996) No increase in micronuclei frequency in cultured blood lymphocytes from a group of filling station attendants. Mutat Res 367: 161-167.

Plappert U, Barthel E, Raddatz K, Seidel HJ (1994) Early effects of benzene exposure in mice. Hematological versus genotoxic effects. Arch Toxicol 68(5): 284-290.

Polychronakis I, Dounias G, Makropoulos V, Riza E, Linos A (2013). Work-related leukaemia: a systematic review. J Occup Med Toxicol 8: 14. Doi: 10.1186/1745-6673-8-14.

Price PS, Rey TD, Fontaine DD, Arnold SM (2012) A reanalysis of the evidence for increased efficiency in benzene metabolism at airborne exposure levels below 3 p.p.m. Carcinogenesis 33: 2094-2099.

Price P, Rey T, Fontaine D, Arnold S (2013) Letter to the editor in response to 'Low-dose metabolism of benzene in humans: science and obfuscation' Rappaport *et al* (2013). Carcinogenesis 34: 1692-1696

Qu Q, Shore R, Li G, Jin X, Chen LC, Cohen B, Melikian AA, Eastmond D, Rappaport SM, Yin S, Li H, Waidyanatha S, Li Y, Mu R, Zhang X, Li K (2002) Haematological changes among Chinese workers with a broad range of benzene exposures. Am J Ind Med 42: 275-285.

Qu Q, Shore R, Li G, Jin X, Chen LC, Cohen B, Melikian AA, Eastmond D, Rappaport S, Li H, Rupa D, Waidyanatha S, Yin S, Yan H, Meng M, Winnik W, Kwok ES, Li Y, Mu R, Xu B, Zhang X, Li K (2003a) Validation and evaluation of biomarkers in workers exposed to benzene in China. Res Rep Health Eff Inst 115: 1-72; discussion 73-87.

Qu Q, Cohen BS, Shore R, Chen LC, Li G, Jin X, Melikian AA, Yin S, Yan H, Xu B, Li Y, Mu R, Zhang X, Li K (2003b) Benzene exposure measurement in shoe and glue manufacturing: a study to validate biomarkers. Appl Occup Environ Hyg 18: 988-998.

Raabe GK, Wong O (1996) Leukaemia mortality by cell type in petroleum workers with potential exposure to benzene. Environ Health Perspect 104(Suppl 6): 1381-1392.

Rappaport SM, Kim S, Lan Q, Li G, Vermeulen R, Waidyanatha S, Zhang L, Yin S, Smith MT, Rothman N (2010) Human benzene metabolism following occupational and environmental exposures. Chem Biol Interact 184: 189-195.

Rappaport SM, Kim S, Lan Q, Vermeulen R, Waidyanatha S, Zhang L, Li G, Yin S, Hayes RB, Rothman N, Smith MT (2009) Evidence that humans metabolize benzene via two pathways. Environ Health Perspect 117: 946-95.

Rappaport SM, Johnson BA, Bois FY, Kupper LL, Kim S, Thomas R (2013a) Ignoring and adding errors do not improve the science. Carcinogenesis 34: 1689-1691

Rappaport SM, Kim S, Thomas R, Johnson BA, Bois FY, Kupper LL (2013b) Low-dose metabolism of benzene in humans: science and obfuscation. Carcinogenesis 34:2-9.

Rauma M, Boman A and Johanson G (2013) Predicting the absorption of chemical vapours. Advanced Drug Delivery Reviews 65:306-14.

Rekhadevi PV, Rahman MF, Mahboob M, Grover P (2010) Genotoxicity in filling station attendants exposed to petroleum hydrocarbons. Ann Occup Hyg 54: 944-54.

Rekhadevi PV, Mahboob M, Rahman MF, Grover P (2011) Determination of genetic damage and urinary metabolites in fuel filling station attendants. Environ Mol Mutagen 52: 310-318.

Rhomberg L, Goodman J, Tao G, Zu K, Chandalia J, Williams PRD, Allen B (2016) Evaluation of acute nonlymphocytic leukaemia and its subtypes with updated benzene exposure and mortality estimates. J Occup Environ Med 58: 414-420.

Richardson DB (2008) Temporal variation in the association between benzene and leukaemia mortality. Environ Health Perspect 116: 370-374.

Richter RO, Kerger BD, Hoyt S and Fedoruk MJ (2013) Total Aromatic Content in Petroleum Solvents Modifies Headspace Benzene Vapor Concentrations: Implications for Exposure Assessments 19 (2):354-373.

Rinsky RA, Hornung RW, Silver SR, Tseng CY (2002) Benzene exposure and hematopoietic mortality: a long-term epidemiologic risk assessment. Am J Ind Med 42: 474-480.

Rinsky RA, Smith AB, Hornung R, Filloon TG, Young RJ, Okun AH, Landrigan PJ (1987) Benzene and leukaemia. An epidemiologic risk assessment. N Engl J Med 316: 1044-1050.

Rinsky RA, Young RJ, Smith AB (1981) Leukaemia in benzene workers. Am J Ind Med 2: 217-245.

Rivedal E, Witz G, Leithe E (2010) Gap junction intercellular communication and benzene toxicity. Chem Biol Interact 184: 229-232.

Roma-Torres J, Teixeira JP, Silva S, Laffon B, Cunha LM, Méndez J, Mayan O (2006) Evaluation of genotoxicity in a group of workers from a petroleum refinery aromatics plant. Mutat Res 604: 19-27.

Rothman N, Haas R, Hayes RB, Li GL, Wiemels J, Campleman S, Quintana PJ, Xi LJ, Dosemeci M, Titenko-Holland N, *et al* (1995) Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. Proc Natl Acad Sci U S A 92: 4069-4073.

Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN, Hayes RB (1996) Hematotoxicity among Chinese workers heavily exposed to benzene. Am J Ind Med 29(3): 236-246.

Roller M, Akkan Z, Hassauer M, Kalberlah F (2006) Risikoextrapolation vom Versuchstier auf den Menschen bei Kanzerogenen. Fb 1078 Bundesanstalt für Arbeitsschutz und Arbeitsmedizin Dortmund/Berlin/Dresden, Wirtschaftsverlag NW Bremerhaven.

Romanian Government Decision No. 1218/2006 completed and modified (2015)

Rushton L, Romaniuk H (1997) A case-control study to investigate the risk of leukaemia associated with exposure to benzene in petroleum marketing and distribution workers in the United Kingdom. Occup Environ Med 54: 152-156.

Rushton L, Schnatter AR, Tang G, Glass DC (2014) Acute myeloid and chronic lymphoid leukaemias and exposure to low-level benzene among petroleum workers. Br J Cancer 110: 783-787.

Sabatini L, Barbieri A, Indiveri P, Mattioli S, Violante FS (2008) Validation of an HPLC-MS/MS method for the simultaneous determination of phenylmercapturic acid, benzylmercapturic acid and o-methylbenzyl mercapturic acid in urine as biomarkers of exposure to benzene, toluene and xylenes. J Chromatogr B Analyt Technol Biomed Life Sci 863: 115-122.

Scherer G, Engl J, Urban M, Gilch G, Janket D, Riedel K (2007) Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. Regul Toxicol Pharmacol 47: 171-183.

Schettgen T, Alt A, Musiol A, Kraus T (2008) Die Hintergrundbelastung der Allgemeinbevölkerung gegenüber Benzol und Toluol-vollautomatisierte Bestimmung von S-Phenylmercaptursäure (SPMA) und S-Benzylmercaptursäure (S-BMA) im Urin mittels LC/MS/MS. 48. Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e. V., Hamburg, 653-655. www.dgaum.de/fileadmin/PDF/Tagungsbaende/DGAUM_Tagungsband_2008.pdf.

Schettgen T, Ochsmann E, Alt A, Kraus T (2010) A biomarker approach to estimate the daily intake of benzene in non-smoking and smoking individuals in Germany. J Expo Sci Environ Epidemiol 20: 427-433.

Schnatter AR, Armstrong TW, Nicolich MJ, Thompson FS, Katz AM, Huebner WH, Pearlman ED (1996) Lymphohaematopoietic malignancies and quantitative estimates of exposure to benzene in Canadian petroleum distribution workers. Occup Environ Med 53: 773-781.

Schnatter AR, Rosamilia K, Wojcik NC (2005) Review of the literature on benzene and leukaemia subtypes. Chem-Biol Interact 153-154: 9-21.

Schnatter RA, Glass DC, Tang G, Irons RD, Rushton L (2012) Myelodysplastic syndrome and benzene exposure among petroleum workers: an international pooled analysis. J Natl Cancer Inst 104: 1724-1737.

Schnatter RA, Kerzic PJ, Zhou Y, Chen M, Nicolich MJ, Lavelle K, Armstrong TW, Bird MG, Lin L, Fu H, Irons RD (2010) Peripheral blood effects in benzene-exposed workers. Chem Biol Interact 184: 174-181.

SCOEL (1991) Recommendation from the scientific committee on occupational exposure limits for benzene. SCOEL/SUM/140. European Commission, December 1991. http://www.google.de/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjf1cvy8MvVAhWMLFAKHS7MDNwQFggIMAA&url=http%3A%2F%2Fec.europa.eu%2Fsocial%2FBlobServlet%3FdocId%3D7423%26langId%3Den&usg=AFQjCNGwWKxQNunegRz_SKymbGX4y1yD-A.

SCOEL (2006) Addendum to SCOEL/SUM/140 (SCOEL 1991), Recommendation of the Scientific Committee on Occupational Exposure Limits: Biological Limit Values for Benzene,

February 2006. http://www.google.de/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjf1cvy8MvVAhWMLFAKHS7MDNwQFggIMAA&url=http%3A%2F%2Fec.europa.eu%2Fsocial%2FBlobServlet%3FdocId%3D7423%26langId%3Den&usg=AFQjCNGwWKxQNunegRz_SKymbGX4y1yD-A.

SEER (2017). US National Cancer Institute, Surveillance, Epdiemiology and End Results Porgram. Cancer Stat Facts. https://seer.cancer.gov/statfacts/ (accessed 9 Nov 2017).

Seniori Constantini A, Quinn M, Consonni D, Zappa M (2003) Exposure to benzene and risk of leukaemia among shoe factory workers. Scand J Work Environ Health 29: 51-59.

Seow WJ, Pesatori AC, Dimont E, Farmer PB, Albetti B, Ettinger AS, Bollati V, Bolognesi C, Roggieri P, Panev TI, Georgieva T, Merlo DF, Bertazzi PA, Baccarelli AA (2012) Urinary benzene biomarkers and DNA methylation in Bulgarian petrochemical workers: study findings and comparison of linear and beta regression models. PloS One 7(12): e50471.

Sha Y, Zhou W, Yang Z, Zhu X, Xiang Y, Li T, Zhu D, Yang X (2014) Changes in poly(ADP-ribosyl)ation patterns in workers exposed to BTX. PLoS One 9(9): e106146.

Shen M, Lan Q, Zhang L, Chanock S, Li G, Vermeulen R, Rappaport SM, Guo W, Hayes RB, Linet M, Yin S, Yeager M, Welch R, Forrest MS, Rothman N, Smith MT (2006) Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis. 27(10): 2083-2089.

Siegel PD, Saxena RK, Saxena QB, Ma JK, Ma JY, Yin XJ, Castranova V, Al-Humadi N, Lewis DM (2004) Effect of diesel exhaust particulate (DEP) on immune responses: contributions of particulate versus organic soluble components. J Toxicol Environ Health A 67: 221-231.

Silver SR, Rinsky RA, Cooper SP, Hornung MW, Lai D (2002) Effect of follow-up time on risk estimates: a longitudinal examination of the relative risks of leukaemia and multiple myeloma in a rubber hydrochloride cohort. Am J Ind Med 42: 481-489.

Smith MT (2010) Advances in understanding benzene health effects and susceptibility. Annu Rev Public Health 31: 133-148.

Smith MT, Jones RM, Smith AH (2007) Benzene exposure and risk of non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 16: 385-391.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM,Caldwell JC, Kavlock RJ, Lambert PF, Hecht SS, Bucher JR, Stewart BW, Baan RA, Cogliano VJ, Straif K (2016) Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environ Health Perspect 124(6): 713-721.

