Committee for Risk Assessment

RAC

Opinion on scientific evaluation of occupational exposure limits for

Benzene

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Adopted

9 March 2018

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON THE EVALUATION OF THE OCCUPATIONAL EXPOSURE LIMITS (OELs) FOR BENZENE

Commission request

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 the Committee for Risk Assessment (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.

I PROCESS FOR ADOPTION OF THE OPINION

Following the above request from the European Commission, the Executive Director of ECHA in the mandate of 12 May 2017[3], requested RAC to draw up an opinion on the evaluation of the scientific relevance of occupational exposure limits (OELs) for benzene with a deadline of 26 March 2018.

Chemical name(s): **Benzene**

EC No.: 200-753-7 (Benzene)

CAS No.: 71-43-2 (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[4] on **10 October 2017** and interested parties were invited to submit commentsby **7 November 2017**.

RAC developed its opinion on the basis of the proposal submitted by ECHA. During the preparation of the RAC opinion, the ECHA proposal was further developed as a Background

[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/10162/13641/rac_mandate_for_oels_for_nickel_en.pdf/647788e7-24d2-ff4f-93a0-7d87fdfae28a

[4].https://echa.europa.eu/echas-executive-director-requests-to-the-committees-previous-consultations

Document (BD) to ensure alignment. In addition, stakeholders were able to provide comments on the RAC opinion during the evaluation process.

The RAC opinion includes a recommendation to the Advisory Committee on Safety and Health at Work (ACSH) in line with the relevant Occupational Safety and Health legislative procedures and in the format used by SCOEL.

II ADOPTION OF THE OPINION OF THE RAC

Rapporteurs, appointed by RAC: **Ruth Moeller** and **Bert-Ove Lund.**

The RAC opinion was adopted by **consensus** on **09 March 2018.**

Assessment of the Scientific Relevance of OELs for benzene

RECOMMENDATION

The opinion of RAC for the assessment of the scientific relevance of Occupational Exposure Limits (OELs) for benzene is set out in the table below and in the following summary of the evaluation.

SUMMARY TABLE

The table summarises the outcome of the RAC evaluation to derive limit values for the inhalation route and the evaluation of the need for a skin notation to protect against dermal exposure.

Derived Limit Values[5]

OEL as 8-hour TWA[6]:	0.05 ppm (0.16 mg/m^3)[7]
STEL	not established
BLV:	0.7 µg benzene/L urine 2 µg S-phenylmercapturic acid (SPMA)/g creatinine (sampling: end of exposure or end of working shift)
BGV:	0.3 µg benzene/L urine 0.5 µg S-phenylmercapturic acid (SPMA)/g creatinine

Carcinogenicity Classification/Categorisation

CLP Harmonised classification for carcinogenicity	Carc 1A; H350 (May cause cancer)
SCOEL Categorisation of carcinogens[8]	Not assigned by SCOEL[9]

Notations

Notations:	'Skin'

[5] The naming conventions of limit values and notations used here follow the 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL 2013; version 7) and the Joint ECHA/RAC – SCOEL Task Force (2017b). [https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf/db8a9a3a-4aa7-601b-bb53-81a5eef93145].

[6] The OEL is based on genotoxicity in workers, specifically: chromosomal damage (aneugenicity and clastogenicity).

[7] To facilitate comparison with the SCOEL (1991) opinion and the current Binding OEL on benzene, ppm was maintained as the leading unit.

[8] See Appendix 1 of the ECHA BD for details on the "SCOEL classification of carcinogens".

[9] In 1991, when SCOEL evaluated benzene, the scheme was not yet in place.

RAC OPINION

Background

This evaluation takes into account previous reviews by international scientific expert bodies and recent scientific literature focussing on human data and the mode of action of carcinogenicity of benzene, in particular:

- the SCOEL Recommendation (SCOEL, 1991),
- the evaluation by the German Committee for hazardous substances (AGS, 2012),
- the evaluation of the U.S. *Agency for Toxic Substances and Disease Registry* (ATSDR, 2007 and 2015),
- the Dutch Council's Expert Committee on Occupational Safety (DECOS, 2014),
- the International Agency on Cancer Research (IARC 2012),
- the French Agency for Food, Environment and Occupational Health and Safety (ANSES, 2014),
- Concawe (1999, 2002, 2006, 2012),
- EU Risk Assessment Report (2008).

