Recommendation from the Scientific Committee on Occupational Exposure Limits for benzene

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Occupational Exposure Limits for benzene

8 hour TWA	:	< 1.0 ppm (< 3.25 mg/m^3)
STEL(15min)	:	
Additional classification	:	"skin"

Substance   identification:

Benzene

Synonyms: Annulene, benzin, benzine, benzole, bicarburet of hydrogen,
carbon

oil, coal naphtha, cyclohexdatriene, fenzen, mineral naphtha, motor benzol, nitration benzene, phene, phenyl hydride, pyrobenzol, pyrobenzole.

EINECS N°:   200-753-7 (Benzene pure)

EEC N°:   601-020-00-8   Classification F; R 11; Carc. Cat. 1; R45
                                                         T; R 48/23/24/25

CAS N°:   71-43-7

MWt: 78.1

Conversion factor (20° C, 101kPa):   3.25 mg/m^3 = 1 ppm

1. Occurrence/use

Benzene is a highly volatile, colourless liquid with an aromatic odour. It forms a highly flammable and explosive mixture with air at 1.4 to 8.0% by volume. It has a MPt of 5.5°C, BPt of 80.1° C and vapour pressure of lOkPa at 20°C. The odour threshold in air is 1-12 ppm (3.2 - 39 mg/m^3) (Sandmeyer, 1981).

Benzene occurs in very low concentrations in the natural environment. However, it is a natural component of crude oil (up to 0.49%) and can be formed during heuting and incomplete combustion of organic material. It occurs widely as a constituent of refined and unrefined petroleum, of unrefined natural gas and of light oil recovered from coal carbonisation gases.

Benzene is one of the highest volume substances with a production volume of about 5 million tons in Western Europe. The main part of the produced benzene - about 90% -is derived from crude oil using processes such as catalytic reforming (20%), toluene hydrodealkylation (20%) and pyrolysis of naphtha and gas oil (50%). The main source in former times, coal carbonisation, now provides less than 10% of the benzene production.

The main uses of benzene are as constituent of petrol (up to 5% v/v) and as a raw material in the chemical industry for the production of ethylbenzene, styrene, cumene, cyclohexane, nitrobenzenes, alkylbenzenes, maleic anhydride and chlorobenzenes.

The main sources of exposure for the general public are smoking, passive smoking, auto-related activities and - to a lesser extent - emissions from industry and consumer products. Although the mean indoor level of < 4.6 ppb (15 µg/m^3) is about twice the mean outdoor level, considerable variations of outdoor levels (0.3 - 46 ppb; 1 - 150 µg/m^3) have been measured.

Occupational exposures and uses have been much reduced during recent decades and most of the published literature seems to support the view that the majority of personal exposure (probably around 90%) is below 1 ppm (3.25 rng/m^3). There are of course some reports of exposures greater than 1 ppm (3.25 mg/m^3) but usually from poorly controlled processes and practices or on a short-term basis to permit specific operations (IARC, 1982; WHO, 1987; BUA, 1988; CONCAWE, 1987; ATSDR, 1988; Wallace, 1989; BIA, 1991).

2. Health Significance

The most likely route of exposure to benzene is inhalation. Absorption through the lungs is rapid. The proportions absorbed are inversely related to the exposure concentration. The mean absorption in humans at exposure concentrations of 50 - 100 ppm (162.5 - 325 mg/m^3) is about 40 - 50% of the inhaled dose (ATSDR, 1988). Dermal absorption of liquid benzene has been shown in animals and humans, but is difficult to quantify because of conflicting experimental data. However, dermal exposure to liquid benzene may contribute significantly to the body burden at current workplace exposure concentrations (OSHA, 1987).

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After uptake, benzene is rapidly distributed into the different tissues, crosses the blood-brain, the placental and the gonadal barrier and can be found in different organs, bone marrow included, positively related to the fat content.

The metabolism of benzene shows qualitatively no substantial differences between humans and animals.

Quantitatively, the amounts of metabolites, the extent of metabolism and the nature of phenol conjugates depend on the species, strain, and doses applied. Benzene is oxidized by the cytochrome P-450-dependent mixed function oxidase system. The main metabolizing organ is the liver. However, the microsomal cytochromes P-450 are not confined to the liver, but are ubiquitous in all tissues, including the bone marrow.

The metabolism of benzene to phenol and the subsequent formaton of water-soluble phenyl conjugates (glucuronides, sulfates) is considered as the detoxification pathway.

Further oxidation of phenol to dihydroxybenzenes, their oxidation to quinones and the ring opening reaction to the reactive t,t-muconaldehyde is considered to be the toxic pathway (Yardley-Jones et al., 1991).

The pathways of detoxication of benzene in rodents are of low affinity and high capacity, whereas the pathways leading to the electrophilic, putative toxic metabolites, appear to be of high affinity and low capacity (Sabourin et al. 1989). This means that the relative amount of "toxic" metabolites increases when exposure levels are lowered. Whether this also applies to humans is presently not known.

Animal Toxicity

Benzene shows low acute toxicity via inhalation [LC_{50} in rodents (mice and rats): 10000 ppm (32500 mg/m^3)].

Repeated exposure (up to 14 days) of mice revealed haematotoxic effects at exposure levels as low as 10 - 20 ppm (32.5 - 65 mg/m^3) (LOAEL).
Most sensitive effects were on circulating lymphocytes (depression) and granulopoietic stem cells (Toft et al., 1982; Rozen et al., 1984; Cronkite et al., 1989). Longer-term repeated inhalation exposure (> 14d; > 90d) of rodents induced similar haematotoxic effects as seen with repeated short-term exposure but, in some experiments, at a somewhat higher exposure level. This may be interpreted as a positive reactive response of the haematopoietic system. However, a reduction of circulating cells (red blood cells, lymphocytes) together with a depression of progenitor cells (Baarson et al., 1984) and a significant altered response to phenylhydrazine induced anaemia (Dempster and Snyder, 1989) was reported when mice were exposed to 10 ppm (32.5 mg/m^3) benzene over 178 and 70 days. These results may be indicative of a LOAEL of 10 ppm (32.5 mg/m^3) with respect to the haematopoietic system in animals.

In contrast to former evaluations (Ashby, 1985) where benzene has been regarded as conclusively non-mutagenic in bacterial test systems, more recent investigations show that at least some metabolites of benzene (e.g. benzene-trans-1,2- dihydrodiol, t, t-

muconaldehyde) are able to induce point mutations in bacteria (Glatt et al. 1989, Glatt and Witz, 1990; Henschler, 1991). In addition, using appropriate modifications (e.g. closed systems, longer preincubation time) it is possible to show the indirect mutagenic property of benzene itself in the Ames-test (Glatt et al., 1989).

Together with the results of in vitro and in vivo direct DNA-binding studies using ^{3}H or ^{14}C-labelled benzene, there is growing evidence that benzene should be considered as a mutagen.

Extensive studies in vivo and in vitro have shown the clastogenic properties of benzene/benzene metabolites. The in vivo induction of sister chromatid exchange in bone marrow cells, as well as in peripheral blood lymphocytes, and the induction of micronuclei in bone marrow polychromatic erythrocytes and in peripheral normochromatic erythocytes in rats and mice, exposed to gaseous benzene, seem to be early and sensitive toxicological endpoints.

Although the direct relationship between sister-chromatid exchange (SCE) and micronucleus (MN) induction and obvious adverse effects, e.g. cancer, is not clear up to now, these effects are considered as adverse in their own right as they are the result of the direct interaction of benzene/benzene-metabolites with chromosomes at different stages of the cell cycle leading to abnormal cells and indicating a genetic activity.