Sram RJ, Svecova V, Rossnerova A (2016) Systematic review of the use of the lymphocyte cytokinesis-block micronucleus assay to measure DNA damage induced by exposure to polycyclic aromatic hydrocarbons. Mutat Res 770(Pt A): 162-169.

Steinmaus C, Smith AH, Jones RM, Smith MT (2008) Meta-analysis of benzene exposure and non-Hodgkin lymphoma: biases could mask and important association. Occup Environ Med 65: 371-378.

Stenehjem JS, Kjaerheim K, Bratveit M, Samuelsen SO, Barone-Adesi F, Rothman N, Lan Q, Grimsrud TK (2015) Benzene exposure and risk of lymphohaematopoietic cancers in 25 000 offshore oil industry workers. Br J Cancer 112: 1603-1612.

Sterz K, Köhler D, Schettgen T, Scherer G (2010) Enrichment and properties of urinary pre-S-phenylmercapturic acid (pre-SPMA). J Chromatogr B Analyt Technol Biomed Life Sci 878: 2502-2505.

Sul D, Lee E, Lee MY, Oh E, Im H, Lee J, Jung WW, Won N, Kang HS, Kim EM, Kang SK (2005) DNA damage in lymphocytes of benzene exposed workers correlates with trans,trans-muco nic acids and breath benzene levels. Mutat Res 582: 61-70.

Surrallés J, Autio K, Nylund L, Järventaus H, Norppa H, Veidebaum T, Sorsa M, Peltonen K (1997) Molecular cytogenetic analysis of buccal cells and lymphocytes frombenzene-expose d workers. Carcinogenesis 18(4): 817-823. Swaen GM, van Amelsvoort L, Twisk JJ, Verstraeten E, Slootweg R, Collins JJ, Burns CJ (2010) Low level occupational benzene exposure and haematological parameters. Chem Biol Interact 184: 94-100.

Swaen GMH, Scheffers T, De Cock J, Slangen J, Drooge H (2005) Leukaemia risk in caprolactam workers exposed to benzene. Ann Epidemiol 15: 21-28.

Swaen GMH, Tsai SP, Burns CG (2010) Meta-analysis on benzene exposure and non-Hodgkin's lymphoma. Occup Environ Med 67: 286-287

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (2008) World Health Organization classification of tumours. Pathology and genetics of tumours of hematopoietic and lymphoid tissues, 4th edition. IARC, Lyon.

Testa A, Festa F, Ranaldi R, Giachelia M, Tirindelli D, De Marco A, Owczarek M, Guidotti M, Cozzi R (2004) A multi-biomarker analysis of DNA damage in automobile painters. Environ Mol Mutagen 46: 182-188.

Thomas R, Hubbard AE, McHale CM, Zhang L, Rappaport SM, Lan Q, Rothman N, Vermeulen R, Guyton KZ, Jinot J, Sonawane BR, Smith MT (2014) Characterization of changes in gene expression and biochemical pathways at low levels of benzene exposure. PloS ONE 9(5): e91828.

Tranfo G, Pigini D, Paci E, Marini F, Bonanni RC (2017) Association of exposure to benzene and smoking with oxidative damage to nucleic acids by means of biological monitoring of general population volunteers. Environ Sci Pollut Res Int 24: 13885-13894.

Tsai SP, Fox EE, Ransdell JD, Wendt JK, Waddell LC, Donnelly RP (2004) A haematology surveillance study of petrochemical workers exposed to benzene. Regul Toxicol Pharmacol 40: 67-73.

Tuo J, Loft S, Thomsen MS, Poulsen HE (1996) Benzene-induced genotoxicity in mice *in vivo* detected by the alkaline comet assay: reduction by CYP2E1 inhibition. Mutat Res 368(3-4): 213-219.

US EPA [Environmental Protection Agency] (2003) Integrated Risk Information System (IRIS). Chemical Assessment Summary. Benzene; CASNR 71-43-2. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0276_summary.pdf#nameddest=rfc

US NIOSH [The National Institute for Occupational Safety and Health] (2014) S-Benzylmercapturic acid and S-phenylmercapturic acid in urine, NIOSH Manual of Analytical Methods (NMAM), Fifth Edition, https://www.cdc.gov/niosh/docs/2014-151/pdfs/methods/8326.pdf, Accessed 13.11.2017

US NIOSH [The National Institute for Occupational Safety and Health] (2017) Benzene. https://www.cdc.gov/niosh/npg/npgd0049.html. Accessed 04.07.2017

US OSHA [Occupational Safety and Health Administration] (2017) Benzene. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10042&p_table=standards, accessed 04.07.2017

Uzma N, Kumar BS, Hazari MA (2010) Exposure to benzene induces oxidative stress, alters the immune response and expression of p53 in gasoline filling workers. Am J Ind Med 53: 1264-1270.

Van Sittert NJ, Boogaard PJ, Beulink GD (1993) Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. Br J Ind Med 50: 460-469.

Verma DK, Johnson DM, Shaw ML and des Tombe K (2001) Benzene and Total hydrocarbons exposures in the downstream petroleum industries. AIHAJ 62 (2):176-194.

Vermeulen R, Li G, Lan Q, Dosemeci M, Rappaport SM, Bohong X, Smith MT, Zhang L, Hayes RB, Linet M, Mu R, Wang L, Xu J, Yin S, Rothman N (2004) Detailed exposure assessment for a molecular epidemiology study of benzene in two shoe factories in China. Ann Occup Hyg 48: 105-116.

Violante FS, Sanguinetti G, Barbieri A, Accorsi A, Mattioli S, Cesari R, Fimognari C, Hrelia P (2003) Lack of correlation between environmental or biological indicators of benzene exposure at parts per billion levels and micronuclei induction. Environ Res 91: 135-142.

Vlaanderen J, Lan Q, Kromhout H, Rothman N, Vermeulen R (2011) Occupational benzene exposure and risk of lymphoma subtypes: A meta-analysis of cohort studies incorporating three study quality dimensions. Environ Health Perspect 119: 159-167.

Vlaanderen J, Land Q, Kromhout H, Rothman N, Vermeulen R (2012) Occupational benzene exposure and risk of chronic myeloid leukaemia: A meta-analysis of cohort studies incorporating study quality dimensions. Am J Ind Med 55: 779-785.

Vlaanderen J, Portengen L, Rothman N, Lan Q, Kromhout H, Vermeulen R (2010) Flexible meta-regression to assess the shape of the benzene-leukaemia exposure-response curve. Environ Health Perspect 118: 526-532.

Vlaanderen J, Vermeulen R, Heederik D, Kromhout H (2008). Guidelines to evaluate human observational studies for quantitative risk assessment. Environ Health Perspect 116: 1700-1705.

Watanabe KH, Bois FY, Daisey JM, Auslander DM, Spear RC (1994) Benzene toxicokinetics in humans: exposure of bone marrow to metabolites. Occup Environ Med 51(6): 414-420.

Weaver VM, Buckley T, Groopman JD (2000) Lack of specificity of trans,trans-muconic acid as a benzene biomarker after ingestion of sorbic acid-preserved foods. Cancer Epidemiol Biomarkers Prev 9: 749-755.

Weisel CP (2010) Benzene exposure: an overview of monitoring methods and their findings. Chem Biol Interact 184: 58-66.

Wetmore BA, Struve MF, Gao P, Sharma S, Allison N, Roberts KC, Letinski DJ, Nicolich MJ, Bird MG, Dorman DC (2008) Genotoxicity of intermittent co-exposure to benzene and toluene in male CD-1 mice. Chem Biol Interact 173: 166-178.

Whysner J, Reddy MV, Ross PM, Mohan M, Lax EA (2004) Genotoxicity of benzene and its metabolites. Mutat Res 566: 99-130.

Williams P, Paustenbach D (2003). Recosntruction of benzene exposure for the Pliofilm cohort (1936-1976) using Monte Carly techniques. J Toxicol Environ Health A. 66(8):677-781.

Williams PRD, Panko JM, Unice K, Brown JL, Paustenbach DJ (2008) Occupational Exposures Associated with Petroleum-Derived Products Containing Trace Levels of Benzene. J Occup Environ Hyg 5: 565-574.

Williams PR, Sahmel J, Knutsen J, Spencer J, Bunge AL (2011) Dermal absorption of benzene in occupational settings: estimating flux and applications for risk assessment. Crit Rev Toxicol 41(2): 111-142.

Wong O (1987) An industry wide mortality study of chemical workers occupationally exposed to benzene. II dose-response analyses. Br J Ind Med 44: 382-395.

Wong O (1995) Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene. Ocuup Environ Med 52: 380-384.

Xing C, Marchetti F, Li G, Weldon RH, Kurtovich E, Young S, Schmid TE, Zhang L, Rappaport S, Waidyanatha S, Wyrobek AJ, Eskenazi B (2010) Benzene exposure near the U.S. permissible limit is associated with sperm aneuploidy. Environ Health Perspect 118: 833-839.

Xiong F, Li Q, Zhou B, Huang J, Liang G, Zhang L, Ma S, Qing L, Liang L, Su J, Peng X, Li Q, Zou Y (2016) Oxidative Stress and Genotoxicity of Long-Term Occupational Exposure to Low Levels of BTEX in Gas Station Workers. Int J Environ Res Public Health 13(12) pii: E1212.

Yang B, Lv J, Cheng W, Zhou Lf, Ye Y, Sun Y, Feng Nn, Wang Q, Jin R, Sun P, Cheng Z, Xia Zl (2012) Micronucleus occurrence in Chinese workers occupationally exposed to benzene. Europ J Oncol 17: 71-78.

Ye LL, Zhang GH, Huang JW, Li Y, Zheng GQ, Zhang DT, Zhou LF, Tao XD, Zhang J, Ye YJ, Sun P, Frank A, Xia ZL (2015) Are polymorphisms in metabolism protective or a risk for reduced white blood cell counts in a Chinese population with low occupational benzene exposures? Int J Occup Environ Health 21: 232-240.

Yoon HS, Lee KM, Kang D (2014) Intercorrelation between Immunological Biomarkers and Job Stress Indicators among Female Nurses: A 9-Month Longitudinal Study. Front Public Health 2: 157.

Zhang GH, Ji BQ, Li Y, Zheng GQ, Ye LL, Hao YH, Ren JC, Zhou LF, Xu XW, Zhu Y, Xia ZL (2016) Benchmark Doses Based on Abnormality of WBC or Micronucleus Frequency in Benzene-Exposed Chinese Workers. J Occup Environ Med 58: e39-44.

Zhang GH, Ye LL, Wang JW, Ren JC, Xu XW, Feng NN, Zhou LF, Ru JG, Hao YH, Tian W, Sun P, Au WW, Christiani DC, Xia ZL (2014) Effect of polymorphic metabolizing genes on micronucleus frequencies among benzene-exposed shoe workers in China. Int J Hyg Environ Health 217: 726-732.

Zhang L, Lan Q, Guo W, Hubbard AE, Li G, Rappaport SM, McHale CM, Shen M, Ji Z, Vermeulen R, Yin S, Rothman N, Smith MT (2011) Chromosome-wide aneuploidy study

(CWAS) in workers exposed to an established leukemogen, benzene. Carcinogenesis 32: 605-612.

Zhang L, Lan Q, Ji Z, Li G, Shen M, Vermeulen R, Guo W, Hubbard AE, McHale CM, Rappaport SM, Hayes RB, Linet MS, Yin S, Smith MT, Rothman N (2012) Leukaemia-related chromosomal loss detected in hematopoietic progenitor cells of benzene-exposed workers. Leukaemia 26: 2494-2498.