However, in addition to these international reviews, the Background Document prepared by ECHA extensively reviewed primary literature from the last ten years and earlier in critical areas such as genotoxicity and haematotoxicity which were subsequently taken into account by RAC in its evaluation. Account has also been taken of the comments provided by interested parties during the public consultation.

Key conclusions of the evaluation

- A mode-of-action-based threshold for chromosomal damage (aneugenicity and clastogenicity) in workers can, in the view of RAC, be used to establish an OEL for carcinogenicity;

- The limit so derived, will avoid exposures that induce chromosomal damage in workers, is considered to have no significant residual cancer risk and will also avoid other adverse effects;

- The leading genotoxic effects, aneugenicity and clastogenicity, are considered to be of secondary nature, i.e. acting indirectly and to follow a non-linear threshold-mechanism. Various studies show induction of adverse chromosomal damage in benzene-exposed workers from different working environments. Primary DNA reactivity of benzene and/or its metabolites seems of little importance. Both benzene and its various reactive metabolites have been shown to exhibit genotoxicity *in vitro* and/or *in vivo* in animals; benzene has a harmonised classification as Muta 1B (H340) according to the Classification, Labelling and Packaging Regulation (EC) 1272/2008 (CLP);

- An extensive human database is available and epidemiological studies of populations occupationally exposed to benzene consistently demonstrate an excess leukaemia cancer risk, in particular for acute myeloid leukaemia (AML), a disease of the bone marrow progenitor cells. Benzene has a harmonised classification as Carc. 1A (H350) according to CLP. The carcinogenic potential is supported by animal data;

- The metabolism of benzene is inherently complex. The first step is the oxidation to benzene oxide by cytochrome P-450, mainly CYP2E1, then via several pathways numerous reactive and toxic metabolites and also reactive oxygen species (ROS) are formed. It can be assumed that benzene will also be metabolised directly in the bone marrow target organ to toxic metabolites with accompanied redox cycling and reactive radical formation;

- The major and most sensitive target organs of benzene are the bone marrow and the haematological system. Benzene affects virtually all peripheral blood cell types, as seen by haematological suppression in workers and experimental animals, due to bone marrow toxicity. An OEL based on chromosomal damage will also avoid exposure causing haematological suppression;

- Benzene can be measured in the air at very low concentrations using standardised methods. Considering a substantial dermal uptake of benzene, air measurements can be complemented with urinary measurements of either benzene as such or the metabolite S-phenylmercapturic acid with sampling at the end of exposure or the end of working shift[10].

- Absorption via the dermal route could make a substantial contribution to total body burden, and thus a skin notation is warranted.

Carcinogenicity and mode of action

Benzene is a human carcinogen based on epidemiological data, providing clear evidence for a causal association between exposure to benzene and acute myeloid leukaemia (AML)/ acute non-lymphocytic leukaemia. There is also evidence of an association between benzene exposure and the pre-leukemic stage Myelodysplastic Syndromes (MDS) and a positive association has been observed between exposure to benzene and acute lymphocytic leukaemia, chronic lymphocytic leukaemia, and multiple myeloma (IARC 2012).

The metabolism of benzene results in the generation of numerous reactive and toxic metabolites, including phenol, hydroquinones, benzoquinones, catechol, benzenetriol, and muconaldehyde, as well as in the generation of reactive oxygen species (ROS). The metabolism of benzene is an important determinant for its toxicity: some of these metabolic processes are mediated via enzymes located in bone marrow cells, where production of semiquinone radicals and benzoquinone via myeloperoxidases accompanied by oxygen radical formation through redox cycling is suggested as a key step in the carcinogenicity of benzene. The main and most sensitive target organs for both carcinogenicity and repeated dose toxicity are the bone marrow and the haematological system. The mechanism(s) of benzene toxicity seem to be a multi-factorial and complex process, not yet fully understood; several modes of action (MoA) are possible, and they could also act synergistically:

- Benzene is metabolised to various reactive (and genotoxic) metabolites that are thought to lead to the effects described below;

[10] Smoking status (smoking history and exposure through passive smoking) has to be considered, due to an average internal background concentration for smokers of about 0.8 to 2 µg benzene/L urine.