The experiments of Tice et al. (1984) and Erexson et al. (1986) show the induction of chromosomal damage (SCE, MN) in rats and mice from benzene exposures as low as 1 -10 ppm (3.25 - 32,5 mg/m^3). This range is presently regarded as representative of the LOAEL for genotoxic effects in rats and mice.

Carcinogenic properties of benzene by inhalation exposure have been shown in a variety of studies on different strains of mice and on Sprague-Dawley rats (ATSDR, 1988). The main target organ was the Zymbal gland, but other carcinogenic-related endpoints were also described, including acute and chronic myelogenous leukemia. The lowest concentration tested was 100 ppm (325 mg/m^3) and this was effective in inducing neoplasia in several organs of rats and mice. However the design of the inhalation studies does not allow the construction of a reliable dose-cancer effect relationship. The most informative carcinogenicity study is the gavage study on B6C3F1 mice and F344/N rats performed within the National Toxicology Program (NTP, 1986) and reviewed by Huff (1989).

Groups of 60 male F344/N rats were administred 0, 50, 100, or 200 mg/kg benzene in corn oil by gavage, 5 days/week for 103 weeks. Groups of 60 female F344/N rats and 60 B6C3F1 mice of each sex were administered 0, 25, 50, 100 mg/kg also for 103 weeks. Most of the primary tumours found in rats (Zymbal gland, oral cavity, skin, uterus) were significantly increased relative to vehicle controls and showed at least a dose related trend (p < 0.05). As in rats, Zymbal gland tumours were also the most prominent tumours in mice. In addition, a dose-related, significant increase of bone marrow hyperplasia and malignant lymphoma was noted in mice. The lowest effective dose applied was 25 mg/kg and this induced a treatment-related statistically significant increase in tumours in B6C3F1 mice. No induction of tumours by dermal application is known.

Benzene induces tumours in both sexes of different strains of rats and mice on multiple sites by several routes of administration. The evidence for carcinogenicity of benzene to animals is regarded as "sufficient" (IARC, 1987).

On the basis of the NTP (1986) carcinogenicity studies on rats and mice, three risk estimations [using conventional (Crump and Allen, 1984) and more sophisticated pharmacokinetically based approaches (Bailer and Hoel, 1989. Beliles and Totman, 1989)] resulted in excess risks of 1-5 cases per 1000 exposed to an average benzene concentration of 1 ppm over 40 years. This indicates that the assumptions used and the methods applied did not influence the outcome of the risk estimates drastically, if the relevant exposure range was close to the doses applied. The results of the pharmacokinetically based assessments are more valid than the calculations from Crump and Allen. However, the inherent variance of the data bases used, and the upper confidence limits calculated (up to 17 cases/1000) do not enable a more accurate excess risk to be determined than the range given above (1-5).

Human Toxicity

The lethal human dose is approximately 10 g/person. The main target organ is the CNS. Sublethal CNS effects resulting from a single exposure are reversible. No acute systemic poisoning nor fatalities have been reported from dermal exposure to liquid benzene (Sandmeyer, 1981).

Exposure to 50 ppm (160 mg/m^3) may be regarded as a LOAEL (headache, lassitude, weariness) and 25 ppm (80 mg/m^3) as a NOAEL for acute effects in humans. No short-term (up to 14 days) toxicity data for humans could be identified. Experience from case reports shows that long term exposure induces similar effects in humans to those seen in animals. In general, severe haematotoxic and myelotoxic effects have been related to exposure levels of about 200 ppm (650 mg/m^3). Adverse effects are documented at exposures of 40 - 70 ppm (130 - 162.5 mg/m^3) (HSE, 1982).

More detailed studies (Townsend et al., 1978; Kipen et al., 1989) support the view that no haematopoietic effects can be seen in humans at prolonged exposure below 25 - 30 ppm (81 - 98 mg/m^3).

A cross-sectional study of haematological effects at chronic low-level exposure [< 0.1 ppm (0.32 mg/m^3)] over ten years did not show any differences to the non-exposed control group. Smoking was identified as the most important confounder with respect to WBC-counts (Collins et al., 1991).

In contrast to these negative findings, a shift to lower WBC-counts at a mean exposure concentration of about 0.6 ppm (1.8 mg/m^3) was recently shown in a preliminary report of an epidemiological study on the influence on blood cells of low level exposure to benzene (Van Damme, 1991).

Although this is a preliminary report, which has to be confirmed by further analysis, it must be noted that the internally consistent trend to higher (normal) WBC-counts demonstrated along the time scale of the continuously reduced exposure gives a good indication that the result is not due to chance. As the effect was a four fold increase in the frequency of abnormal WBC-counts below 4000 counts/µl, a LOAEL of 0.6 ppm (1.8 mg/m^3) must be taken into account if these results are confirmed. Aplastic anaemia, a non-malignant but severe haematologic disease, is frequently related to higher benzene

concentrations. However, some isolated cases show that death due to anaemia can also occur as a result of exposure levels as low as 5 ppm (16 mg/m³) (e.g. Bond et al., 1986).

At present it is not possible to establish a LOAEL or NOAEL for haematotoxic effects in humans, but there are indications that these levels will be below the already suggested NOAEL of 25 - 30 ppm (81 - 98 mg/m³) (BUA, 1988).

In humans benzene frequently induces numerical and structural chromosomal aberrations in bone marrow cells and peripheral lymphocytes following long-term exposure to high levels.

The results of studies on structural chromosomal aberrations in peripheral lymphocytes of workers exposed to less than 25 ppm (81 mg/m³) mainly below 10 ppm (32.5 mg/m³) are less clear. However, taking into account the size of the groups investigated, the statistical analysis performed, the levels of significance calculated, the size and the (restricted) quality of control groups used, the study of Killian and Daniel (1978; cited in OSHA, 1987), reanalyzed by Picciano (1979; cited in OSHA, 1987) is the most conclusive of the low-level studies. This study demonstrated a highly significant (p < 0.001) increase of "double damage" (breaks + rings, dicentrics, translocations, exchange figures) in peripheral lymphocytes of 53 workers exposed to benzene levels < 10 ppm [32 mg/m³]. The demonstration of a linear exposure-response relationship of this "double damage" in the range of 1 - 10 ppm (3.2 - 32 mg/m³) shows not only the quantitative relationship, but indicates the qualitative reliability of the study by making uncontrolled high long-term exposures before the study period less probable and showing that short peak-exposures might not influence this relationship drastically. Together with the study of Yardley-Jones (1990) and the less reliable studies indicating chromosomal damage in peripheral human lymphocytes at exposure levels at or below 10 ppm (32 mg/m³), a LOAEL in the range of 1 - 10 ppm (3.2 - 32 mg/m³) might be appropriate.

Numerous cases have been reported of individuals exposed to benzene or mixtures containing benzene who developed leukemia. The most common form of leukemia seen was acute myelogenous leukemia. Besides the acute myelogenous leukemia and its variants, some cases of chronic myelogenous leukemia (CML) and acute and chronic forms of lymphatic leukemia (ALL, CLL) as well as some lymphomas have been noted (IARC, 1982). From the numerous epidemiological studies performed, some found no indication of increased leukemia mortality (Tsai et al., 1983; Rushton et al., 1981a; Thorpe, 1974, Hurley et al., 1991), others provided no persuasive evidence of a causal relationship between benzene and lymphocytic leukemia (e.g. Linos et al., 1980; Arp et al., 1983 and Checkoway et al., 1984), and a few provided a clear rekuiomhip between benzene exposure and leukemia (e.g. Girard and Revol, 1970; Decoufld et al., 1983; Bond et al., 1986; Wong, 1987a, b; Yin et al., 1987a, b; Rinsky et al., 1987). The evaluation of the "negative studies" shows, that none of these studies would be classified as negative according to the requirements of the "Criteria for the validity of human studies" (SEG/KEY/9).