Appendix 1. Tables

Table 34: Recent occupational benzene exposure assessment studies gathered from the literature

Work area / Occupation	n	Mean, µg / m³	Median, µg / m³	Range, µg / m³	Location	Reference
Upstream petroleum industry-offshore						
Modes of operation and tasks on a production vessel in the Norwegian sector of the North Sea	139	1398	65 (GM)	3 (LOD)-54440	Norway/ the North Sea	Kirkeleit et al 2006
Ordinary activity						
Shutdown	71	65	13 (GM)	LOD-715		
Tank work	26	99	33 (GM)	LOD-683		
	42	94453	910 (GM)	13-54440		
Offshore oil and gas production operations-North Sea	241	91% of the samples <163		65 (LOD)-1632 (99th percentile)	United Kingdom/ the North Sea	HSE 2000
Refinery 1,					Sweden, 2009-2011	Almerud *et al* 2017
Refinery process technicians	132	15		10-23		
Outdoor process technicians	108	21		13-34		
Process area	71	10		6-16		
Harbour and tank park	37	75		35-160		
Indoor process technicians	24	4		3-5		
Maintenance workers	67	6		4-9		
Process area	41	4		3-5		
Harbour and tank park	20	13		6-30		
Laboratory workers	25	5		3-6		
Engineers	41	5		3-7		
Safety and emergency staff	14	5		4-7		
Refinery 2						
Outdoor process technicians	66	14		8-23		

Work area / Occupation	n	Mean, µg / m³	Median, µg / m³	Range, µg / m³	Location	Reference
Process area	51	13		7-24		
Tank park	15	165		5-48		
Laboratory workers	11			3-21		
Engineers	17			2-10		
Refinery 1 workers	43	610		230-1600***	Sweden, 2011 and 2013	Akerstrom *et al* 2016
Refinery employees	27	430		140-1300***		
Contractors	16	1200		150-9500***		
Refinery 2 workers	26	960		7-4500		
Refinery employees	13	870		23-4500		
Other occupations	13	1100		7-3400		
Oil harbour workers	34	310		80-1200***		
Jetty workers	20	470		96-2300***		
Dockworkers	14	170		12-2400***		
Sewage tanker drivers	16	360		68-1900***		
Oil refinery workers	32	25			Italy	Campagna *et al* 2012
Oil refinery					Egypt	Hosny *et al* 2017
Site 1, average		1816				
Tank farm		1373				
Pump station		1779				
Lab complex		3717				
Site 2, average		305				
Tank farm		420				
Lab		92				
Site 3, average		395				
Tank farm		411				

Work area/Occupation	n	Mean, µg/m³	Median, µg/m³	Range, µg/m³	Location	Reference
Tank drivers						
Fuel tank drivers	17	280	246	7-1020	Italy	Lovreglio *et al* 2016
Fuel tank drivers	18	307	247	7-1017	Italy	Lovreglio *et al* 2014
Tank farm	8	11		2-27	Germany	Breuer *et al* 2013
Filling tank vehicles (tank farm)	8	24*	33		Germany, 2013-2014	Breuer *et al* 2015
Retail/marketing						
Gasoline station workers	89	59		5-284 (5-95 percentile)	Italy	Campo *et al* 2016
Gasoline station (including office, pump area and garage)	10	5		1-13	Germany	Breuer *et al* 2013
Gasoline station						
Indoor	17	4*	4		Germany, 2013-2014	Breuer *et al* 2015
Outdoor	5	5*	3			
Filling station attendants	13	20	14	5-53	Italy	Lovreglio *et al* 2016
Filling station attendants	24	23	20	4-66	Italy	Lovreglio *et al* 2014
Service-station attendants	28		40	8-260	Italy	Fracasso *et al* 2010
Gasoline pump maintenance workers	21		24	5-515		
Service station workers						
1995	21	736		272-1603	Spain	Periago and Prado 2005
2000	28	241		115-453		
2003	19	163		36-564		
Gasoline station attendants		132		64-2207	Brazil	Moro *et al* 2017
Gasoline station						
Attendants				2-2900	Not reported	Edokpolo *et al* 2014

Work area / Occupation	n	Mean, µg / m³	Median, µg / m³	Range, µg / m³	Location	Reference
Maintenance workers				51-540		
Customers refueling				150-4900		
Gasoline station – refuelling in fuel bay / workshop		4/5		4-5	South Africa	Moolla *et al* 2015
Repairing workshop	12	250		7-1500	Germany	Breuer *et al* 2013
Maintenance work on motor vehicles (repairing workshop)	6	332*	750		Germany, 2013-2014	Breuer *et al* 2015
US Air force personnel					USA	Krieg *et al* 2012
Administrative personnel e.g. medical technicians and military police	139	4		1-61		
Fuelling aircraft / maintaining fuel storage facilities	38	137		1-1854		
Maintenance work inside fuel tanks	115	876		3-6629		
Chemical industry						
Chemical industry	19	110		<2-830	Germany	Breuer *et al* 2013
Petrochemical industry						
Operators	145	45	10	<3-90	Italy	Carrieri *et al* 2010 and 2012
Outdoor operators	173	35	9	2-895		
Petrochemical industry workers	33		28	2-594	Italy	Fracasso *et al* 2010
Other industry						
Shoe factory						
Smaller factory	116	71000	47000*		China	Vermeulen *et al* 2004
Larger factory	2667	11000	4200*			

Work area / Occupation	n	Mean, µg / m³	Median, µg / m³	Range, µg / m³	Location	Reference
Exposure from exhaust of vehicles/equipments						
Traffic policemen	70		19	13-31	Italy, 2001-2002	Angelini *et al* 2011
Landscaping work with gasoline-engined equipment	60	10*	10		Germany, 2013-2014	Breuer *et al* 2015
Forest workers using chainsaw	80	71	45		Italy	Neri *et al* 2016
Databases						
MEGA-database (1998-2002)						
Transfer / filling up of gasoline	27	<100**		<100-3400	Germany	DGUV 2007e
Cleaning of / in tanks and vessels	100	<100**		<100-2200		
Repair / maintenance / test bench	114	<100**		<100-700		
Foundry	43	<100**		<100-1200		
Laboratories	14	<100**		<100-800		
FIOH database (2004-2007); contains different areas of work	83	90	2		Finland	FIOH 2010

*GM=geometric mean; **detection limit is 0.1 mg/m³; ***95% CI

Table 35: Benzene in urine of workers exposed to benzene concentrations below 10 ppm

Type of workers	N, smoking	Benzene in air (ppm / m^3) ±SD (range)	Benzene in air (mg / m^3) ±SD (range)	Benzene in urine (µg / L)	Reference
Shoe factory workers, China	10 (SM, NS)	7.6±2.2		102±2.1[2)]	Marchetti *et al* 2012
	10 (SM, NS)	3.0±3.4		11.6±1.6[2)]	
	10 (SM, NS)	1.0±2.6		2.8±1.7[2)]	
	11 (SM, NS)	control		0.1±1.3[2)]	
Shoe factory workers, China	16 (76% SM)	7.6±2.3		50±3.1[2)]	Ji et al 2012 ; Xing *et al* 2010
	17 (76% SM)	1.0±2.6		4.2±2.5[2)]	
	33 (73% SM)	control		0.1±1.8[2)]	
Shoe factory workers, China	18 (11% SM)	2.64±2.70		66±139[1)]	Zhang *et al* 2012
	14 (21% SM)	0.04±0.00		0.25±0.61[1)]	
Shoe factory workers, China	110 (SM, NS)	2.85±2.11		86.0±130[1)]	Lan et al 2004
	109 (SM, NS)	0.57±0.24		13.4±18.3[1)]	
	140 (SM, NS)	<0.04		0.382±1.24[1)]	
Tank workers	10 (31% SM)	0.23±2.89	0.75±9.39	2.1±0.3[2)] [27.0±3.44 nmol/L]	Hopf *et al* 2012
Controls	18 (29% SM)	-	-	0.067±0.175[2)] [0.86±2.24 nmol/L]	
Petrochemical workers, refinery	131 NS	0.246±0.060	0.076±0.018	3.68±14.5[1)] [47.10±186.00 nmol/L] (no controls)	Ong *et al* 1996
Fuel tank driver	19 (58% SM)	0.1±0.1	0.306±0.266	2.96±3.00[1)]	Lovreglio *et al* 2014
Fuel filling station attendants (M), Italy	24 (50% SM)	0.007±0.005	0.023±0.017	0.60±0.72[1)]	
Controls	31	0.001	0.0046±0.0026	1.23±2.63[1)]	

Type of workers	N, smoking	Benzene in air (ppm / m^3) ±SD (range)	Benzene in air (mg / m^3) ±SD (range)	Benzene in urine (µg / L)	Reference
Filling station attends	12 (3 SM, 9 NS)	0.056±0.052	0.181±0.169	0.76±0.38[1)]	Lagorio *et al* 1998
Controls	11	-	-	0.23±0.10[1)]	
Refinery blue collar workers	33 NS	0.043 (0.02-0.678)	0.14 (0.06-2.2)	0.308 (0.110-1.471)[3)]	Fustinoni *et al* 2011
Controls	65 NS	0.001 (<0.001-0.005)	0.004 (0.001-0.016)	0.090 (0.051-0.373[3)]	
Workers in metallurgical coke production plant	93 (39% SM)	0.007±0.006 (personal sampling) 0.013±0.011 (stationary sampling)	0.023±0.019 (personal sampling) 0.043±0.036 (stationary sampling)	1.32±3.54[1)] 0.12 (<0.02-28.97)[4)]	Lovreglio *et al* 2017
Gasoline station attendants	46 NS	0.019 (0.003-0.147)	0.061 (0.011-0.478)	0.342 (0.042-2.836)[4)]	Fustinoni *et al* 2005
Traffic policemen, Italy	49 NS	0.007 (0.003-0.12)	0.022 (0.009-0.36)	0.151 (0.025-0.943) [4)]	
Controls	33 NS	0.002 (<0.002-0.0035)	0.006 (<0.006-0.115)	0.133 (<0.015-0.409) [4)]	
Gasoline station attendants	89 (34% SM)	0.018 (0.001-0.087)	0.059 (0.005-0.284)	0.339 (0.090-2.749)[3)]	Campo *et al* 2016
Controls	90 (34% SM)	0.001 (<0.001-0.006)	0.004 (0.001-0.018)	0.157 (0.054-2.554)[3)]	
Oil refinery workers	19 NS	0.010 (0.004-0.038)	0.030 (0.012-0.123)	0.267 (0.151-0.557)[5)]	Campagna *et al* 2012
Control	51 NS	0.002 (<0.001-0.003)	0.006 (0.001-0.009)	0.120 (0.067-0.176)[5)]	
Policemen, Italy	80 NS	0.002 (0.0001-0.003)	0.006 (0.0003-0.009)	0.160 (0.13-0.19)[5)]	Manini *et al* 2008
Controls	none				
Taxi drivers, Italy	21 NS	0.002±0.001	0.006±0.002	0.44±1.79[2)]	Manini *et al* 2006
Controls	none				

Abbreviations: NS: non-smoker; SM: smoker

4) mean±SD; 2) GM±GSD; 3) median, 5^{th}-95^{th} percentile; 4) median, minimum-maximum; 5) median, interquartile range

Table 36: SPMA in urine of workers exposed to benzene concentrations below 10 ppm

Type of workers	N (smoking)	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	SPMA in urine (µg/g creat)	Comment on measurement of SPMA	Reference
Shoe manufacture, Wuhan, China (no controls)	16 (SM, NS)	6.64±0.41[1)]	21.55±1.33[1)]	347.83±1.78[1)]	Acidification of urine, HPLC-MS	Lv *et al* 2014
	15 (SM, NS)	2.43±0.33[1)]	7.90±1.08[1)]	96.49±1.95[1)]		
	24 (SM, NS)	0.99±0.54[1)]	3.05±1.76[1)]	43.56±1.95[1)]		
Service station attendants, India (no controls)	200	0.43-0.46	1.1-1.3	7.8±0.34[2)] [9.40±0.41 µg/L]	Acidification of urine, GC-MS	Rekhadevi *et al* 2011
		0.40-<0.43	>1.3-1.4	6.9±0.02[2)] [8.32±0.28 µg/L]		
		0.34-<0.40	>1.4-1.5	5.6±0.61[2)] [6.71±0.74 µg/L]		
Benzene factory workers, Estonia	12 (75% SM)	0.400 (0.030-8.80)	1.3 (0.1-28.6)[1)]	7.9 (0.3-221)[1)]	Acidification of urine, HPLC	Marcon *et al* 1999
Cokery workers, Estonia	5 (40% SM)	0.308 (0.154-0.524)	1.0 (0.5-1.7) [1)]	8.5 (2.1-28.9)[1)]		
Controls	8 (63% SM)	-	-	1.2 (0.3-8.6)[1)]		
Fuel tank driver (M), Italy	19 (58% SM)	0.100±0.100	0.306±0.266[2)]	2.94±3.24[2)]	Acidification of urine, HPLC-ESI-MS/MS	Lovreglio *et al* 2014
Controls	31 (52% SM)		0.0046±0.0026	0.65±1.00[2)]		
Petrochemical industry operators (no controls)	97 NS	0.017 (0.001-0.280)[2)]	0.055 (0.003-0.91)	1.14 (<0.06-18.63)[2)]	Acidification of urine, HPLC-MS/MS	Carrieri *et al* 2010
Petrochemical workers (no controls)	22 NS	0.011±0.004	0.037±0.012 (0.002-0.894)[2)]	0.88±0.39 (0.05-18.63)[2)]	Acidification of urine, HPLC-MS/MS	Carrieri *et al* 2012
Petrochemical workers (no controls)	103 NS	0.011±0.012 (0.001-0.090)	0.0368±0.04 (0.004-0.292)[2)]	0.84±1.67 (0.05-14.39)[2)]	Acidification of urine, HPLC	Mansi *et al* 2012