- reactive oxygene species (ROS) are formed in the bone marrow, which might contribute to transformation of cells and genetic damage (Hartwig 2010, McHale *et al.* 2012);
- a strong role of genetic damage in leukaemia is recognised, benzene and/or its metabolites cause chromosomal aberrations in peripheral blood lymphocytes of chronically exposed workers. Even low exposure levels around 1 ppm are associated with aneuploidy and micronuclei in occupationally exposed workers, and in highly exposed workers have been associated with higher levels of chromosomal changes commonly observed in AML (McHale *et al.* 2012, ECHA BD table 21);
- error prone DNA repair has been suggested to be involved in leukemic transformation of cells based on mechanistic studies (Hartwig 2010);
- recent evidence suggests that benzene can also cause epigenetic alterations and affect the bone marrow niche regulation, but the mechanisms and implications of those alterations deserve further investigations (McHale *et al.* 2012);
- immunosuppression presents a risk factor for cancer, and immunological alterations including humoral and cellular suppression have been reported for benzene-exposed workers (Minciullo *et al.* 2014[11]) and might play a role in clonal evolution of leukemic cells due to escape from immunosurveillance;
- haematotoxicity/haematological suppression, specific bone marrow toxicity is suggested by the range of cell types and lineages affected (ECHA BD table 18);
- additional factors discussed include changes in genexpression and disease relevant biochemical pathways (McHale *et al.* 2011[12]), general cytotoxicity triggering regenerative cell proliferation, apoptosis, and AhR receptor-mediated effects on gene transcription and cellular proliferation/cell cycle progression (Hirabayashi *et al*. 2010).

At least some of these effects are likely to occur in humans at low exposure levels (≤ 1 ppm), in particular genotoxicity (clastogenicity and aneugenicity), oxidative damage, immunotoxicity, altered gene expression, and receptor-mediated effects. Haematotoxicity is expressed as haematological suppression affecting virtually all blood cell types in benzene exposed workers, likely due to effects on progenitor and/or stem cells. Haematotoxicity has been suggested to play a role in benzene leukemia since persistent cytopenias and other blood disorders frequently precede the onset of leukaemia in patients developing AML secondary to exposure to benzene or alkylating agents.

RAC notes that the scientific evidence is still lacking which would allow the conclusion that haematotoxicity is the causal triggering event in benzene-associated leukaemia. It is challenging to connect the carcinogenicity of benzene to one specific MoA. However, genotoxicity in the haematological system is likely to precede haematotoxicity and carcinogenicity. 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*". RAC considers that an exposure limit protecting against the

[11] Minciullo *et al.,* 2014: Cytokine network involvement in subjects exposed to benzene. J Immunol Res. 2014: 937987.

[12] McHale *et al.,* 2011: Global gene expression profiling of a population exposed to a range of benzene levels. Environ Health Perspect. 2011, 119(5); 628-640.

leading genotoxic effects of benzene, i.e. chromosomal aberrations, which will also avoid exposure causing haematological suppression and other adverse effects, can be considered to be of no significant residual cancer risk.

There is evidence that benzene induces micronuclei, chromosomal aberrations, aneuploidy, sister chromatid exchange, and DNA strand breaks *in vitro*, in experimental animals, and in humans (Whysner *et al.* 2004, ECHA BD tables 21-25). However, the leading genotoxic effects are considered to be clastogenicity and aneugenicity. Indirect oxidative DNA damage induced by benzene-related redox cycling and ROS formation also seem to play an important role (McHale *et al.* 2012) and can be related to various lesions including DNA base modifications, single and double strand breaks, DNA-protein crosslinks or abasic sites. Double strand breaks, e.g. caused by ROS, appear important in benzene toxicity as their repair may be highly error prone (Hartwig 2010). In the past, also a role for topoisomerase II inhibition in chromosomal damage was discussed (DECOS 2014, Whysner *et al.* 2004).