The validity of these studies is mainly reduced because of poor exposure description, the heterogeneity of the groups studied, problems with classification, lack of information on employment and too low exposure levels. These drawbacks, together with the large confidence intervals of the SMR and RR reported makes these studies uninformative.

The most informative epidemiologic studies are from Ott et al. (1978) in the updated form of Bond et al. (1986), Wong (1987a, b), Yin et al. (1987a, b) and Rinsky et al. (1987).

The study of Ott/Bond gave a reliable indication that myeloid leukemia was induced by exposure to benzene levels in the range of 1 -10 ppm (3.2 - 32 mg/m³) (RR = 4.4; 95% CI: 1.2 - 11). Although the reported exposure profile has been criticized (CEFIC, 1991), no information has been given that OSHA's (1987) estimation of the cumulative exposure of the cases was inaccurate.

The study of Wong revealed no statistically significant increases of leukemia and lymphatic or haematopoietic cancer using the general public as control. Compared with an internal control group, which is preferred, increased risks were noted for lymphatic and haematopoietic cancers (e.g. RR = 4.66; p = 0.03; all exposed workers). A relationship between exposure to benzene and leukemia could also be established (p = 0.039). RR could not be given as there were no leukemia cases in the control group.

The study of Wong (1987a, b) gives evidence of a positive relationship between benzene and lymphatic and haematopoietic cancer, including leukemia - although no acute myelogenous cancer was involved - at cumulative exposure levels corresponding to 180 - 720 ppm-months [15 - 60 ppm-years]. Analysis of the exposure pattern and response suggests that cumulative exposure is more important than maximum peak exposures. Due to the diagnostic overlap and principal considerations of the etiology of lymphomas and leukemias it may be appropriate to combine lymphoma and leukemia in the analysis of benzene induced cancers.

Yin et al. (1987a, 1987b, 1989) analyzed a cohort of 28,460 benzene exposed workers from 233 factories. They identified 30 leukemia cases whose cumulative exposure varied between 10 and 5000 ppm-years. Twenty % (6/30) of the cases had a cumulative lifetime exposure at or below 30 ppm-years and a TWA exposure between 2 and 10 ppm. The SMR was 5.74 (p < 0.01). The average latency period was 11.4 years. A significant increase in lung cancer of benzene exposed non-smokers was also reported (SMR = 2.3; p < 0.01).

Although this study gives further evidence for the relationship of exposure to benzene, some reservations concerning the accuracy of these data may be justified because of the composition of the cohort investigated and the related difficulties with exposure assessment.

The Rinsky et al. (1987) retrospective cohort study is generally agreed to be the most carefully performed study available today. This "Pliofilm" cohort studied was made up of rubber-chloride workers from two plants.
The first study (Infante et al., 1977) was updated twice (Rinsky et al., 1981; Rinsky et al. 1987).
The cohort showed a statistically significant increase in deaths from all lymphatic and haematopoietic neoplasms (15 observed; 6.6 expected; SMR = 227).
This increase was mainly due to the excess number of deaths from leukemia (9 observed; 2.7 expected; SMR = 337; 95% CI: 154 - 641) and from multiple myeloma (4 observed, 1 expected;SMR = 409; 95% CI: 110 - 1047).
The cohort analysis showed a progressive increase of SMR's with increasing exposure to benzene.
The cumulative exposure varied from 0.1 to 640 ppm-years for the cases observed. This indicates again the great variety in the susceptibility of the individuals exposed to benzene.

Based on Rinsky's data from the first update of the Pliofilm cohort (Rinsky et al. 1981), Crump and Allen (1984) constructed individual exposure profiles and estimated the cumulative benzene exposure in ppm-years for each worker. Applying a linear relative risk model on

these data, Crump and Allen estimated an excess risk for leukaemia of 6.6 cases/ 1000 exposed for 40 ppm-years.

The risk assessment of Rinsky et al. (1987) was based on a case control analysis of the most recent update of the Pliofilm cohort. The individual cumulative exposure estimations were lower than those of Crump and Allen, because they did not take into account that exposure in the early years - where no reliable measurements have been made - exceeded those of more recent times. Using a log linear model (conditional logistic regression) - which was selected for the best fit with the exposure-response relationship - Rinsky et al. estimated 5.3 excess leukaemia deaths per 1000 workers exposed to 45 ppm-years. Brett et al. (1989) used Crump and Allen's exposure assessments, Rinsky's data from the cohort analysis, redefined the control group used and estimated on this basis with a conditional logistic regression model 0.5 excess leukaemia deaths per 1000 workers exposed to 45 ppm-years.

Other risk assessments on the basis of the studies of Ott et al. (1978), Bond et al. (1986), and Wong (1987a; 1987b), although less reliable than those mentioned before, show a remarkable external consistency of the risk estimates based on independent data sets. The summarized 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 (see Bertazzi and Zocchetti, 1991). As the Pliofilm cohort provides the strongest evidence for a benzene exposure related induction of myelocytic leukaemia, assessments based on these data are preferred. Although some preference may be given to the approach of Brett et al. (1989), no definitive conclusion can be drawn at present, because factors as:

- measurement strategy used
- peak exposure
- dermal absorption/exposure
- dose absorbed via inhalation
- internal exposure
- other types of cancers (lymphoma, multiple myeloma) induced

that cannot yet be precisely quantified may alter the risk estimates in either direction.

Therefore, the estimated 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.

Benzene conc.,, 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

Final Evaluation and Recommendation

Benzene induces neoplasms in both sexes of different strains of rats and mice on multiple sites (including lymphomas and leukaemias) by several routes of administration (inh., i.g., oral). The lowest effective dose applied was 25 mg/kg and induced statistically significant increased tumors in B6C3F1 mice (NTP, 1986). The evidence for carcinogenicity to animals is now regarded as "sufficient" (IARC, 1987). Benzene is also a known human carcinogen (IARC, 1987). Clear evidence from epidemiological studies (e.g. Rinsky et al., 1987; Yin et al., 1987; Bond et al., 1986) is available showing a causal relationship of myelogenous leukaemia and benzene exposure. Growing evidence from epidemiological studies indicates that benzene may also induce lymphomas and multiple myelomas (e.g. Wong, I987a; Rinsky et al., 1987; DScoufle et al., 1983).

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, 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). 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.

As 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), a skin notation is suggested.

At present no method for biological monitoring can be recommended because the available test methods lack sensitivity and specificity at the appropriate level.

No STEL was considered necessary.

Key Bibliography

Arp, E.W., Wolf, P.H. Checkoway, H. (1983): Lymphocytic leukemia and exposures to benzene and other solvents in the rubber industry. J. Occup. Med. 25, 59-602

Ashby, J., de Serres, F.J., Draper, M., Ishidate, M, Margolin, B.H., Matter, B.E. and Shelby, M.D. (esd.) (1985): Evaluation of Short-Term Tests for Carcinogens. Report on the International Programme on Chemical Safety's Collaborative Study on in vitro Assays, Elsevier, Amsterdam, 11-174.

ATSDR (Agency for Toxic Substances and Diseases Registry), U.S. Public Health service. Toxicological Profile for Benzene, TP-1988/03

Austin, H., Delzett, E., and Code, P. (1988): Benzene and leukaemia. A review of the literature and a risk assessment. Amer. J. Epid., 127. 419-439.

Baarson, K.A., Snyder, C.A., and Albert, R.E. (1984): Repeated exposure of C57 Bl mice to inhaled benzene at lOppm markedly depressed erythropoetic colony formation. Toxicol. Lett. 20, 337-373.

Bailer, A.J. and Hoel, D.G. (1989): Metabolite-based internal doses used in a risk assessment of benzene. Env. Health Perspect. 82, 177-184.

Beliles, R.P. and Totman, L.C., (1989): Pharmacokinetically based risk assessment of workplace exposure to benzene. Regul. Toxicol. Pharmacol. 9, 186-95.