Type of workers	N (smoking)	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	SPMA in urine (µg/g creat)	Comment on measurement of SPMA	Reference
Workers in metallurgical coke production plant	93 (39% SM)	0.007±0.006 (personal sampling) 0.013±0.011 (stationary sampling)	0.023±0.019 (personal sampling) 0.043±0.036 (stationary sampling)	0.84±0.97[2)]	Acidification of urine; LC-MS/MS	Lovreglio *et al* 2017
Policemen (no controls)	80 NS	0.002 (0.001-0.003)	0.006 (0.0003-0.009)[3)]	0.42 (0.20-1.07)[3)]	Acidification of urine, LC-MS/MS	Manini *et al* 2008

Abbreviations: F: female; M: male; NS: non-smoker; SM: smoker

4) GM±GSD (range); [2)] arithmetic mean±SD (range); [3)] median

Table 37: ttMA in urine of workers exposed to benzene concentrations below 10 ppm

Type of workers	N (smoking)	Benzene in air (ppm) ±SD (range)	Benzene in air (mg/m³) ±SD (range)	ttMA in urine (µg/g creat)	Reference
Shoe factory workers, China	10	7.6±2.2		13000±1500[1)] [15.6±1.8 mg/L]	Marchetti *et al* 2012
	10	3.0±3.4		4500±2583[1)] [5.4±3.1 mg/L]	
	10	1.0±2.6		1417±1333[1)] [1.7±1.6 mg/L]	
Shoe factory workers, China	16	7.6±2.3		13333±1333[1)] [16.1±1.6 mg/L]	Ji *et al* 2012 ; Xing *et al* 2010
	17	1.0±2.6		1583±1583[1)] [1.9±1.9 mg/L]	
Service station attendants, India	200	0.43-0.46		143±7.73[1)] [171±9.28 µg/L]	Rekhadevi *et al* 2011
		0.40-<0.43		119±6.45[1)] [143±7.74 µg/L]	
		0.34-<0.40		103±3.6[1)] [123±4.31 µg/L]	
Fuel tank driver (M), Italy	19 (58% SM)	0.1±0.1	0.306±0.266	134±94	Lovreglio *et al* 2014
Gasoline station attendants (M), Brazil	20 (40% SM)	0.043 (0.021-0.680)	0.139 (0.068-2.207)	424 (287-548)[1)] [509 (344-658) µg/L]	Moro *et al* 2017
Gasoline station attendants (F), Brazil	20 (10% SM)	0.038 (0.020-0.206)	0.124 (0.064-0.670)	448 (138-838)[1)] [538 (165-993) µg/L]	
Gasoline station attendants (M), Brazil	43 NS	0.023 (0.015-0.396)	0.076 (0.050-1.285)	326 (189-454)	Moro *et al* 2013
Gasoline pump maintenance workers	12 NS	0.025 (0.002-0.051)	0.080 (0.008-0.165)	109.6 (13.4–242.5)	Fracasso *et al* 2010
Service station attendants	15 NS	0.019 (0.004-0.08)	0.063 (0.012-0.260)	103.5 (30.0–418.0)	Fracasso *et al* 2010
Gasoline attendants	46 NS	0.019 (0.003-0.147)	0.061 (0.011–0.478)	49 (<10–581)	Fustinoni *et al* 2005
Petrochemical industry operators	97 NS	0.017 (0.001-0.280)[2)]	0.055 (0.003-0.91)	74.7	Carrieri *et al* 2010

Type of workers	N (smoking)	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	ttMA in urine (µg/g creat)	Reference
Petrochemical workers	103 NS	0.011±0.012 (0.001-0.090)	0.0368±0.04 (0.004-0.292)[2)]	64±58 (15–465)	Mansi *et al* 2012
Petrochemical industry operator	15 NS	0.010 (0.001-0.183)	0.033 (0.002–0.594)	109 (49–3800)	Fracasso *et al* 2010
Oil refinery workers	19 NS	0.009	0.030 (0.012-0.123)	35 (26-66) median	Campagna *et al* 2012
Fuel filling station attendants (M), Italy	24 (50% SM)	0.007±0.005	0.023±0.017	85±33	Lovreglio *et al* 2014
Traffic policemen	49 NS	0.007 (0.003-0.097)	0.022 (0.009–0.316)	82 (<10–416)	Fustinoni *et al* 2005
Bus drivers	106 NS	0.006 (<0.002-0.028)	0.021 (<0.006–0.092)	57 (<10–536)	
Traffic policewomen	48 NS	0.005±0.003	0.017±0.010	62.0±59.8	Ciarrocca *et al* 2012b
Police drivers (F)	21 NS	0.006±0.003	0.019±0.009	61.8±59.8	
Traffic policemen	62 NS	0.004	0.0125	63.0	Ciarrocca *et al* 2012a
Police drivers (M)	22 NS	0.004	0.0116	47.7	
Traffic wardens (M, F), Italy	15 (SM, NS)	0.004±0.003	0.014±0.010	89±103[1)] [107±123 µg/ L urine]	Violante *et al* 2003
Policemen	80 NS	0.002 (0.001-0.003)	0.006 (0.0003-0.009)[3)]	38.6 (31.7–51.6)	Manini *et al* 2008
Taxi drivers	21 NS	0.002±0.001	0.006±0.002	before shift: 105±67 after shift: 122±70	Manini *et al* 2006

Abbreviations: F: female; M: male; NS: non-smoker; SM: smoker

4) assuming 1.2 g creatinine/L urine

Table 38: Studies investigating haematological effects in workers

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Result	Findings	Reference
Data from Health surveillance programms						
Controls	3227					Tsai *et al* 2004
Shell employees Deer Park and Norco, USA	1200	0.14 (0.005-1.3; since the year 1988) 0.60 (0.1-5.7; for the years 1977-1988)		–	No effects on WBC, LYM, RBC, Hb, HCT, MCV, PLT	
Controls	1059 M				EOS (µL): 181.92	Swaen *et al* 2010
Dow employees Terneuzen, Netherlands	701 M	0.22 (0.01-1.85)		–	No effects on Hb, HCT, WBC, LYM, NEU, BAS, MONO; small effect on EOS	
		<0.5			EOS (µL): 185.45	
		0.5-1.0			EOS (µL): 168.23	
		>1.0			EOS (µL): 167.22	
Controls	268					Collins *et al* 1991
Monsanto employees	200	0.09* (0.01-1.40) * value calculated based on exposure estimates for 26 job descriptions		–	No effects on, RBC, Hb, PLT, increased values for WBC and MCV	
Controls	553					Collins *et al* 1997
Workers in medical/ industrial hygiene system	387	0.55 (0.01-88) with <5% >2 ppm		–	No effect (abnormal values) on LYM	
Benzene-exposed workers identified from the Korean Special Health	10,702				No effects (abnormal values) on WBC, RBC in females, PLT, NEU, LYM; RBC count (below normal lower limit of normal) in males decreased:	Koh *et al* 2015a, b
		0.002 (<0.01)		–	Ref.	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	Result	Findings	Reference
Examination Database		0.04 (≤0.01-<0.1)		–	Decreased RBC OR 0.41 (95% CI 0.27-0.61)	
		0.21 (≤0.1-<0.5)		–	Decreased RBC OR 1.32 (95% CI 0.96-1.81)	
		2.61 (≤0.5-<5.95)		LOAEC	Decreased RBC OR 2.12 (95% CI 1.52-2.95)	
Shoe manufacturing workers						
Workers in Tianjin shoe factories	16 F	0.14±0.04			Result not reliable due to inappropriate controls	Qu *et al* 2003a
	51	controls			RBC (x10^{10}/L): 463±52 WBC (x10^6/L): 6,671±1,502 NEU (x10^6/L): 4,006±1,108	Qu *et al* 2002, 2003a
	54	3.07±2.9		+	RBC (x10^{10}/L): 403±62 WBC (x10^6/L): 6,383±1,330 NEU (x10^6/L): 3,377±868	
	36	5.89±4.8			RBC (x10^{10}/L): 396±57 WBC (x10^6/L): 6,089±1,455 NEU (x10^6/L): 3,491±1,121	
	29	17.4±15.5			RBC (x10^{10}/L): 404±51 WBC (x10^6/L): 6,103±1,560 NEU (x10^6/L): 3,501±1,314	
	11	50.6±55.4			RBC (x10^{10}/L): 391±39 WBC (x10^6/L): 4,727±548 NEU (x10^6/L): 2,480±451	
	140	controls				Lan *et al* 2004
	109	0.57±0.24		+	WBC, GRA, LYM, B cells, Mono, PLT (for detailed results see Table 17)	
	110	2.85±2.11				
	31	28.73±20.74				
	42	<0.04 (controls)			WBC (/µL): 6454.8±1746.5	McHale *et al* 2011
	29	0.3±0.9		+	WBC (/µL): 5524.1±1369.2	
	30	0.8±0.8		+	WBC (/µL): 5510.0±1170.7	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	Result	Findings	Reference
	11	7.2±1.3		+	WBC (/µL): 5418.2±1376.8	
	13	24.7±15.7		+	WBC (/µL): 5176.9±1326.8	
Workers from six shoe factories in Zhejiang Province, China	385	2.0 (0.7-17.8)				Ye *et al* 2015
	220	controls			WBC (x10^9): 6.47±1.40	
	24	<1.0	<3.25	+	WBC (x10^9): 5.57±1.79*	
	149	<1.8	<6.0	(+)	WBC (x10^9): 6.01±1.47 n.s.	
	212	≥1.8	≥6.0	+	WBC (x10^9): 5.27±1.54**	
	96	≥1.5 ppm-y	≥5.02 mg/m^3-years	–	WBC (x10^9): 6.17±1.58	
	96	>6.1 ppm-y	>19.90 mg/m^3-years	+	WBC (x10^9): 5.63±1.54**	
	96	>9.8 ppm-y	>31.81 mg/m^3-years	+	WBC (x10^9): 5.45±1.81**	
	97	>18.2 ppm-y	>59.00 mg/m^3-years	+	WBC (x10^9): 5.19±1.20**	
Workers in Wenzhou shoe factories, China	317	1.60 (0.8-12.09)				Zhang *et al* 2016
	94	controls		–	WBC (x10^9/L): 6.48±1.42	
	65	3.55 ppm-y		–	WBC (x10^9/L): 6.14±1.60	
	65	6.51 ppm-y		–	WBC (x10^9/L): 6.14±1.33	
	65	10.72 ppm-y		+	↓WBC (x10^9/L): 5.76±1.57**	
	65	20.02 ppm-y		+	↓WBC (x10^9/L): 6.04±1.87**	
	65	40.71 ppm-y		+	↓WBC (x10^9/L): 5.70±1.60**	
Workers in industries other than shoe factories						
Controls	110					Huang *et al* 2014
Petrochemical workers, Shanghai, China	121	0.08-4.8 AM: 0.67; 0.56; 0.82 (mean AM calculated: 0.68)	0.25-15.70 (1.4% >10) AM: 2.24; 1.83; 2.65	–	No effects on WBC, Hb, PLT	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Result	Findings	Reference
Workers in five factories, Korea	61	0.268±0.216 (0.005-2.032)		–	No effects on RBC, Hb, MCV, MCH, WBC, PLT; MCHC Sign. Decreased	Kang *et a l* 2005 ; Sul *et al* 2005
	26	<0.1				
	28	0.1-1.0				
	7	1-3				
Workers in five factories (rubber, shoes, insulation, pharmaceutical) in Shanghai	928	<1		(+)	Odds rations (95% confidence interval) WBC 2.49 (0.31, 20.0) RBC: 10.8 (1.41, 82,5) MCV: 5.65 (0.63, 51.1) PLT: 2.18 (0.24, 19.8)	Schnatter *et al* 2010
		1-<10		—	WBC: 1.92 (0.23, 15.7) RBC: 5.13 (0.66, 39.9) MCV: 5.91 (0.75, 46.5) PLT: 1.76 (0.2., 15.2)	
		≥10		+	WBC: 4.07 (0.51, 32.4) RBC: 16.0 (2.11, 121) MCV: 17.1 (2.35, 134.1) PLT 4.54 (0.56, 36.7)	
Controls	50 (76% M)	0.02±0.09			WBC (10^3/mm³) : 8.32±2.37 EOS (10^3/mm³) : 0.10±0.12 BAS (10^3/mm³) : 0.03±0.07 RBC (10^6/mm³) : 4.96±0.45 MCV (mm³) : 83.56±9.67	Pesatori *et al* 2009
Petrochemical workers, Bulgaria	106 (85% M)	0.3±0.2		—	WBC (10^3/mm³) : 8.15±1.88 EOS (10^3/mm³) : 0.08±0.14 BAS (10^3/mm³) : 0.06±0.10 RBC (10^6/mm³) : 5.06±0.50 MCV (mm³) : 85.70±7.72	
	47 (92% M)	4.9±5.3		—	WBC (10^3/mm³) : 8.33±1.92 EOS (10^3/mm³) : 0.06±0.13 BAS (10^3/mm³) : 0.08±0.13 RBC (10^6/mm³) : 5.07±0.51 MCV (mm³) : 86.9±7.20	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	Result	Findings	Reference
Controls	50 (84% SM)				BAS : 0.34 (0.82) EOS : 1.22 (1.40) MONO : 1 (1.68) LYM : 41.2 (9.29) NEU : 55.3 (8.90)	Seow *et al* 2012
Petrochemical workers, Bulgaria	158 (89% SM)	0.46 (0.19-23.9 ppm			BAS : 0.78 (1.29) p<0.006 EOS : 0.84 (1.49) MONO : 1.35 (1.47) LYM : 42.1 (9.44) NEU : 53.8 (9.46)	
Workers exposed to gasoline						
Controls	28	0.013 (0.010-0.016)	0.042 (0.034-0.052))			Moro *et al* 2015
Gasoline station attendants (M), Brazil	60	0.044 (0.018-0.680)	0.144 (0.058-2.207)	+	↓RBC ($10^6/mm^3$): 4.9±0.1 vs 5.2±0.1 ↓Hb (g/dL): 14.6±0.1 vs 15.1±0.1 ↑NEU (%): 57.4±0.9 vs 52.0±1.5	
Controls (M)	20	0.013 (0.010-0.015)	0.043 (0.033-0.050)			Moro *et al* 2017
Gasoline station attendants (M), Brazil	20	0.043 (0.021-0.68)	0.139 (0.068-2.207))	+	↓RBC ($10^6/mm^3$): 4.9±0.1 vs 5.2±0.1 ↓Hb (g/dL): 14.5±0.3 vs 15.1±0.1 ↑NEU (%): 56.7±1.8 vs 51.3±1.7	
Controls (F)	20	0.014 (0.013-0.014)	0.045 (0.043-0.047)			
Gasoline station attendants (F), Brazil	20	0.038 (0.02-0.2)	0.124 (0.064-0.670)	+	↑WBC ($10^3/mm^3$): 8.3±0.4 vs 6.5±0.3 ↓LYM (%):31.5±1.7 vs 37.0±1.9 ↓EOS (%): 1.3±0.2 vs 3.2±0.6	
Workers exposed to engine emissions						
Controls	36	0.001±0.0003	0.004±0.001			Maffei *et al* 2005
Traffic policemen (M+F; 36% SM), Italy	49	0.0075±0.0044	0.024±0.014	–	No effects on Hb, Hct, PLT, WBC, LYM, NEU	
Traffic policemen (69% M; 25% SM), Italy	112	0.005±0.003	0.017±0.010	+	↓LEU, ↓NEU, ↓LYM (correlation with blood benzene); due to missing control and insufficinet control for smoking, study not	Casale *et al* 2016