Benzene and its metabolites have been shown to be mutagenic *in vitro* and the induction of gene mutations seems to be possible also *in vivo* in animals, however, this is of low magnitude. Only negligible binding of benzene to DNA was shown in 32P-postlabeling studies at carcinogenic exposure levels in rats, also no concordance of target organs between DNA-binding studies and the comparable carcinogenicity bioassays was demonstrated (see review by Whysner *et al.* 2004). No benzene-oxide DNA adducts in mice or humans have been found (Zarth *et al.* 2014[13]). Two indirect mechanisms known to cause DNA mutations however, oxidative stress and error-prone DNA repair, are associated with benzene exposure (McHale *et al.* 2012). Overall, it is indicated that benzene is only weakly effective in directly inducing DNA mutations and a significant role of adduct formation in benzene leukemia is unlikely (see also DECOS, 2014).

Human studies have investigated, in particular, frequencies of micronuclei (MN) and chromosomal aberrations in peripheral lymphocytes of benzene exposed workers (ECHA BD table 21). However, the bone marrow stem cells might show a higher sensitivity to the genotoxic insult. In animals, a recent NTP-NIEHS study (French *et al.* 2015) investigated MN induction in male Diversity Outbred mice (4-week inhalation exposure, 6 hrs/day, 5 days/week) to 1-100 ppm benzene. In peripheral blood reticulocytes, MN frequency was statistically significantly increased post-exposure in the 100 ppm groups, while in bone marrow derived reticulocytes MN frequency increased dose-dependently with increasing exposure to 1, 10, and 100 ppm benzene. MN in immature reticulocytes are indicative of bone marrow exposure and the different sensitivity of bone marrow-derived and peripheral reticulocytes may be due to the fact that peripheral blood contains different generations of erythrocytes and that some of them may have been subject to apoptosis. An older study (Erexson *et al.* 1986) reports MN induction in bone marrow polychromatic erythrocytes at 1 ppm benzene inhalation exposure for 6 hours in Sprague-Dawley rats. Benzene leukemia is a disease of the bone marrow progenitor cells.

In the view of RAC, 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

[13] Zarth et al., 2014: Analysis of the benzene oxide-DNA adducts 7-phenylguanine by liquid chromatography-nanoelectrospray ionization-high resolution tandem mass spectrometry-parallel reaction monitoring: application to DNA from exposed mice and humans. Chem Biol Interact. 2014; 215: 40-45.

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

Cancer Risk Assessment and Derived Limit Values

Benzene has often been characterised as a genotoxic carcinogen for which fully protective, health based limit values cannot be derived. However, RAC is of the opinion that a mode-of-action (MoA-) based threshold can be established, based on the weight of evidence of the large volume of human data from the workplace.

This threshold is based on the leading mutagenicity effects of benzene, i.e. aneugenicity and clastogenicity, which are considered likely to be early lesions decisively contributing to cancer and critical trigger events in benzene leukaemia. Reliable genotoxicity data / endpoints associated with human disease should be given most weight when conducting a risk assessment, and these include data on clastogenicity and aneugenicity (MacGregor *et al.* 2015[14]). The occurrence of micronuclei in lymphocytes is a biomarker of a genotoxic event and is often seen in cancer as a manifestation of chromosomal instability (Bonassi *et al.* 2011[15]).

These leading genotoxic effects of benzene are unlikely to result from primary DNA reactivity of the compound or its metabolites. Non-DNA reactive agents that disrupt the mitotic machinery, such as aneugens, are commonly assumed to follow a non-linear, i.e. a threshold mechanism (MacGregor *et al.* 2015; EFSA, 2005[16]). Antioxidant defence mechanisms protect at low exposure levels and, thus, a non-linear mode of oxidative DNA damage can equally be assumed. Thresholds therefore are likely to exist, but are presumably low and difficult to identify.

Chromosomal damage is reported for benzene-exposed workers with LOAECs estimated for peripheral blood lymphocytes from concentrations of about 1 ppm (ECHA BD table 21; Xing *et al.* 2010, Ji *et al.* 2012, Marchetti *et al.* 2012, Qu *et al.* 2003a, Zhang *et al.* 2011, 2012, 2014, 2016, Testa *et al.* 2005, Major *et al.* 1994). Some reports also suggest clastogenic and aneugenic effects below 1 ppm, the most relevant studies showing effects at concentrations of around 0.5 ppm in petroleum refinery workers (Kim *et al.* 2008, 2010). Further studies in the range of 0.1 to < 1 ppm show borderline or no effects but have shortcomings in particular due to the limited number of subjects which could hamper a (clear) detection of benzene-related effects above the mutational background (ECHA BD table 21, Carere *et al.* 1995, 1998, Lovregio *et al.* 2014, Pitarque *et al.* 1996). In the range below 0.1 ppm, no relevant effects are reported in the more reliable studies reviewed (ECHA BD table 21, Bukvic *et al.* 1998, Fracasso *et al.* 2010, Basso *et al.* 2011, Sha *et al.*

[14] IWGT, 2015: IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk. Mutation Research 783 (2015) 66-78.