Bertazzi, P.A., and Zocchetti, C. (1991): Quantitative estimates of leukaemia risk associated with benzene exposure. SEG/CDO/C1, Supplement F.

BIA (1991): Berufsgenossenschaftliches Institut für Arbeitssicherheit (Sankt Augustin, Germany) "Arbeitsumweltdossier Benzol", submitted to the Commission of the European Communities on 7 Oct 1991.

Bond, G.G., Me Laren.F.A., Baldwin, C.L. and Cook, R.R., (1986): An update of mortality among workers exposed to benzene. Br. J. Ind. Med., 43, 685-691.

Brett, S.M., Rodricks, J.V., and Chinchilli, V.M. (1989); Review and update of leukaemia risk potentially associated with occupational exposure to benzene. Env. Health Pespect. 82, 267-281.

Social Europe

BUA (Beratergremium fur umweltrelevante Altstoffe) eds., 1988: Benzol, BUA-Stoffbericht N° 24, Weinheim; Basel; Cambridge; New York, VCH-Verlagsgesellschaft 1988.

CFFIC (Conseil Europeen des Federations de l'Industrie Chimique). 1984: Criterium document on Benzene, submitted to SEG

Checkoway, H. Wilcosky, T., Wolf, P. et al. (1984): An evaluation of the associations of leukemia and rubber industry solvent exposures. Am. J. Ind. Med. 5, 239-49

Collins, J.J., Conner, P., Friedlander, B.R. et al (1991): A study of the hematologic effects of chronic low-level exposure to benzene. J. Occup. Med. 23, 619-626.

CONCAWE (1987). A survey of exposures to gasoline vapour. Report N° 4/87, The Hague, 1987.

Cronkite, E.P., Drew, R.T., Inone, T., Hirabayashi, Y., and Bullis, J.E. (1989): Hematotoxicity and carcinogenicity of inhaled benzene. Env. Health Persp. 82, 97-108.

Crump, K., and Allen, B. (1984): Quantitative estimates of risk of leukaemia from occupational exposure to benzene. Occupational Safety and Health Administration, Docket H-059B.

Decouflg, P., Blattner, W.A., Blair, A., (19£3): Mortality among chemical workers exposed to benzene and other agents. Environ. Res. 30, 16-25.

Dempster, A.M., and Snyder, C.A. (1989): The ability of the murine erythrocytes to respond to hemolytic doses of phenylhydrazine is significantly impaired by exposures to 10 ppm benzene. Toxicol. Lett. 48, 249-257.

Erexson, G.L., Wilmer, J.L., Steinhagen, W.H., and Kilgerman, A.D. (1986): Induction of cytogenetic damage in rodents after short-term inhalation of benzene: Environ. Mutagen. 8, 29-40

Girard, P.R., Revol, L. (1970): La frequence d'une exposition benzenique au cours des hemopathies graves. Nouv. Rev. Fr. Hematol. 10(4), 477-484

Glatt, H. and Witz, G. (1990): Studies in the induction of gene mutations in bacterial and mammalian cells by the ring-opened benzene metabolites trans, trans-muconaldehyde and trans, trans-muconic acid. Mutagenesis 5, 263-266.

Glatt, H., Padykula, R., Berchtold, G.A., Ludewig, G. Platt, K.L., Klein, J. and Oesch, F. (1989): Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. Environ. Health. Perspect. 82, 81-89.

Social Europe

Henschler, D., Eder, E., Epe, B and Schiffmann, D. (1991): Genotoxic and cell-transforming properties of (trans,trans)-muconaldehyde. Mut. Res. 248, 35-43.

HSE (Health and Safety Executive) (1982): Toxicity Review N° 4, "Benzene". Her Majesty's Stationary Office, London.

Huff, J.E., Haseman, J.K., De Marini, D.M., Eustis, S., Maronpot, R.R., Peters, A.C., Persing, R.L., Chrisp, C.E., and Jacobs, A.C. (1989): Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F1-mice. Env. Health Persp. S2, 125-163

Hurley, J.F. Charrie, J.W., and Mac Laren, W. (1991): Exposure to benzene and mortality from leukemia (ICD 204-207): results from coke oven and other coal products workers. Letter to the editor of Brit. J. Ind. Med., March 1991.

IARC (1987): IARC Monographs on the Evaluation of Carcinogenic Risks to Hurmans. Supplement 7. Overall evaluations of carcinogenicity. An updating of IARC Monographs Volumes 1 to 42. Lyon, 120-122.

IARC (1982): Monographs on the Evaluation of Carcinogenic Risks to Humans, "Some Industrial Chemicals and Dyestuffs". Lyon, I ARC, 29, 93-148.

Infante, P.P. Rinsky, R.A., Wagener, J.K. et al. (1977): Leukemia in benzene workers. Lancet 2, 76-78

Killian, D.J., and Daniel, R.L. (1978): Cytogenetic study of workers exposed to benzene in the Texas Division of Dow Chemical USA, February 29, 1978. OSHA Doc. No. 11-059. Exhibit No. 230 X-2. Occupational Safety and Health Administration, Washington, DC (1978).

Kipen, H.M., Cody, R.P., and Goldstein, B.D. (1989): Use of longitudinal analysis of peripheral blood counts to validate historical reconstructions of benzene exposure. Environ. Health Perspect. 82, 199-206.

Linos, A., Kyle, R.A., O'Fallon, W.M. et al. (1980): A case-control study of occupational exposures and leukemia. Int. J. Epidemiol. 9, 131-5

NTP (National Toxicology Program) (1986): Technical Report on the toxicology and carcinogenesis studies of benzene in F344/N rats and B6C3F1 mice (gavage studies). NTP TR 289.DHHS (NIH), Pub No. 86-2545. Research Triangle Park, NC.

OSHA (1987): Occupational Safety and Health Administration: 29 CFR Part 1910. Occupational Exposure to Benzene: Final rule. Part II. Department of Labor. Fed. Reg. 52, No. 176, 34460-34578 (Sept. 11, 1987).

Ott, M.G. Townsendhorosend, J.C., Fishbach, W.A., and Langner, R.A. (1978): Mortality among individuals occupationally exposed to benzene. Arch, environ. Health, 33, 3-10.

Picciano, D.J. (1979): Cytogenetic study of workers exposed to benzene. Environ. Res. 19, 33-39.

Picciano, D.J., (1980): Monitoring industrial populations by cytogenetic procedures. In: Proceedings of a workshop on methodology for assessing reproductive hazards in the workplace, pp. 293-306. P.P. Infante and M.S. Locator, Eds. U.S. Government Printing Office. Washington, D.C.

Rinsky, R.A.; Smith, A.B., Hornung, R., Fillaon, T.G., Young, R.J., Okun, A.H., and Landrigan, P.J (1987): Benzene and leukemia. An epidemiologic risk assessment. N. Engl. J. Med. 316. 1044-1050.

Rinsky, R.A., Young, R.J. and Smith, A.B. (1981): Leukemia in benzene workers. Am.J. Ind. Med. 2, 217-245

Rozen, M.G., Snyder, C.A. and Albert, R.I., (1984): Depressions in B-and T-lymphocyte mitogen induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol. Letters 20, 343-349.

Rushton, L.; Alderson, M.R. (1981b): A case-control study to investigate the association between exposure to benzene and deaths from leukemia in oil refinery workers. Br. J. Cancer, 43, 77-84

Rushton, L.; Alderson, M.R. (1981a): An epidemiologic survey of eight oil refineries in Britain. Br. J. Ind. Med., 38, 225-234

Sabourin, P.J., Bechtold, W.E., Griffith, W.C., Birnbaum, L.S., Lucier, G., and Henderson, R.F. (1989): Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 99, 421-44.