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	Result	Findings	Reference
Police drivers (43% M; 38% SM)	69	0.003±0.002	0.010±0.007		suitable to investigate haematological effects at environmental benzene concentrations	
Police motorcyclists (100% M; 22% SM)	9	-	-			
Policemen with other outdoor activities (72% M; 40% SM)	26	-	-			

Abbreviations:↓: reduced; ↑: increased; +: positive; −: negative; BAS: basophiles; BZ: benzene; F: female; GRA: granulocytes; Hb: haemoglobin; HCT: haematocrit; LEU: leucocytes; LYM: lymphocytes; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; M: male; MCHC: mean corpuscular haemoglobin concentration; MONO: monocytes in WBC; MPV: mean platelet volume, NEU: neutrophils; NS: non-smoker; PLT: platelet count; PDW: platelet volume distribution width; P-LCR: platelet large cell ratio; RCB: red blood cell count; SM: smoker; WBC: white blood cell count; *: statistically significant $p \leq 0.05$; **: statistically significant $p \leq 0.01$

Table 39: Genotoxic effects in shoe factory workers

Type of workers	N	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Shoe and glue factories in Tianjin, China									
Controls	51 (31% SM)	0.004±0.003						CA: 1.78±2.1 Aneuploidy: 3.0±2.7	Qu *et al* 2003a
Workers	16 (0% SM)	0.14±0.004					+	Result not reliable; inappropriate controls	
	73 (36% SM)	2.26±1.35					+	CA: 2.99±2.1 Aneuploidy: 7.3±7.2	
	33 (55% SM)	8.67±2.44						CA: 2.74±2.0 Aneuploidy: 8.1±9.5	
	8 (0% SM)	19.9±3.1						CA: 2.14±2.1 Aneuploidy: 5.8±5.1	
	19 (38% SM)	51.8±43.3						CA: 3.69±2.5 Aneuploidy: 3.9±3.5	
Controls (M, 73% SM)	33	<LOD		0.1±1.8 µg/L urine		(not measured)			Xing *et al* 2010
Workers (M, 76% SM)	17	1.0±2.6		4.2±2.5 µg/L urine		1.9±1.9 mg/L	+	Aneuploidy in sperm (concentration dependent)	
	16	7.6±2.3		50±3.1 µg/L urine		16.1±1.6 mg/L	+		
Controls (M, 73% SM)	33	<LOD		0.1±1.8 µg/L urine		(not measured)			Ji *et al* 2012
Workers (M, 76% SM)	17	1.0±2.6		4.2±2.5 µg/L urine		1.9±1.9 mg/L	+	Aneuploidy (gain of chromosome 21 in PBL, gain of sex chromosomes in sperms)	
	16	7.6±2.3		50±3.1 µg/L urine		16.1±1.6 mg/L	+		

Type of workers	N	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Controls (M)	11 (SM, NS)	<LOD		0.1±1.3 µg/L urine		(not measured)			Marchetti *et al* 2012
Workers (M)	10 (SM, NS)	1.0±2.6		2.8±1.7 µg/L urine		1.7±1.6 mg/L	+	sperm Struct. aberrations: IRR 1.42 (95% CI: 1.10, 1.83) ; 1p36.3 deletions : IRR 4.31 (95% CI: 1.18, 15.78)	
	10 (SM, NS)	3.0±3.4		11.6±1.6 µg/L urine		5.4±3.1 mg/L	+	Struct. aberrations: IRR 1.44 (95% CI: 1.12, 1.85) ; 1p36.3 deletions : IRR 6.02 (95% CI: 1.69, 21.39)	
	10 (SM, NS)	7.6±2.2		102.4±2.1 µg/L urine		15.6±1.8 mg/L	+	Struct. aberrations: IRR 1.75 (95% CI: 1.36, 2.24) ; 1p36.3 deletions : IRR 7.88 (95% CI: 2.21, 28.05)	
Controls (12M, 15 F)	27 (37% SM)	0.035							Zhang *et al* 2011
Workers	22 (9% SM)	4.95±3.61					(+)	Aneuploidy in PBL (only for trisomy 10)	
	25 (28% SM)	28.3±20.1					+	Aneuploidy in PBL (monosomy and trisomy)	

Type of workers	N	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Controls	14 (21% SM)	0.04		0.25±0.61 µg/L urine					Zhang *et al* 2012
Workers	18 (11% SM)	2.64±2.70		66.39±138.5 µg/L urine			+	Chromosomal loss (chromosomes 7 and 8) in PBL	
	10 (50% SM)	24.19±10.6		897.7±874.6 µg/L urine			+		
Shoe factories in Wenzhou, China									
Controls	197 (10% SM)							MN: 1.92±1.44	Zhang *et al* 2014
Workers	385 (24% SM)	2.0 (0.8-18)	6.4 (2.6-57.0)				+		
	24	<1	<3.25				+	↑MN: 3.29±1.40	
	149	<1.8	<6.00				+	↑MN: 3.11±1.92	
	212	≥1.8	≥6.00				+	↑MN: 3.45±1.91	
Controls	94 (0% SM)	0						MN: 1.81±1.13	Zhang *et al* 2016
Workers in Wenzhou shoe factories, China	317 (12% SM)	1.60 (0.8-12.09)					+		
	65	3.55 ppm-y						MN: 2.63±1.76**	
	65	6.51 ppm-y						MN: 2.95±1.59**	
	65	10.72 ppm-y						MN: 3.09±2.07**	
	65	20.02 ppm-y						MN: 3.35±1.99**	
	65	40.71 ppm-y						MN: 3.91±2.04**	

Type of workers	N	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Other shoe factories, China									
Controls	30					0.14±0.00		MN: 2.64±1.67 ‰ ,	Liu *et al* 1996
Shoe factory workers, China	35	0.75±0.73	2.46±2.42			0.19±0.01		MN: 3.98±1.77 ‰	
Paint workers	24	31.7±15.5	103±50			13.00±172		MN: 7.89±1.28 ‰	
Shoe factory workers	28	131±56	424±181			59.5±1.85		MN: 8.15±1.45 ‰	

Abbreviations: CA: chromosomal aberration, F: female; M: male; MN: micronucleus; NS: non-smoker; PBL: peripheral blood lymphocytes; SM: smoker

Table 40: Genotoxic effects in industrial workers (other than shoe factories)

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Controls	37								Testa *et al* 2005
Car-painters	25	3.1±5.4	9.99±17.6				+	↑ CA in PBL	
Controls	42								Major *et al* 1994
Benzene destillers, Hungary	42	2.2 (0.3-15)	7 (3-20)					↑CA in PBL ↑SCE in PBL White blood cell count and haematocrit not changed	
Benzene plant, Estonia		1.25; 0.83; 1.13	4.06; 2.71; 3.67				–	MN (numerical abnormalities of chromosome 9) / PBL (38 exposed workers; 71% smokers/ 13 controls; 31%smoker) and buccal cells (18 exposed workers; 50% smokers/ 15 controls; 33% smokers)	Surrallés *et al* 1997
Coke oven plant, Estonia		0.34; 0.04	1.09; 0.13						
Controls	8 (63% SM)			11.9 (<5-34) nmol/L blood	1.2 (0.3-8.6)	0.9 (<5-5.4) µmol/L		↑CA (1 & 9) in PBL only in benzene factory workers for which exposure up to 8.8 ppm is reported	Marcon *et al* 1999
Cokery workers, Estonia	5 (40% SM)	0.308 (0.154-0.524)	1.0 (0.5-1.7)	51.4 (37-83) nmol/L blood	8.5 (2.1-28.9)	3.5 (<0.5-8.6) µmol/L	–		
Benzene factory workers, Estonia	12 (75% SM)	0.400 (0.030-8.80)	1.3 (0.1-28.6)	41.2 (12-358) nmol/L blood	7.9 (0.3-221)	2.9 (<0.5-155) µmol/L	+		
Controls	31							MN, nucleoplasmic bridge, nuclear budding / PBL	Jamebozorgi *et al* 2016
Petrochem. workers, Iran	47	<1					–		