[15] Bonassi et al.: 2011: Micronuclei in lymphocytes is a biomarker of a genotoxic event and manifestation of chromosomal instability often seen in cancer. Mutagenesis. 2011 Jan; 26(1):93-100.

[16] EFSA, 2005: Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. The EFSA Journal (2005) 282, 1-31.

2014), suggesting a NOAEC for chromosomal damage in peripheral lymphocytes in the range of 0.1 ppm. However, as the LOAEC is considered more reliable in this case, RAC has chosen to estimate a NOAEC for genotoxicity based on extrapolation from the LOAEC.

Considering a weight-of-evidence based estimated human LOAEC of 1 ppm for chromosomal damage in peripheral lymphocytes of workers, acknowledging an animal LOAEC of 1 ppm for increased frequency of micronuclei in mouse bone marrow reticulocytes and rat bone marrow polychromatic erythrocytes (Erexson *et al.* 1985, French *et al.* 2015), and using assessment factors (AF) following ECHA Guidance to account for uncertainties, **RAC has derived an OEL of 0.05 ppm for chromosomal damage in bone marrow**.

A reduced **AF of 2** is considered warranted for intraspecies variability due the uncertainties in the data base caused by the existence of some (less robust) studies suggesting human LOAECs below 1 ppm. In addition, the toxicokinetics of benzene are complex and there are several polymorphisms involved likely leading to variation in degree of toxicity (ECHA BD chapter 7.1.1).

A dose-response-related **AF of 10**, according to the ECHA guidance R.8., is considered appropriate by RAC for the following reasons:

1) a LOAEC to NOAEC extrapolation in peripheral lymphocytes with a default factor of 3, the remaining AF accounts for the following concerns:

2) the bone marrow cells may exhibit a higher sensitivity to the genetic insult, and measurements in peripheral blood cells likely underestimate the severity of the effects in the bone marrow (as shown by animal experiments), and

3) the adversity of the genetic effect as an early decisive lesion for cancer per se and the severity of the resulting leukaemia.

The MoA of genotoxic carcinogens includes irreversible steps such as the fixation of DNA lesions into permanent and heritable mutations. Benzene is a human genotoxic carcinogen and leukemia is a disease of the bone marrow. RAC considers an overall AF of 10 for dose-response and severity justified and proportionate.

As a comparison, other approaches were used by RAC to assess what levels alternative points of departure in the human data as well as the animal data would yield in terms of limits. If starting with a NOAEC (which would be less well supported by the data) of 0.1 ppm for chromosomal damage in human peripheral lymphocytes, this would still require an AF for extrapolation to bone marrow stem- and progenitor cells and to account for the severity of the effects. Thus the resulting OEL protective of the target organ bone marrow would be well below 0.1 ppm and below the proposed OEL of 0.05.

Alternatively, considering animal data on its own to select a point of departure, a LOAEC of 1 ppm in bone marrow derived reticulocytes (against a LOAEC of 100 ppm in peripheral cells) in male DO mice (French *et al.* 2015) 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 translate to a $BMDC_{(worker)10}$ of 0.1 ppm). Then, considering interspecies variability in toxicokinetics (DECOS 2014, ATSDR 2007) and toxicodynamics, an animal-derived OEL starting from effects in rodent bone marrow cells would again be well below 0.1 ppm and below the proposed OEL of 0.05.

Bone marrow toxicity of benzene manifests itself in haematological suppression with the reduction of one or more blood cell types in workers as evidenced by a variety of studies involving thousands of workers overall from different work environments. In a weight-of-evidence approach and taking into account the reviewed studies and their reliability, LOAECs for haematotoxicity in workers are in the range of 2 ppm and above[17], but not seen as the leading effect by RAC. The OEL of 0.05 ppm recommended by RAC based on chromosomal damage is also protective against haematotoxicity. 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).