Sabourin, P.J., Chen, B.T., Lucier, G., Birnbaum, L.S., Fisher, E., and Henderson, R.F. (1987): Effect of dose on the absorption and excretion of [^{14}C] benzene administered orally or by inhalation in rats and mice. Toxicol. Appl. Pharmacol. 87, 325-336.

Sandmeyer E.E., (1981): Aromatic hydrocarbons. In: Patty's Industrial Hygiene and Toxicology. Vol. 2, 3rd rev. ed., Clayton, G.D., Clayton, F.E.,(eds),. New York: Interscience Publishers, pp. 3253-3283.

SEG/KEY/9: Criteria for the validity of human studies. July 1991

Social Europe

Thorpe, J.J. (1974): Epidemiologic survey of leukemia in persons potentially exposed to benzene. J. Occup. Med. 16, 375.

Tice, R.R., Sawey, M.J., Drew, R.T. and Cronkite, E.P. (1984): Benzene-induced micronuclei in the peripheral blood of mice: A retrospective analysis. Environ. Mutagen. 6, 421

Tice, R.R., Vogt, F.F. and Costa, D.L. (1981): Effect of sex, strain, age and route of exposure in benzene-induced sister chromatid exncange (SCE) in murine bone marrow. Environ. Mutagen. 3, 338-339

Toft, K., Oloffson, T., Tunek, A. and Berlin, M., (1982): Toxic effects on mouse bone marrow caused by inhalation of benzene. Arch. Toxicol., 51, 295-302.

Townsend, J.C., Ott, M.G., and Fishbeck, W.A. (1978): Health exam findings among individuals occupationally exposed to benzene. J. Occup. Med. 20, 543-548.

Tsai, S.P., Wen, C.P., Weiss, N.S. et al. (1983): Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J. Occup. Med. 25, 685-692.

Van Damme, K., Casteleyn, L., Van der Auwera, and Chellini, E. (1991): The influence of low levels of exposure to benzene on the blood cell counts: an epidemiological study. Preliminary report to the Belgian Ministry of Science.

Wallace, L.A. (1989): Major sources of benzene exposure. Environ. Health Perspect. 22, 165-169.

WHO (World Health Organisation) (1987): Air quality guidelines for Europe. WHO Regional Publications. European Series No. 23 - Kopenhagen: WHO 1987.

Wong, O. (1987a): An industry wide mortality study of chemical workers occupationally exposed to benzene. I. General results. Br.J. Ind. Med. 44, 365-381.

Wong, O. (1987b): An industry wide mortality study of chemical workers occupationally exposed to benzene. II. Dose-response analysis. Br.J. Ind. Med. 44, 382-395.

Yardley-Jones, A., Anderson, D., and Parke, D.V. (1991): The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. Br.J. Ind. Med. 48, 437-444

Yardley-Jones, A., Anderson, D., Lovell, D.P. and Jenkinson, P.C. (1990): Analysis of chromosomal aberrations in workers exposed to low level benzene. Br. J. Ind. Med. 47, 48-51.

Yin, S.N., Li, G.L., Tain, F.D. et al. (1989): A retrospective cohort study of leukemia and other cancers in benzene workers. Environ. Health Perspect. 82, 207-213

Social Europe

Yin, S.N., Li, G.L., Tain, F.D. et al. (1987a): Leukemia in benzene workers: A retrospective cohort study. Br. J. Ind. Med., 44, 124-128.

Yin, S-N., Li,Q., Lin, Y., Tian, F., Du, C., and Jin, C. (19875): Occupational exposure to benzene in China. Br. J. Ind. Med. 44, 192-195.

Addendum to SUM/140, February 2006

Recommendation of the Scientific Committee on Occupational Exposure Limits: Biological Limit Values for Benzene

Benzene induces neoplasm in different strains of rats and mice at multiple sites, including lymphomas and leukaemias. In humans, it is haematotoxic and induces leukaemias. In 1991, SEG/SCOEL has recommended to keep human occupational exposures to benzene well below 1.0 ppm (3.25 mg/m³). As absorption of liquid benzene through the skin may contribute substantially to the amount absorbed at exposure levels below 1 ppm, a skin notation had been suggested.

Metabolism and kinetics

Benzene is mainly taken up by inhalation. The blood-air equilibrium is attained after about 30 minutes, and approx. 50% of benzene inhaled under this equilibrium is retained in the body (14).

According to Low (24), elimination of benzene from various tissues is biphasic; at the beginning with a shorter half-life between 2.4 and 2.8 hours and then, in a slower phase dependent on the dose, with a half-life between 11 and 29 hours. At concentrations between l and 10 ml/m3 there is a marked accumulation of the substance, however. In persons exposed long-term, elimination can therefore last for up to 150 hours (9).

In addition to being exhaled unchanged, benzene is metabolised in the liver. The main product of metabolism is phenol, which is eliminated renally in the form of its glucuronide or sulphate at a level of up to 45 % of the total amount of benzene absorbed (3). The biological half-life amounts to about 5 hours (38). By far the greatest amount is eliminated within 24 hours. Only after benzene exposures above 10 ml/m³ does phenol accumulate in the course of the working week.

In a first stage, benzene is mainly oxidised in the liver to benzene epoxide, which can then react further in many ways. Transformation to the main metabolite, phenol, and to diphenols, which are mostly eliminated in the form of conjugates, mainly takes place spontaneously. Coupling to glutathione leads to the formation of 5-phenylmercapturic acids, which are eliminated with the urine (34). Transformation via epoxide hydrolase yields a dihydrodiole, which can be further degraded to catechol, o-quinone, 1,2,4-trihydroxybenzene and trans,trans-muconic acid (20). Mercapturic acids are also formed (25). Furthermore, ring-expansion to benzene oxide (oxepin) is under discussion (16).

Benzene metabolism considered
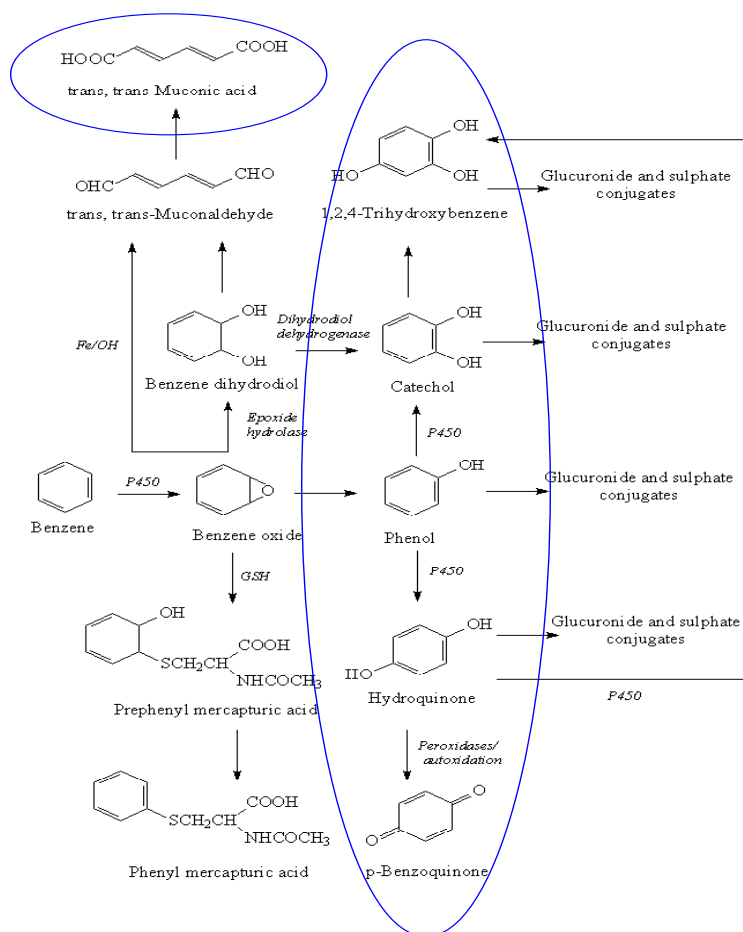
The reactions named above produce intermediates which are toxic or lead to metabolites via further metabolic stages which are responsible for the toxicity and carcinogenicity of benzene; in particular, benzoquinone and *trans-trans*-muconic aldehyde (18). It was shown experimentally that benzene also reacts with haemoglobin to form adducts. This could not, however, be confirmed in humans. However, *S*-phenylcysteine in albumin was identified as a coupling product (8).