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Controls	76 (38% SM)							↑CA in PBL (more smokers and heavy smokers in exposed group)	Kim *et al* 2004
Coke oven plant workers, Korea	82 (49% SM)	0.56 (0.01-0.74)	1.8 (0.0-2.4)				+		
Controls	33								Kim et al 2008
Petroleum refinery workers, Korea	108	0.51 (0.004-4.25)					+	↑MN and CA in PBL	
Controls	10 (30% SM)	–						↑MN in PBL, aneuploidy chromosomes 7 and 9	Kim *et al* 2010
Workers exposed directly to benzene, Korea	30 (40% SM)	0.51					+		
	18	<1.5 ppm-year					+		
	12	≥1.5 ppm-year					+		
Controls	93 (30% SM)							MN: 4.3	Yang *et al* 2012
Workers from Anhui Province, China	219 (50% SM)	<0.17	<0.6				+	↑MN increased depending on WBC count: normal WBC: 17.1 unstable WBC: 23.6 Low WBC: 29.2	
Controls (M)	34 NS								Basso *et al* 2011
	16 SM								
	46 NM						–		

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m^3)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Petroleum refinery workers (M), Italy	33 SM	0.029±0.034 (<0.01-0.25)	0.093±0.11 (<0.001-0.81)				–	No effect on MN (CBMN assay without FISH) /PBL	
Controls	130								Sha *et al* 2014
Decorators	132	0.009	0.03				–	No effects on MN (CBMN assay without FISH) /PBL; since respiratory masks were used, the result is not suitable for a quantitative evaluation of the dose-response	
Painters	129	0.06 (0.04-0.10)	0.21 (0.12-0.32)				–		
Workers in five factories, Korea (56 M, 5 F)	61 (54% SM)	0.268±0.216 (0.005-2.03)				1.02±0.45 (0.24–2.77) mg/g creat	+	↑DNA damage (Comet) in PBL correlated with ttMA excretion and benzene concentration	Sul *et al* 2005
-Printing	4 (25% SM)							1.41±0.41	
-Shoe-making	7 (14% SM)							1.34±0.53	
-Nitro-benzene	9 (44% SM)	up to 2						1.82±1.10	
-MDA	18 (55% SM)							1.19±0.29	
-Carbomer	17 (65% SM)	up to 2						2.05±0.54	
-BTX production	6 (50% SM)							1.98±0.29	
Controls (office workers)	100 NS	<0.003	<0.01		0.68 (0.14-2.26)				Li *et al* 2017

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Workers in petrochemical plant, China	96 NS	0.034 (0.003-0.27)	0.11 (0.01-0.89)		1.76 (0.33-8.65)		+	↑ DNA damage (Comet), no sign. difference in blood cell counts	
Controls (M)	26 NS	0.002 (0.001-0.00.003)	0.005 (0.002-0.011)		1.9 (0.3-9.6)	79 (3-460) µg/g creat			Fracasso *et al* 2010
	25 SM	0.002 (0.001-0.005)	0.008 (0.004–0.016)		2.30 (0.50–10.08)	88.60 (13.30–445.00)			
Petrochemical industry operators (M), Italy	15 NS	0.01 (0.001-0.18)	0.033 (0.002-0.594)		8.7 (0.5-13.2)	108 (49-380) µg/g creat	+	↑ DNA damage (Comet) in PBL	
	18 SM	0.007 (0.002-0.148)	0.023 (0.006–0.482)		8.60 (0.40–35.60)	139.00 (56.00–422.00) µg/g creat	+		

Abbreviations: CA: chromosomal aberration, F: female; M: male; MN: micronucleus; NS: non-smoker; PBL: peripheral blood lymphocytes; SM: smoker

Table 41: Genotoxic effects in workers exposed to gasoline

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Finding	Reference
EUROPE									
Controls	24								Carere *et al* 1995
Workers exposed to petroleum fuels, Italy	23	0.46±0.14	1.5±0.7				–	No effect on MN in PBL; slight (p=0.066) increase in CA in PBL	
Control	12			158±39 µg/g crea.		82.5±20.2			Carere *et al* 1998
Fuel filling station attendants, Italy	12	0.1±0.01	0.32±0.03	518±85 µg/g creat.		116.3±25.6	–	No effects on CBMN /FISH in PBL	
Controls	43								Pitarque *et al* 1996
Fuel filling station attendants, Spain	50	0.28±0.04	0.91±0.14				–	No effect on MN in PBL	
Controls	19								Bukvic *et al* 1998
Fuel filling station attendants, Italy	21	0.72					–	No effect on MN in PBL	
Controls (M)	26 NS	0.001 (0-0.003)	0.005 (0.002-0.011)		1.9 (0.3-9.6)	79 (3-460) µg/g creat			Fracasso *et al* 2010
	25 SM	0.002 (0.001-0.005)	0.008 (0.004–0.016)		2.30 (0.50–10.08)	88.60 (13.30–445.00) µg/g creat			
	11 NS				5.1 (1.6-7.2)		–	No CA in PBL	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Finding	Reference
Service station attendants (M), Italy		0.019 (0.004-0.08)	0.063 (0.012-0.260)			103 (30-418) µg/g creat	+	DNA damage (comet assay) in PBL	
	13 SM	0.009 (0.002-0.021)	0.029 (0.008–0.068)		7.20 (3.88–15.00)	127.00 (42.00–256.00)	–	No CA in PBL	
							+	DNA damage (comet assay) in PBL	
Gasoline pump maintenance workers (M), Italy	12 NS	0.025 (0.002-0.051)	0.080 (0.008-0.165)		2.0 (0.2-10.5)	109 (13-242) µg/g creat	–	No DNA damage (comet assay) in PBL	
	9 SM	0.002 (0.001-0.159)	0.009 (0.002–0.515)		1.05 (0.62–6.76)	65.90 (20.20–225.00)	+	DNA damage (comet assay) in PBL	
Controls (M)	31 (52% SM)	0.001±0.001	0.004±0.002	1.23±2.63 µg/L urine	0.65±1.00	93±132 µg/g creat	–	CA: 3.7±1.9 MN: 7.3±2.7	Lovreglio *et al* 2014
Fuel filling station attendants (M), Italy	24 (50% SM)	0.007±0.005	0.023±0.017	0.60±0.72 µg/L urine	0.77±0.76	85±33 µg/g creat	–	CA: 4.0±1.9 MN: 8.0±0.3	
Fuel tank driver (M), Italy	19 (58% SM)	0.1±0.1	0.306±0.266	2.96±3.00 µg/L urine	2.94±3.24	134±94 µg/g creat	–	CA: 3.3±1.4 MN: 8.6±2.7	
Controls (M)	20 (45% SM)	0.001	0.005±0.003	0.54±0.99 µg/L urine	0.39±0.49	92±156 µg/g creat		No effect on DNA damage (comet assay) and DNA repair capacity	Lovreglio *et al* 2016
Fuel filling station attendants (M), Italy	13 (54% SM)	0.006	0.020±0.016	0.73±0.88 µg/L urine	0.65±0.58	86±38 µg/g creat	–		

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Finding	Reference
Fuel tank driver (M), Italy	17 (59% SM)	0.086±0.077	0.280±0.249	3.04±3.08 µg/L urine	3.07±3.3	124±95 µg/g creat	–		
Workers exposed to JP-8 jet fuel, USA (39% SM)	139	0.001±0.002	0.004±0.006				–	No DNA damage (Comet) after 4 h shift (but before shift)	Krieg *et al* 2012
	38	0.042±0.12	0.137±0.400				–		
	115	0.33±0.45	0.875±1.480				–		
Controls (M)	22 NS	0.013 (0.010-0.016)	0.042 (0.034-0.052)			74 (47-121) µg/g creat			Moro *et al* 2013
Gasoline station attendants (M), Brazil	43 NS	0.023 (0.015-0.396)	0.076 (0.050-1.285)			326 (189-454) µg/g creat	+	↑ MN in buccal cells, ↑ DNA damage (comet) in whole blood, oxidative damage	
Controls (M)	22					117±439 µg/g creat			Göethel *et al* 2014
Gasoline station attendants (M), Brazil	43					439.8±97 µg/g creat	+	No sign. effect on MN but DNA damage (comet) in whole blood	
Taxi drivers (M), Brazil	34					Not analyzed	+		
Control (M)	20 (0% SM)	0.013 (0.001-0.015)	0.043 (0.033-0.050)			149 (104-236) µg/L			Moro *et al* 2017
Gasoline station attendants (M), Brazil	20 (40% SM)	0.043 (0.021-0.680)	0.139 (0.068-2.207)			509 (344-658) µg/L	+	Correlation between ttMA and: ↑ DNA damage (comet), ↑ MN in buccal cells	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Finding	Reference
Control (F)	20 (0% SM)	0.014 (0.013-0.014)	0.045 (0.043-0.047)			189 (77-335) µg/L			
Gasoline station attendants (F), Brazil	20 (10% SM)	0.038 (0.020-0.206)	0.124 (0.064-0.670)			538 (165-993) µg/L	+	Correlation between ttMA and: ↑ DNA damage (comet), ↑ MN in bucal cells	
Controls		0.005-0.01		2.82±1.45 ppb in blood					Pandey *et al* 2008
Fuel filling station attendants, India		0.1-0.25		7.94±1.45 ppb in blood				↑ DNA damage (comet), ↑ MN in PBL	
Controls	200 (50% SM)	0.04 (0.037-0.053)	0.133 (0.120-0.173)	2.12 (1.01-4.00) ppb in blood					Rekhadevi *et al* 2010
Fuel filling station attendants, India	200 (54% SM)	0.40 (0.33-0.46)	1.322 (1.137-1.494)	5.18 (3.01-8.34) ppb in blood			+	↑Comet, MN in PBL	
Controls	200			0.6 µg/L urine	4.98 µg/L	40.90 µg/L		CA /PBL (%): 2.39 MN /buccal cells (‰): 2.36	Rekhadevi *et al* 2011
Service station attendants, India	200	0.34-<0.40		8.89±1.41 µg/L	6.71±0.74 µg/L	122.85±4.31 µg/L	+	CA /PBL (%):3.48±0.91* MN /buccal cells (‰): 2.74±0.44*	
		0.40-<0.43		11.74±0.54 µg/L	8.32±0.28 µg/L	142.58±7.74 µg/L		CA /PBL (%):6.86±0.34* MN /buccal cells (‰): 4.00±0.03*	
		0.43-0.46		13.44±0.54 µg/L	9.40±0.41 µg/L	171.23±9.28 µg/L		CA /PBL (%):8.03±0.18* MN /buccal cells (‰): 5.16±0.37*	

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Finding	Reference
Controls	52 (31% SM)							MN / buccal cells; DNA damage in whole blood, oxidative stress	Xiong *et al* 2016
Refueling workers, China	200 (29% SM)	0.018	0.059				+		

Abbreviations: CA: chromosomal aberration, F: female; M: male; MN: micronucleus; NS: non-smoker; PBL: peripheral blood lymphocytes; SM: smoker

Table 42: Genotoxic effects in workers exposed to engine emissions

Type of workers	n	Benzene in air (ppm)±SD (range)	Benzene in air (mg/m³)±SD (range)	Benzene in blood or urine	SPMA in urine (µg/g creat)	ttMA	Result	Findings	Reference
Chemical laboratory workers (M, F)	47 (SM, NS)							MN: 5.76±3.11	Violante *et al* 2003
Traffic wardens (M, F), Italy	15 (SM, NS)	0.004±0.003	0.014±0.010	0.66±0.99 µg/L urine)		106.9±123.17 mg/ L urine	–	No effect on MN; MN: 4.70±2.63	
Office workers (41 M, 17 F)	58 (SM, NS)	0.001	0.004					MN frequency was mainly modulated by age and gender, not by chemical or smoking	Leopardi *et al* 2003
Traffic wardens (100 M, 34 F), Italy	134 (SM, NS)	0.003	0.010				–		
Indoor workers (15 F, 21 M)	36 (36% SM)	0.001±0.0003	0.004±0.001					MN: 4.83±1.84	Maffei *et al* 2005
traffic policemen (20 F, 29 M), Italy	49 (35% SM)	0.008±0.004	0.024±0.014				+	MN: 7.06±2.87 ↑ MN frequency (increasing with years of employment); no effect haematological parameters	
City employees (11 F, 29 M), Italy	40 (38% SM)	0.001 (0.001-0.002)	0.003 (0.001–0.008		0.15 (0.15-0.34			↑ MN frequency (adjusted for age and sex)	Angelini *et al* 2011, 2012
Traffic policemen (31 F, 39 M), Italy	70 (29% SM)	0.006 (0.004-0.019)	0.019 (0.013–0.031		0.38 (0.25–0.70)		+		

Abbreviations: CA: chromosomal aberration, F: female; M: male; MN: micronucleus; NS: non-smoker; PBL: peripheral blood lymphocytes; SM: smoker

Table 43: Summary of the most relevant cohort studies and nested case-control studies therein assessing the association between occupational exposure to benzene and risk of leukaemia or its subtypes.