Since the proposed limit value relies on a mode of action-based threshold for the leading genotoxic effects, which are the likely critical trigger events in benzene leukemia, some uncertainties may remain as to a residual cancer risk. Certainly, primary DNA reactivity of benzene or its reactive metabolites seems of little importance, but cannot be fully ruled out, thus it is difficult to definitively exclude some remaining risk at lower exposure levels. There is however, a remarkable consistency of published cancer risk estimates based on the higher exposure levels previously encountered in occupational settings, i.e. above 1 ppm. Considering, however, that multiple thresholded MoAs likely contribute to benzene leukaemia development and in view of the overall experimental and epidemiological evidence available supporting a genotoxic-threshold for benzene, the remaining uncertainties are considered to be very low. Given this evidence, estimated excess cancer risks as derived by linear extrapolation can be seen as overly conservative.

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. For the general population, the main sources of benzene exposure are vehicle exhaust and cigarette smoke. Directive 2008/50/EC (EU Parliament and Council Directive 2008) sets a limit value for the protection of human health of 5 µg benzene/m^3 (0.0015 ppm) to improve air quality in the EU. 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). Subsequently, benzene concentrations in urban areas decreased. In some urban areas, the limit value of 5 µg/m^3 (0.0015 ppm) might still be exceeded. At workplaces in Europe, the long-term average exposure to benzene is usually 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 tasks in the range of 0.05 to 0.1 ppm (such as in the petrochemical industry, fuel tank driving, R&D in laboratories) and in the range above 0.1 ppm (e.g. maintenance work in refineries, gasoline pump repair and maintenance, tank cleaning work in petroleum industry) (see ECHA BD table 5-7).

[17] (Pesatori *et al.* 2009, Lan *et al.* 2004, Qu *et al.* 2003a, Koh *et al.* 2015a, Zhang *et al.* 2016, Ye *et al.* 2015, Rothman *et al.* 1996, Schnatter *et al.* 2010), and a NOAEC in the range of 0.5 ppm seems relevant based on extrapolation with a dose-response-related AF of 3-4 (LOAEC to NOAEC), supported by a modelled BMD_5 of 0.43 ppm (Qu *et al.* 2003a, LOA 2017b) and health surveillance studies with similar NOAECs (Koh *et al.* 2015, Tsai *et al.* 2004)

In conclusion, RAC considers that an exposure limit value should not exceed 0.05 ppm (0.16 mg/m³) in order to avoid risk for chromosomal damage in workers. A MoA-based threshold of 0.05 ppm benzene is proposed which can be considered to be associated with no significant residual cancer risk and will also avoid other adverse effects.

STEL

Benzene causes effects in the central nervous system at high concentrations of 300-3000 ppm. Considering an OEL of 0.05 ppm, it is not expected that a concentration of 300 ppm will be reached under normal workplace conditions. Therefore, **no STEL is recommended by RAC.**

Biological Limit Values

In addition to setting an OEL, there might be a need for setting biological limit values. Considering the correlation as published by DFG (2017a, b), an OEL of 0.05 ppm (0.16 mg/m³) would correspond to a 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.

Biological Guidance Values

The DFG published the 95th percentiles for benzene and metabolites in the general population. Based on this information, the following Biological Guidance Values (BGV) are recommended by RAC, that can support interpretation of benzene biomonitoring data of occupational exposed workers:

- **0.3 µg benzene / L urine, and**
- **0.5 µg S-phenylmercapturic acid (SPMA) / g creatinine.**

Biological Monitoring

For assessing occupational exposure, in addition to the air exposure assessment data, biomonitoring may provide additional information including sources and pathways of exposure. In particular, the dermal route can be an important contributor to total benzene exposure in certain situations. The metabolism of benzene generates numerous metabolites, such as benzene oxide, benzene dihydrodiol, 1,4-hydroquinone, 1,2-hydroquinone (catechol), 1,2,4-benzene triol, *trans,trans*-muconaldehyde, *trans,trans*-muconic acid, and S-phenylmercapturic acid. Many of these have half-lives too short to be used for monitoring, but benzene as such, S-phenylmercapturic acid, and *t*,*t*-muconic acid can be reliably measured in urine. However, for low exposure to benzene (< 1 ppm), benzene and S-phenylmercapturic acid in urine seem to be the most reliable biomarkers.