Biological indicators

In addition to the determination of benzene in blood, metabolites formed are candidate parameters for monitoring exposure to benzene. Determination of benzene in blood has the advantage of being highly specific (6). Analytical determination which is based on a head-space technique is comparatively simple and interference-free if contamination can be avoided during sampling and sample preparation. Due to the short biological half-life of benzene in blood, sampling should take place immediately after exposure.

The phenol excretion in urine, however, is not specific for exposure to benzene as it can be influenced by exogenous factors, e.g. components of food or medicines which contain phenyl groups. Angerer (3) demonstrated that in 30 % of the persons in a collective exposed to an average concentration pf 10 ml/m^3 benzene, the phenol concentration was below 35 mg/l, which corresponded to the maximum value of a non-exposed collective. This parameter appears only suitable for benzene monitoring on a collective basis and at a concentration range greatly above the current OELs. It is therefore no longer used.

S-Phenylmercapturic acid, which is formed at amounts of about 0.1 % during the metabolism of benzene, represents a parameter suitable for indicating benzene exposure specifically and sensitively. Due to the small amount excreted, however, analysis is a complicated matter, so that this parameter cannot be determined in all occupational-medical laboratories (28).

Excretion of *trans, trans-muconic* acid in urine has been suggested as a specific indicator metabolite for benzene (13). This metabolite reacts very sensitively to benzene exposure even below l ppm (6).

In addition, oxidative products of benzene degradation could be considered for biological monitoring, such as catechol, hydroquinone, quinone and adducts with guanine or the cysteine of albumin. There is, however, little experience with regard to the validity of these parameters.

Benzene in blood

Unlike the determination of phenolic benzene metabolites in urine, measurement of benzene in blood indicates the internal exposure not only of groups, but also of individuals. According to Docter and Zielhuis (11) and Täuber (45), it should be taken into account that during heavy physical work more benzene is taken up by the organism than during rest or light work. Under steady-state conditions the equilibrium between benzene in the ambient air and benzene in the blood is reached after approx. 0.5 hours. After the end of exposure the concentration of benzene in blood decreases exponentially; the half-life is stated as being 0.5 hours. The method of blood sampling is therefore of great importance when monitoring benzene exposure by determination of the benzene concentration in blood. As the relationships between the benzene concentration in ambient air and the benzene concentration in blood are only valid under steady-state conditions, blood sampling should take place immediately after the end of exposure. The requirements demanded of the sampling technique (3, 12) and storage should be taken into account.

S-Phenylmercapturic acid in urine

The *S*-phenylmercapturic acid concentration in urine increases continuously with exposure to benzene during the working day and reaches its maximum about 2 hours after the end of exposure. Afterwards it decreases again, and at the beginning of the next shift it is within the normal range. It is a metabolite, which indicates benzene exposure specifically and sensitively in the individual, even if benzene exposure is below l ppm (28). In practice it has been shown that there is a good correlation between benzene exposure and the *S*-phenylmercapturic acid excretion in urine at the end of the shift (39).

t,t-Muconic acid in urine

*trans,trans-*Muconic acid is a product of benzene metabolism. About 2 % of the benzene taken up is excreted with the urine as *trans,trans-*muconic acid. During occupational exposure to benzene the level of *trans,trans-*muconic acid in urine increases towards the end of the shift (31). The concentration of *trans,trans-*muconic acid in urine is a sensitive arameter for low level exposure to benzene (12, 13, 33, 39).

Analytical methodology

Benzene in blood

Various gas chromatographie methods are described for the determination of benzene in blood (1, 3). According to Snyder *et al.* (42), blood samples of 0.2-0.5 g are necessary. Up to

0.5 µg benzene/g blood can be detected. The analysis procedures described by Sato *et al.* (35) require 0.02-1 ml blood and have a detection limit of 0.02 µg benzene/ml blood.

According to Angerer (3), for the determination of the blood-benzene concentration 2 ml venous blood are taken and injected into a head-space vial sealed with a rubber stopper which contains about 50 mg ammonium oxalate as an anti-coagulant. After 30 minutes incubation at 60 °C, the benzene concentration is quantified by head-space gas chromatography.

The coefficient of variation for in-series precision is stated as being 2.6 % for this method. The detection limit is below 0.002 µg benzene/ml blood. This procedure has been validated; it is possible to increase the sensitivity of the method by a factor of 10 by enrichment procedures, so that now benzene concentrations in the environmental range are accessible to determination (4, 5). Determination of benzene in blood is highly specific for detection of previous exposure to benzene, provided that contamination during sampling and preparation is avoided.

S-Phenylmercapturic acid in urine

Determination of S-phenylmercapturic acid in urine is carried out using gas chromatography/mass spectrometry. 5-Fluoro-phenylmercapturic acid is added to 5 ml urine as an internal standard. The urine is extracted twice with 5 ml ethyl acetate. After evaporation of the extract, methylation is carried out with diazomethane. The S-phenylmercapturic acid is separated using gas chromatography and detected using mass spectrometry for the masses 194.042 and 212.032. The detection limit is 1 µg/1, in-series precision 8 % and day-to-day precision 10.5 % (28).

After acidification of the urine sample to pH < 2 it can be transported at ambient air temperatures up to 25 °C and can be kept for at least three months at -18 °C.

t,t-Muconic acid in urine

The high-pressure liquid chromatographie procedure of Ducos (12) has proven suitable for the determination of *trans,trans*-muconic acid in urine. A UV detector is used. During this procedure *trans trans*-muconic acid is separated from the urine using an anion exchanger and at the same time enriched.

This procedure described by Ducos (12) has been optimised and tested with regard to its analytical reliability.

Background exposures

Environmentally-related exposure to benzene via air, water, food and passive smoking can be estimated at approx. 0.2 mg/day (14). The benzene level of tobacco smoke fluctuates between 20 and 90 µg per cigarette. If one assumes an average tobacco consumption of 20 cigarettes per day, the theoretical uptake amounts to 0.4 to 1.8 mg benzene per day. The benzene concentration inside cars is considerable. The benzene uptake is estimated at approx. 200 µg per day. This is reflected in the values of the blood-benzene concentration of the normal population. Brugnone *et al.* (10) found in employees from rural areas benzene levels of 200ng/l (variation: 7-1003 ng/1) and in employees from urban areas values of 296 ng/1 (variation: 7-2241 ng/1). The values for smokers and non-smokers were 381 ng/l (variation: 7-2241 ng/l) and 205 ng/1 (variation: 7-924 ng/1). Angerer *et al.* (5) found for German non-smokers without occupational exposure a mean benzene level of 176 ± 62 ng/1, for smokers 211 ± 0.5 ng/l; immediateiy after smoking the concentration was 365 ± 178 ng/1.