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Pliofilm (Rinsky 1981)	748 rubber workers	Estimation based on work histories and air sampling data	Leukaemia mortality	Estimated range 35 -100 ppm	7	SMR 5.6
Pliofilm uodate (Rinsky 1987)	1165 white male rubber workers	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 (0.4-12) 12 (1.3-43) 66 (13-190) 3.4 (1.5-6.4)
Pliofilm update (Wong 1995)	1165 white male rubber workers	Estimation based on work histories and air sampling data	AML mortality	ppm-years 0-40 40-199 200-399 ≥400 Total	 1 0 2 3 6	SMR 1.2 (0.0-0.6) 0 (0.0-14.8) 27 (3.3-98) 98 (20-290) 5.0 (1.8-11)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Pliofilm update and reassessment (Paxton 1996)	1212 male rubber workers	Three different exposure estimates: original Rinsky estimates, Crump and Allen estimates assuming reduced benzene levels over time and Paustenbach estimates normalised to a 40hr week and including inhalation and dermal routes	Leukaemia mortality	ppm-years		SMR
				Rinsky		
				0-5	3	2.0 (0.4-5.8)
				>5-50	3	2.3 (0.5-6.7)
				>50-500	7	6.9 (2.8-14)
				>500	1	20 (0.5-110)
				Crump		
				0-5	1	0.9 (0.02-4.9)
				>5-50	4	3.3 (0.9-8.3)
				>50-500	6	4.9 (1.8-11)
				>500	3	10 (2.1-30)
				Paustenbach		
				0-5	1	1.3 (0.03-7.4)
				>5-50	2	1.8 (0.2-6.5)
				>50-500	4	2.8 (0.8-7.2)
				>500	7	12 (4.8-24)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Pliofilm update (Crump 1996)	See Paxton	Exposure estimates of Paustenbach (1992) applied to follow-up until 1987	AML and al leukaemia mortality	ppm-years		SMR
				AML		
				0-45	0-2	0.0 – 2.4
				45-400	1	2.0
				400-1000	2	9.1
				>1000	5	83
				Total	8-10	5.0 – 6.2
				All leukaemia		
				0-45	3	1.2
				45-400	4	2.7
				400-1000	2	3.1
				>1000	5	28
				Total	14	2.9
Pliofilm update (Rinsky 2002)	1291 exposed and 554 unexposed male and female rubber workers. In this table the results are shown for men only.	Estimation based on work histories and air sampling data	Leukaemia mortality	ppm-years		SMR
				1 ppm-day – 40	6	1.5 (0.5-3.3)
				40-200	4	3.2 (0.9-8.9)
				200-400	2	5.6 (0.6-24)
				≥400	3	24 (4.8-79)
				Total	15	2.6 (1.4-4.2)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Pliofilm update (Silver 2002)	See Rinsky 2002	See Rinsky 2002	Leukaemia mortality	See Rinsky 2002. Results not shown by exposure category	1 in 1950 and 15 in 1996	SMR from 33 (0.8-180) in 1950 to 2.5 (1.4-4.1) in 1996.
Pliofilm update (Richardson 2008)	See Rinsky 2002	Estimation based on work histories and air sampling data	Leukaemia mortality	ppm-years <1 1-<50 50-<250 250-500 ≥500 Cumulative exposure Time since expsoure <10 years 10 to 20 years ≥20 years	 5 3 4 4 1	RR 1.0 reference 0.8 (0.2-3.2) 2.5 (0.6-10.2) 11 (2.3-47) 14 (0.7-120) RR at 10 ppm-years 1.05 (1.02 – 1.08) 1.19 (1.10 – 1.29) 1.05 (0.97 – 1.13) 1.00 (0.90 – 1.05) p for trend 0.001

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Pliofilm update Rhomberg 2016	1696 white male rubber workers	Estimated based on employment history and Monte Carlo techniques	AML and ANLL mortality	ppm-years Quartiles		SMR
				ANLL		
				<2.33	0	0.0 (0.0 – 6.0)
				2.33 – 10.66	0	0.0 (0.0 – 6.0)
				10.67 – 52.75	0	0.0 (0.0 – 5.2)
				>52.76	8	9.5 (4.1 – 19)
				AML		
				<2.33	0	0.0 (0.0 – 7.1)
				2.33 – 10.66	0	0.0 (0.0 – 7.1)
				10.67 – 52.75	0	0.0 (0.0 – 6.2)
				>52.76	6	8.4 (3.1 – 18)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
NCI/CAPM (Hayes 1997	74828 exposed and 35805 unexposed male and female workers employed from 1972 through 1987 in 672 factories in 12 Chinese cities	Estimation based on work histories and benzene measurements	Incidence	ppm-years		RR
			Leukaemia			
				<40	11	1.9 (0.8-4.7)
				40-99	8	3.1 (1.2-8.0)
				≥100	19	2.7 (1.2-6.0)
						p for trend 0.04
			ANLL			
				<40	5	1.9 (0.5-7.0)
				40-99	5	4.3 (1.1-16)
				≥100	11	3.6 (1.1-12)
						p for trend 0.06
			ANLL/MDS			
				<40	7	2.7 (0.8-9.5)
				40-99	7	6.0 (1.8-21)
				≥100	14	4.4 (1.4-14)
						p for trend 0.01

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
NCI/CAPM update (Linet 2015)	73789 exposed and 34504 unexposed male and female workers employed from 1972 through 1987 in 672 factories in 12 Chinese cities	See Hayes 1997. Results by exposure group not provided in this update	Incidence	See Hayes 1997, results not shown by exposure category		RR
			Leukaemia		60	2.5 (1.4-4.9)
			AML		26	2.1 (0.9-5.2)
			CML		13	2.5 (0.8-11)
			ALL		8	5.4 (1.0-99)
			CLL		2	∞ (0.3–∞)
			MDS		8	∞ (1.9–∞)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
UK Petrol (Lewis 1997 and Rushton 1997)	Nested case-control study in a cohort of 23306 distribution workers in petroleum distribution industry.	Based on work history records	Incidence	ppm-years		OR
			Leukaemia	<0.45	22	1.0 reference
				0.45-4.49	47	1.4 (0.8-2.6)
				4.5-44.9	20	2.5 (0.7-3.0)
				≥45	1	1.4 (0.1-13)
			CLL	<0.45	8	1.0 reference
				0.45-4.49	16	1.1 (0.4-2.9)
				4.5-44.9	7	1.2 (0.4-3.9)
				≥45	0	0
			AML	<0.45	7	1.0 reference
				0.45-4.49	15	2.2 (0.8-6.1)
				4.5-44.9	9	2.8 (0.8-9.4)
				≥45	0	0
Australian Health Watch (Glass 2000,2003,2005)	Nested case-control study in a cohort of 17525 Australian petroleum industry workers.	Estimation based on work history and benzene measurements	Leukaemia incidence	ppm-years		OR
				≤1	3	1.0 reference
				>1 – 2	6	3.9 (0.9–17)
				>2 – 4	8	6.1 (1.4-26)
				>4 – 8	3	2.4 (0.4-14)
				>8 – 16	6	5.9 (1.3-27)
				≥16	7	98 (8.8-1100)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Canadian cohort (Schnatter 1996)	Nested case-control study in a cohort of 6672 Canadian petroleum marketing and distribution workers	Estimated based on work histories and historical industrial hygiene surveys	Leukaemia mortality	ppm-years		OR
				0 – 0.45	10	1.0 reference
				>0.45 – 4.5	1	0.4 (0.01-4.1)
				>4.5 – 45	1	0.2 (0.0-1.3)
				>45	2	1.5 (0.2-13)

Pooled analysis (Schnatter 2012, Rushton 2014, Glass *et al* 2014)	Updated nested case-control study of UK Petrol, AHW and Canadian cohorts pooled	Estimated using historical monitoring data	Incidence	ppm-years		OR
			AML	<0.348	20	1.0 reference
				0.348-2.93	19	1.0 (0.5-2.2)
				≥2.93	21	1.4 (0.7-2.9)
			MDS	<0.348	6	1.0 reference
				0.348-2.93	8	1.7 (0.6-5.5)
				≥2.93	15	4.3 (1.3-14)
			CLL	<0.348	24	1.0 reference
				0.348-2.93	32	1.5 (0.8-2.8)
				≥2.93	24	1.1 (0.6-2.0)
			CML	<0.348	4	1.0 reference
				0.348-2.93	16	5.0 (1.5-18)
				≥2.93	8	2.2 (0.6-7.7)
			MPD	<0.348	8	1.0 reference
				0.348-2.93	10	1.3 (0.5 – 3.5)
				≥2.93	12	1.8 (0.7 – 4.7)
			Exposure window 2-20 years before diagnosis			
			CML			
				0	4	1.0 reference
				>0 – 0.143	5	8.2 (0.8 – 86)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
				>0.143 – 1.23	13	32 (2.6 – 390)
				>1.23	6	13 (1.1 – 150)
						p for trend 0.16
			MPD	0	10	1.0 reference
				>0 – 0.143	3	1.0 (0.2 –4.6)
				>0.143 – 1.23	7	2.8 (0.7 – 11)
				>1.23	10	4.2 (1.0 – 19)
						p for trend 0.025
Offshore cohort 1 (Kirkeleit 2008)	27919 Norwegian offshore workers registered from 1981 to 2003 and 366 114 referents from the general population	Estimated	Incidence	ppm		RR
				0.001 – 0.69 ppm		
			AML		6	2.9 (1.3 – 6.7)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Offshore cohort 2 (Stenehjem 2015)	Nested case-control study in a cohort of 24 917 Norwegian male men reporting offshore work between 1965 and 1999	Estimated based on a job time –exposure matrix	Incidence	ppm-years		HR
			AML	Unexposed	2	1.0 reference
				>0.001- 0.037	2	1.4 (0.2-11)
				>0.037 – 0.123	1	0.9 (0.1 – 9.3)
				0.124 – 0.948	5	4.9 (0.9 – 27)
						p for trend 0.052
				never	2	1.0 reference
				ever	8	2.2 (0.5 – 10)
			CLL			
				Unexposed	1	1.0 reference
				>0.001- 0.037	4	6.2 (0.7 – 54)
				>0.037 – 0.123	2	3.1 (0.3 – 34)
				0.124 – 0.948	5	6.7 (0.8 – 60)
						p for trend 0.212
				never	1	1.0 reference
				ever	11	5.4 (0.7 – 41)
Dow Chemical (Ott 1978)	workers in chlorobenzol alkylbenzol and ethylcellulose production	Estimated based on industrial hygiene measurements and work histories	Leukaemia mortality	ppm 2-9	1	1 case of leukaemia vs. 0.9 expected, no SMR calculated