Benzene in urine is a suitable biomonitoring parameter for which sensitive analytical methods are available (ECHA BD table 12). S-phenylmercapturic acid (SPMA) in urine is

also a suitable biomonitoring parameter for which sensitive analytical methods are available (ECHA BD table 13). However, for reliable results that can be correlated with benzene exposure in the air, acid hydrolysis of the urine sample and a detection with appropriate chromatographic methods like LC/MS/MS are required. BLV corresponding to the proposed OEL of 0.05 ppm and BGV as reference for benzene and SMPA in urine are recommended by RAC (see above).

A critical point for the measurement of benzene is its short half-life and its high volatility. Hence, sampling of urine is recommended at the end of exposure or end of shift (DFG, 2017[18]). Appropriate sampling and storage of urine samples is required (samples should be kept cold and hermetically sealed). 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 (see ECHA BD chapter 6.1 and 6.2).

The German DFG (2017a,b) published a correlation between benzene concentrations in air and urine, and benzene in urine is concluded to be a suitable biomarker for monitoring exposure as low as 0.03 ppm benzene in the air and above. The 95th percentile for benzene in urine for the general population was determined to be 0.3 µg/L in a metropolitan area (Campagna *et al.* 2014). Arnold *et al.* (2013) reported urinary benzene levels for non-smoking general population of 0.10 to 0.25 µg/L. An OEL of 0.05 ppm corresponds to a urinary concentration of approximately 0.7 µg/L, which is above the general population (non-smoking) background. Due to increased urinary benzene concentration in the range of 0.2 to 0.80 µg/L due to smoking, smoking status (smoking history and whether individuals are exposed to passive smoke), needs to be considered.

Urinary *trans,trans*-Muconic acid (ttMA) is not recommended anymore for benzene biomonitoring because it is not sensitive enough at low exposure levels.

Air Monitoring

For the measurement of benzene in the air well established methods are available that detect benzene at concentrations well below 0.01 ppm and down to 0.0006 ppm (0.002 mg/m^3) (ECHA BD table 11). **Thus, at the proposed limit value, no measurement difficulties are foreseen.**

Notations

The dermal route can be an important contributor to total benzene exposure in certain situations, such as immersion of the skin in a solution or when the airborne concentration of benzene is very low, this is suggested even for products with contamination levels of less than 0.1% benzene (i.e. the labelling concentration limit) (Kalnas *et al.* 2000, Williams *et al.* 2011).

Based on the experimental skin absorption data for benzene the steady state absorption rate range has been estimated to be 200-400 µg/cm^2*h (Williams *et al.* 2011). In relation to an OEL of 0.05 ppm, this rate exceeds by far the critical absorption value (CAV) of 0.08 µg/cm^2*h (ECETOC 1998). Jakasa *et al.* (2015) calculated the dermal uptake with 5.85% at an OEL of 1 ppm (3.2 mg/m^3).

[18] DFG, 2017: List of MAK and BAT Values 2017: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 53

SCOEL (1991) suggested 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) and Annex III of Directive 2004/37/EC currently lists a 'skin notation'.

RAC therefore recommends to maintain the 'skin' notation for benzene.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is prepared by the European Chemicals Agency (ECHA).

Annex 2 Comments received on the ECHA proposal, response to comments provided by the ECHA Dossier Submitter and RAC (excluding confidential information).

Note (December 2018)

Please note that **a correction** was needed in the paragraph on Cancer Risk Assessment and Derived Limit Values (p.10):

Alternatively, considering animal data on its own to select a point of departure, a LOAEC of 1 ppm in bone marrow derived reticulocytes (against a LOAEC of 100 ppm in peripheral cells) in male DO mice (French *et al.* 2015) 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 translate to a $BMDC_{(worker)10}$ of 0.1 ppm). Then, considering interspecies variability in toxicokinetics (DECOS 2014, ATSDR 2007) and toxicodynamics, an animal-derived OEL starting from effects in rodent bone marrow cells would again be well below 0.1 ppm and below the proposed OEL of 0.05.