In an investigation of 10 male and 10 female non-smokers and 20 male and 20 female smokers, phenylmercapturic acid concentrations of 0.2 µg/g creatinine (non-smokers) and 5 µg/g creatinine (smokers) were determined (29). The differences between smokers and non-smokers are considerably greater than those mentioned above. According to van Sittert *et al.* (39), excretion of *S*-phenyl-mercapturic acid in smokers or non-smokers was below the analytical detection limit of 1-5 µg/g creatinine.

t,t-Muconic acid is also excreted in urine by persons not exposed to benzene occupationally. The reasons for this must include the environmental exposure to benzene in industrialised countries. In addition, other substances, such as sorbic acid, taken up with food, are being discussed as possible source for the excretion of *t,t*-muconic acid. The *t,t*-muconic acid concentrations occurring in the urine of the normal population are quoted at values between not detectabte and 3 mg/l. Concentrations in the urine of the normal population are given with mean values between 0.13 and 1.11 mg/l (6, 7, 12, 13, 27, 33).

Evaluation

Benzene in blood

From the published air/blood quotients of 1:8 to 1:10 a median value of approx. 280 µg benzene/1 blood would be calculated to correspond to the air value of 10 ppm. The empirically determined values (2, 3) correspond with these calculated values with regard to their order of magnitude.

S-Phenylmercapturic acid (S-PMA) in urine

Van Sittert *et al.* (39) reported about 12 studies with employees from 10 factories who were exposed to benzene during the production and maintenance of machines in chemical plants and refineries. Thirty-one male persons were chosen for the evaluation of a relationship between the benzene level in the air and the excretion of *S*-phenylmercapturic acid (S-PMA) in urine. The benzene concentration at the workplace was determined with active personal dosimeters. The substances were determined gas chromatographically with a flame ionisation detector. At the end of the shift urine was sampled from the male test persons and quantitative determination of *S*-phenylmercapturic acid twas performed using gas chromatography/mass spectrometry.

The following correlation between the benzene concentration in the air (up to 2 ppm) and the excretion of phenylmercapturic acid in urine was deduced from the values measured:

Benzene [ppm] = 0.068 x S-PMA [µg/g creatinine] + 0.14 [r **= 0.963**]

The values from this investigation are shown in Table 1.

Table 1: Relationship between the benzene air exposure and *S*-PMA excretion (39)

Benzene air concentration (mg/m³; eight-h TWA)	S-PMA urine concentration (µg/g creatinine; mean and 95% CI)
1.0	12(10-14)
2.3	27 (24-30)
3.0	42 (38-16)
3.25 (1 ppm)	46 (41-50)
4.0	56 (52-62)

For BLV evaluation the relationship found by van Sittert *et al.* (39) can be taken as a basis. Sampling time is end of exposure or end of shift.

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trans,trans-Muconic acid in urine

Ducos *et al.* (13) investigated the excretion of *Irans, trans-muconic* acid in workers exposed to benzene (n = 23) in 3 factories several times. These persons were exposed to benzene concentrations between 0. 1 and 75 ppm. The air was sampled using personal air sampling. In these investigations in total 105 pairs of values were obtained (benzene/air-*trans,trans-muconic* acid/urine). There was a highiy significant correlation (p < 0.001) between the benzene concentration in the air and the *trans,trans-muconic* acid excretion in urine. According to this, an increase in the benzene concentration in air by 1 ppm corresponds to an increase in the *trans,trans-muconic* acid concentration in urine of l mg/l. It must be taken into account that excretion of *trans,trans-muconic* acid in non-occupationally exposed persons can be as much as 1 mg/l urine.

The following values have been caiculated on the basis of the relationship between the benzene concentration in ambient air and the excretion of *Irans, trans-nmconic* acid in urine:

Table 2

Benzene (ml/m^3)	*trans,trans-Muconic* acid in urine (mg/l)
0.3	
0.6	1.6
0.9	—
1.0	2
2	3
4	5
6	7

Interpretation of the data

The determination of benzene in whole blood allows conclusions to be drawn about the internal exposure to benzene in individual workers. When interpreting the data, the amount of heavy physical work must be taken into consideration. The time of sampling also plays a decisive role. Blood should be sampled immediately after the end of exposure.

For the determination of *S*-phenylmercapturic acid it is sufficient to acidify urine sampled at the end of the shift with hydrochloric acid to a pH value <2. It can be transported at ambient air temperatures up to 25 °C and kept at -18 °C for several months. Analysis can, however, only take place in laboratories equipped with a mass spectrometer.

Environmental influences affect the excretion of *S*-phenylmercapturic acid only to a limited extent. Smoking can represent exposure to 0.1 ppm benzene.

In the general population the amount of *trans,trans*-muconic acid excreted varies. The values are usually below 1 mg/l. Sorbic acid, which is added to various foods as a preservative, is suspected of being transformed into muconic acid.

As a result of this "normal excretion", *trans, trans*-muconic acid is diagnostically only a sufficiently reliable indicator of individual exposure for benzene concentrations of more than 1 ppm. In investigations with collectives, however, excretion of muconic acid also indicates very sensitively benzene concentrations below 1 ppm.

This means that with benzene air concentrations above 1 ppm, all 3 discussed parameters are suitable for evaluation of individual exposure to benzene, whereas below 1 ppm only determination of benzene in blood and *S*-phenylmercapturic acid in urine allow reliable conclusions to be drawn.

References

1. Angerer, J.: Quantitative Bestimmung von Lösungsmitteln im Blut mit Hilfe der gaschromatographischen Dampfraumanalyse, Arbeitsmed. Sozialmed. Präventivmed. *11*,116-118 (1976)

2. Angerer, J., Lehnert, G.: Biologische Grenzwerte für kanzerogene Arbeitsstoffe - Beispiel Benzol, in; Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin e.V., 22. Jahrestagung, Gentner Verlag, Stuttgart 427-431 (1983)

3. Angerer, J.: Prävention beruflich bedingter Gesundheitsschäden durch Benzol, Toluol, Xylole und Ethylbenzol, Schriftenreihe "Arbeitsmedizin, Sozialmedizin, Präventivmedizin", Gentner Verlag, Stuttgart (1983)

4. Angerer, J., Hörsch, B.: Determination of aromatic hydrocarbons and their metabolites in human blood and urine, J. Chromatogr, *580,* 229-255 (1992)

5. Angerer, J. Scherer, G., Schaller, K.H., Müller, J.: The determination of benzene in human blood as an indicator of environmental exposure to volatile aromatic compounds. Fresenius J. Anal. Chem. *339, 740* (1991)

6. Angerer, J., Rauscher, D., Schaller, K.H., Letzel, S., Popp, W., Müller, G., Rüdiger, H.: Die t.t-Muconsäure als Untersuchungsparameter beruflich bzw. ökologisch bedingter Benzolexpositionen, in: Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. 33. Jahrestagung, Gentner Verlag, Stuttgart, 191-195 (1993)

7. Bechtold, W.E., Lucier, G., Birnbaum, L.S., Yin, S.N., Li, G.L., Henderson, R.F.: Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene, Am. Ind. Hyg. Assoc. J. *52(11),*473-478 (1991)

8. Bechtold, W.E., Willis, J.K., Sun, J.D., Griffith, W.C., Reddy, T.V.: Biological markers of exposure to benzene - S-phenylcysteine in albumin. Carcinogenesis *13,* 1217-1220 (1992)

9. Bittersohl, G.: Der gegenwärtige Stand der Kenntnisse über die Benzenwirkung - Metabolismus, Z. gesamte Hyg. *36,* 135-136 (1990)

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

11. Docter, HJ., Zielhuis, R.L.: Phenol excretion as a measure of benzene exposure. Ann. Occup. Hyg. *10,* 317-326 (1967)

12. Ducos, P., Gaudin, R., Robert, A., Francin, J.M., Maire, C.: Improvement in HPLC analysis of trans,trans-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. Int. Arch. Occup. Environ. Health 62, 529-534 (1990)

13. Ducos, P., Gaudin, R., Bei, J,, Maire, C., Francin, J. M., Robert, A., Wild, P.: t,t,-Muconic acid, a reliable biological indicator for the detection of individual benzene exposure down to the ppm level. Int. Arch. Occup. Environ. Health *64,* 309-313 (1992)