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Dow Chemical update (Bond 1986)	594 workers in chlorobenzol alkylbenzol and ethylcellulose production	See Ott 1978	Leukaemia mortality	ppm-months		SMR
				0-500	2	1.7 (no CI calculated)
				500-1000	0	0
				≥1000	1	2.5 (no CI calculated)
				Total	4	1.9 (0.5-4.9)
Dow Chemical update (Bloemen 2004)	2266 workers in chlorobenzol alkylbenzol and ethylcellulose production	See Ott 1978	Mortality	ppm-years		SMR
			Leukaemia and aleukaemia	<28.3	4	0.6 (0.2-1.5)
				28.3-79.1	4	2.0 (0.5-5.1)
				≥79.1	4	2.2 (0.6-5.5)
				Total	12	1.1 (0.6-2.0)
			AML	<28.3	2	0.9 (0.1-3.1)
				28.3-79.1	1	1.5 (0.04-8.2)
				≥79.1	1	1.6 (0.04-9.0)
				Total	4	1.1 (0.3-2.8)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Dow Chemical update (Colllins 2015)	2266 workers in chlorobenzol alkylbenzol and ethylcellulose production	See Ott 1978	Mortality	Ppm-years		SMR
			Leukaemia	0 – 3.9	3	0.6 (0.1 – 1.8)
				4.0 – 24.9	7	1.2 (0.5 – 2.5)
				≥25	10	1.7 (0.9 – 3.2)
			ANLL	0 – 3.9	0	0.0 (0.0 – 2.4)
				4.0 – 24.9	3	1.8 (0.4 – 5.2)
				≥25	2	1.3 (0.2 – 4.7)
			MDS			
				0 – 3.9	0	0.0 (0.0 – 72)
				4.0 – 24.9	0	0.0 (0.0 – 65)
				≥25	1	25 (0.6 – 140)

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Chemical Manufacturers Association (CMA) (Wong 1987)	4602 exposed male workers in US chemical industry and 3074 unexposed males from same company in same period	Estimation based on work history and benzene measurements	Mortality	ppm-months		RR (no CI)
			All lymphatic and haematopoietic cancer	non-exposed	3	1.0 reference
				<180	5	2.1
				180-719	5	3.0
				≥720	5	3.9
						p for trend 0.02
			Leukaemia and aleukaemia	non-exposed	0	Undefined
				<180	2	p for trend 0.01
				180-719	1	
				≥720	3	

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
Solutia/Monsanto plant of CMA cohort (Collins *et al* 2003)	4417 male and female chemical industry workers	Estimation based on work history and benzene measurements	Mortality	ppm-years		SMR
			Leukaemia	<1	2	0.7 (0.1–2.5)
				1-6	4	1.4 (0.4–3.6)
				>6	6	1.7 (0.6-3.8
			ANLL	<1	1	1.4 (0.1-5.1)
				1-6	2	2.7 (0.3-9.9)
				>6	2	2.2 (0.3-8.1)
			CLL	<1	1	1.6 (0.0-8.9)
				1-6	0	0.0 (0.0.5.9)
				>6	1	1.3 (0.0.5.9)
Caprolactam workers (Swaen 2004)	311 men exposed to benzene solvent in caprolactam (Nylon 6 monomer) production in 1951-68.	Estimation based on work history and benzene measurements	Mortality			
			Leukaemia	Total average 159 ppm-years	1	0.86 (0.01 – 4.3)
				Average 3.4	0	0
				Average 68.8	1	2.4 (3.2 – 1200)
				Average 401.5	0	0

Cohort (Reference)	Cohort description	Exposure assessment	Haematological malignancy	Exposure TWA [a]	No of cases/ deaths	Relative risk (95% CI)
EDF-GDF cohort (Guénel 2002)	Nested case-control study in a cohort of 170 000 employed by EDF-GDF	Estimated based on a job-exposure matrix	Leukaemia incidence	ppm-years 0 >0-<1.1 1.1-<5.5 5.5-<16.8 ≥16.8	 48 6 7 5 6	OR 1.0 reference 0.7 (0.3-1.7) 1.4 (0.6-3.5) 1.9 (0.6-5.9) 3.6 (1.1-12) p for trend 0.02
Shoe factory (Seniori Constantini 2003)	1687 male and female workers exposed in shoe factory work compared to general population death rates	Estimated 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 (0.5-15) 2.5 (0.3-9.1) 5.1 (1.4-13) p for trend 0.02

Appendix 2. Lower Olefins and Aromatics REACH Consortium (LOA) Proposal for an Alternative Cancer Risk Extrapolation for Benzene

1.1 Background

Recently, LOA provided suggested cancer risk values for benzene when commenting on RAC's initial background document on an occupational exposure limit values for benzene. The initial RAC document had used references from AGS (2012), Khalade et al 2010 and Vlaanderen to suggest that the risk of benzene is 6.7 x 10(-3) per ppm over a lifetime. The revised opinion only changed this value to a perceived upper estimate, but had no practical effect on the initial opinion. RAC did not acknowledge alternative calculations provided by LOA, thus we present here a more detailed description of those calculations so that they may be more seriously considered in the next revision.

1.2 Calculation of the occupational cancer risk values

A survival analysis, also called 'life-table' analysis, was used to estimate the LOA cancer risk values. Survival analysis is a statistical methodology to describe mortality or survival rates in populations during a specified time. By comparing mortality rates between an exposed population and a non-exposed population, the number of extra deaths that corresponds to a certain exposure level can be estimated. The method enables the calculation of the numbers of leukaemia deaths that occur in specified intervals of time (usually one year intervals) when there is no exposure and the excess deaths resulting from exposure. By summing these excess deaths over the lifetime of a cohort, the excess risk can be calculated. This 'number of extra deaths' serves as a point of departure to estimate cancer risk values. The approach has been used by various bodies in Europe including SCOEL (Zocchetti et al, 2004) and the Health Council of the Netherlands (2013).

The approach will give different risk values depending on (1) the epidemiological dataset used, (2) the model applied to estimate an exposure-response relationship, (3) the life tables applied, and (4) the age to which mortality is analysed. In addition, the type of leukaemia modelled is important. In this case, all leukaemias are used because of the availability of relevant models although acute myeloid leukaemia would be preferable.

The following principles and assumptions were applied:

1. The life-table was derived from the Eurostat database for the EU 28 countries for the years 2008-10. This life-table had already been constructed for a previous project and more up to date rates are now available. However, use of the more recent mortality rates is unlikely to have had a large impact on the risk estimates. The probability of dying during each year of life was determined for all ages up to age 84 in the absence of exposure, and life-tables were constructed for males, females and the whole population.
2. The Eurostat database was also used to obtain mortality rates for leukaemia and all causes of death for 5-year age groups up to age 85 years and for the age group of 85 years and over.
3. For occupational exposure to benzene, it was assumed that exposure of the cohort starts at the age of 20, and lasts until the age of 60 years. Every year, the cohort reduces in size, through death as a result of the cause of death under study and other causes. The cohort was followed until all members had died.
4. Relative rates (RR) for leukaemia were taken from Richardson et al (2008) and used to calculate the extra leukaemia deaths per year due to benzene exposure at each age. Richardson (2008) assessed temporal variation in the impact of benzene on leukaemia rates via exposure time windows using Cox proportional hazards regression models. The study found that a model with three exposure time windows

(< 10 years, 10 to < 20 years, and ≥ 20 years prior) provided a substantially better fit to these data from the Pliofilm study than a simple lifetime cumulative exposure model. The largest magnitude of association was observed for cumulative benzene exposure accrued in the period < 10 years prior to death or withdrawal from follow-up (in other words the most recent 10 years) (RR 1.19 per 10 ppm-years; 95% CI 1.10–1.29), whereas cumulative benzene exposure received 10 to < 20 years prior to death or withdrawal exhibited a smaller, positive association with leukaemia (RR 1.05 per 10 ppm-years; 95% CI 0.97–1.13), and benzene exposures received ≥ 20 years prior to death or withdrawal showed no association with leukaemia. If X_{10} is cumulative benzene exposure accrued in the most recent10 years and $X_{10\text{-}20}$ is cumulative benzene exposure accrued 10 to < 20 years previously, then the overall RR is calculated as:

$$\log RR = (\log(1.19) * X_{10} + \log(1.05) * X_{10\text{-}20})/10.$$

For a worker exposed for 40 years from the age of 20, the model of Richardson et al (2008) predicts that the RR for 1 ppm exposure rises to a peak of 1.25 between the ages of 40 and 60 years, and then falls back to 1.0 by age 80.

Note that this calculation assumes that there is a causal risk due to benzene exposure not only for the most recent 10 years prior to death or withdrawal from follow up, but also from 10 -20 years prior, even though the confidence interval for this latter period includes unity (i.e. the risk is not statistically significant). Thus, the calculation using both the <10 year and 10 - <20 year periods can be considered a conservative estimate which may over-predict excess leukemia risk.

Excess leukaemia deaths for women and men were averaged, so that the calculations describe the average risk for the population. Slightly different answers result from using the lifetable for the whole population, because the proportions of surviving men and women at different ages will differ from that of the averaged life-tables of men and women.

In addition, numbers of excess incident cases of leukaemia were calculated to enable comparisons to be made with the excess risk estimates derived by AGS. The GLOBOCAN 2012 database was used to obtain cancer incidence rates for the EU 28 countries. However, these were only available for the age bands 0-14, 15-39, 40-44, 45-49, …., 70-74, and 75+ years. GLOBOCAN 2012 reported that the cumulative risk of developing leukaemia between the ages of 0 and 74 is 0.7% (both sexes), compared to a cumulative risk of dying from leukaemia between the same ages of 0.3%. The incidence calculation was terminated at age 85 because the lifetable did not have the probability of dying at each age beyond 85, and hence it was not possible to calculate the numbers of incident cases for each year of life after the age of 85. However, there was no increased risk of developing leukaemia after the age of 80, so this is inconsequential for the estimate of excess leukemia risk due to benzene exposure.

The Table shows the excess risk of leukaemia mortality at different exposure levels. The excess risk of leukaemia incidence is also shown for comparison with the AGS risk estimates, although the RRs of Richardson (2008) were obtained from modelling leukaemia deaths.

Table 44: Excess risk of leukaemia mortality and incidence at different exposure levels

	Exposure level			
	0.1 ppm	0.2 ppm	0.5 ppm	1 ppm
Excess risk of leukaemia mortality over whole life	0.35×10^{-4}	0.70×10^{-4}	1.79×10^{-4}	3.73×10^{-4}
Excess risk of developing leukaemia by age 80	0.77×10^{-4}	1.56×10^{-4}	4.01×10^{-4}	8.38×10^{-4}
RAC Draft Opinion November 2017 (Based on AGS, 2012)	6.7×10^{-4}	(1.34×10^{-3})	(3.35×10^{-3})	6.7×10^{-3}

The excess risk estimates are likely overestimates as Williams and Paustenbach (2004) concluded that the benzene exposure estimates used in the study by Richardson (2008) under-predicted benzene exposures for most Pliofilm jobs.

1.3 References to Appendix 2

AGS [Ausschuss für Gefahrstoffe] (2012) Begründung zu Benzol in BekGS 910, Ausgabe: November 2012, Stand: Mai 2012.

Health Council of the Netherlands (2013) 1,3-Butadiene; Health-based calculated occupational cancer risk values. The Hague: Health Council of the Netherlands, publication no. 2013/08.

Khalade A, Jaakkola MS, Pukkala E, Jaakkola JK (2010) Exposure to benzene at work and the risk of leukaemia: a systematic review and meta-analysis. Environ Health 9: 31. doi: 10.1186/1476-069X-9-31.

Richardson DB (2008) Temporal variation in the association between benzene and leukemia mortality. Environ Health Perspect 116(3): 370-374.

Williams PR, Paustenbach DJ (2003) Reconstruction of benzene exposure for the Pliofilm cohort (1936-1976) using Monte Carlo techniques. J Toxicol Environ Health A 66: 677-781.

Vlaanderen J, Portengen L, Rothman N, Lan Q, Kromhout H, Vermeulen R (2010) Flexible meta-regression to assess the shape of the benzene-leukaemia exposure-response curve. Environ Health Perspect 118: 526-532.

Zocchetti C, Pesatori AC, Bertazzi PA (2004) A simple method for risk assessment and its application to 1,3-butadiene. Med Lav 95: 392-409.