14. Eikmann, T., Kramer, M., Goebel, H.: Die Belastung der Bevölkerung durch Schadstoffe im Kraftfahrzeug-Innenraum - Beispiel Benzol, Zbl. Hyg. *193,* 41-52 (1992)

15. Fiserova-Bergerova, V., Vlach, J., Cassady, J.C.: Predictable "individual differences" in uptake and excretion of gases and soluble vapours simulation study, Brit. J. Ind. Med. *37,* 210-214(1980)

16. Goldstein, B.D.: Introduction: Occam's razor is dull, Environ. Health Persp. *82,* 3-6 (1989)

17. Gollmer, L., Graf, H., Ullrich, V.: Characterisation of the benzene monooxygenase system in rabbit bone marrow, Biochem. Pharmacol. *33,* 3597-3602 (1984)

18. Huff, J.E., Hasemann, J.K., DeMarini, D.M., Eustis, S., Maronpot. R.R., Peters, A.C., Persing, R.L., Chrisp, C.E., Jacobs, A.C.: Multiple-site carcinogenicity of benzene in Fisher 344 rats and B6C3F1 mice, Environ. Health Persp. *82* (1989)

19. Hunter, C.G., Blair, D.: Benzene: Pharmacokinetic studies in men. Ann. Occup. Hyg. *]5,* 193-199(1972)

20. Inoue, O., Seiji, K., Nakatsuka. H., Watanabe, T., Yin, S.N., Li, G.L., Cai, S.X., Jin, C., Ikeda, M.: Excretion of 1,2,4- benzenetriol in the urine of workers exposed to benzene. Brit. J. Ind. Med. *46,* 559-556 (1989)

21. Jowa, L., Witz, G., Snyder, R., Winkle, „ Kalf, G. F.: Synthesis and characterisation of deoxyguanosine benzoquinone adducts, J. Appl. Toxicol. *10,* 47-54 (1990)

22. Koch, R.: Umweltchemikalien, physikalisch-chemische Daten, Toxizitäten, Grenz- und Richtwerte, Umweltverhalten, VCH- Verlagsgesellschaft, Weinheim (1989)

23. Länderausschuss für Immissionsschutz, Arbeitsgruppe "Krebsrisiko durch Luftverunreinigungen", Beurteilungsmasstäbe zur Begrenzung des Krebsrisikos durch Luftverunreinigungen. Abschlussbericht, LAI-Schriftenreihe, Erich Schmidt Verlag, Berlin (1991)

24. Low, L.K., Reddy, G.R., Blackbum, G.R., Mackerer,C.R.: Benzene mechanistic research project. Final report. Mobil Environmental and Health Sciences Laboratory, P.O. box 1209. Princeton NJ 08534-1029, USA, September, l (1991)

25. Lunte, S.M.L., Lunte C. E.: Electroanalytical approaches to understand benzene metabolism. J. Pharmaceutical Biomed. Analysis *8,* 131-136 (3990)

26. Maibach, H.I., Anjo, D.M.: Percutaneous penetration of benzene and benzene contained in solvents used in the rubber industry. Arch. Environm. Health *36,* 256-260 (1981)

27. Melikian, A.A., Prahalad, A.K., Hoffmann, D.: Urinary trans-trans-muconic acid as an indicator of exposure to benzene in cigarette smokers, Cancer Epidemiology, Biomarkers & Prevention 2, 47-51 (1993)

28. Müller, G., Fahnert, R., Müller, Ch., Popp, W., Schmieding, W., Norpoth, K.: Biological Monitoring bei Benzolexpositionen bis zur Höhe des geltenden TRK-Wertes, in: Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. 31. Jahrestagung, Gentner Verlag, Stuttgart. 571-574 (1991)

29. Müller, G., Dibowski, A., Norpoth, K.: Die S-Phenylmerkaptursäure-Ausscheidung im Harn bei Rauchern und Nichtrauchern, in: Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. 33. Jahrestagung, Gentner Verlag, Stuttgart, 467^71 (1993)

30. Nakajima, T., Okuyama, S., Yonekura, I., Sato, A.: Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene. Chem. Biol. Interact. *55,* 23-38 (1985)

Social Europe

31. Popp, W., Müller, G., Angerer, J., Norpoth, K.; Benzol-Belastung und biologisches Monitoring bei Kfz-Mechanikem, in: Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. 33. Jahrestagung, Gentner Verlag, Stuttgart (1993)

32. Rosskamp, E., Seifert, B.: Ausgewählte cancerogene Umweltschadstoffe. Gegenüberstellung der dem Menschen über verschiedene Belastungspfade zugeführte Mengen, Institut für Wasser-, Boden-, Lufthygiene des BGA, Berlin (1991)

33. Ruppert, T., Scherer, G., Rauscher, D., Angerer, J., Adlkofer, F.: Determination of urinary t,t-muconic acid by gas chromatography-mass spectrometry. J. Chromatogr. B. 66, 71-76 (1995)

34. Sabourin. P.J., Bechtold, W.E., Griffith, W.C, Birnbaum, L.S., Lucier, G., Henderson, R.F.: Effect of exposure concentration, exposure rate and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 99, 421-444 (1989)

35. Sato, A., Nakajima, T., Fujiwara, Y.: Determination of benzene and toluene in blood by means of a syringe-equilibration method using a small amount of blood, Brit. J. Ind. Med., 32, 210-214 (1975)

36. Sato, A,, Nakajima, T., Fujiwara, Y., Muruyama, N.: Kinetic studies on sex difference in susceptibility to chronic benzene intoxication - with special reference to body fat content, Brit. J. Ind. Med. 32, 321-328 (1975)

37. Sato, A., Nakajima, T., Koyama, Y.: Dose related effects of a single dose of ethanol on the metabolism in rat liver of some aromatic and chlorinated hydrocarbons, Toxicol. Appl. Pharmacol. 60, 8-15 (1981)

38. Sherwood, R.J., Carter, F.W.G.: The measurement of occupational exposure to benzene vapour. Ann. Occup. Hyg. 13, 125-146 (1970)

39. Sittert, N.J. van., Boogaard, P.J., Beulink, G.D.J.: 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 (1993)

40. Snyder, C.A.: Benzene, Ethels Browning's Toxicity and Metabolism of Industrial Solvents, 2nd edition. Vol. I; Hydrocarbons, R. Snyder (Ed.), Elsevier Science Publishers B.V. 3-37 (1987).

41. Snyder, R., Uzuko, F., Gonasum, L., Bromfield, E., Wells, A.: The metabolism of benzene in vitro. Toxicol. Appl. Pharmacol. 11, 346-360 (1967)

42. Snyder, R., Laskin, S., Goldstein, B.: An extractive method for determination of benzene in blood by gas chromatography. Am. Ind. Hyg. Assoc. J. 36, 833-836 (1975)

43. Snyder, R., Jowa, L.. Witz, G., Kalf, G., Rushmore, T.: Formation of reactive metabolites from benzene. Arch. Toxicol. 60, 61 (1987)

44. Swaen, G., Meijers, J.: Risk assessment of leukaemia and occupational exposure to benzene, Brit. J. Ind. Med. 46, 826-830 (3989)

45. Täuber, U.: Wirkung von Benzol auf Mensch und Tier. Deutsche Gesellschaft für Mineralölwissenschaft und Kohlechemie e.V., Forschungsbericht, 174-76 (1980)

46. Teisinger, J., Bergerova-Fiserova, V., Kurdna, J.: The metabolism of benzene in men, Pracovni Lekarstvi 4,175 (1952)

47. WHO (World Health Organization): Air quality guidelines for Europe. WHO Regional Publications, European Series No. 23 (1987)