European Commission

Recommendation from the Scientific Committee on Occupational Exposure Limits for Nitrogen Dioxide

SCOEL/SUM/53
June 2014

Employment, Social Affairs and Inclusion

Table of Contents

Recommendation from the Scientific Committee on Occupational Exposure Limits for Nitrogen Dioxide

8-hour TWA:	0.5 ppm (0.955 mg/m^3)
STEL (15-min):	1 ppm (1.91 mg/m^3)
BLV:	-
Additional categorisation:	-
Notation:	-

This Recommendation is based on compilations by WHO (1997), DECOS (2004), US EPA (2008), DFG (2005 and 2010), ACGIH (2012) and National Research Council of the National Academies (2012). An additional literature search was performed in December 2013.

1. Substance identification, physico-chemical properties

Chemical name:	Nitrogen dioxide
Synonyms:	Nitrogen peroxide
Molecular formula:	NO_2
EC No.:	233-272-6
CAS No.:	10102-44-0
Molecular weight:	46.01 g/mol
Boiling point:	21.2 °C
Melting point:	-9.3 °C
Vapour pressure (80 °C):	52 kPa
Conversion factors:	1.91 mg/m^3 = 1 ppm
(20 °C, 101.3 kPa)	1 mg/m^3 = 0.524 ppm

EU harmonised classification:

Press. Gas		
Ox. Gas 1	H270	May cause or intensify fire; oxidiser
Skin corr. 1B	H314	Causes severe skin burns and eye damage
Acute Tox. 2	H330	Fatal if inhaled

Depending upon the temperature, nitrogen dioxide is a colourless solid, yellow liquid or reddish-brown gas with an irritating odour. The vapour density is 1.58 times that of air. The odour threshold is 0.1–0.4 ppm (0.2–0.8 mg/m^3) (US EPA 2008, DFG 2005).

2. Occurrence/use and occupational exposure

Nitrogen dioxide (NO_2) is found in ambient air as a product of natural as well as human activities. Production of NO_2 as the final product is limited. Production as a chemical intermediate, particularly in nitric acid and fertiliser production is very large throughout the EU. Occupational exposure may occur in the chemical industry, during

gas welding, in agriculture (silos), in mining (explosives) and from exhaust from combustion engines in confined areas. It is also produced by various industrial emissions and in tobacco smoke. Combined exposure to NO_2 and nitrogen monoxide (NO) is ubiquitous. Oxidation of NO to NO_2 may easily occur in some circumstances (DFG 2005 and 2010).

2.1. Analytical measurement

There are different methods for the measurement of airborne NO_2 (electrochemical cell, chemoluminescence). The lowest detection limit (LDL) of 0.002 ppm is reached by using chemoluminescence. Chemoluminescence measurements are considered to represent the "gold standard" of NO/NO_2 analysis. However, there are restrictions to the applicability of this method in underground mining (especially coal mining) or at workplaces where heat is involved. By contrast, the "*multiwarn"* electrochemical device, which allowed personal air sampling, had an LDL of only 1 ppm, but was suitable for the measurement of even very short exposure peaks (1 min). This instrumentation was used in the German coal mine studies (Dahmann *et al* 2007 and 2009; see Section 3.5.1). However, since the publishing date of these studies technology has further advanced. The "*multiwarn*" is no longer manufactured, and there are now new multi-gas detectors and new electrochemical sensors commercially available. Application of new technology resulted in an electrochemical sensor with a lower detection limit of NO_2 of 0.04 ppm. This sensor can be used in gas detectors that are applied in coal mines in several countries including Europe, as they are ATEX approved for use in mining environments (communication by Dräger Safety AG & Co KGaA, Lübeck/Germany, to SCOEL, dated 18 October 2013).

3. Health significance

The critical effect of NO_2 is irritation of the deep compartment of the respiratory tract in both animals and man. NO_2 is well absorbed via the lungs. It is then likely to be incorporated into intermediary metabolism pathways and does not result in systemic effects.

3.1. Toxicokinetics

NO_2 reacts only slowly in water to form nitric acid and nitrous acid, both of which have irritative to caustic effects (Mücke and Wagner 1998). It was concluded from a study with monkeys with radioactively labelled NO_2 that nitric acid and nitrous acid are formed also in the respiratory tract. The acids or their salts were detected in blood and urine. Experimental studies also showed that NO_2 or its products can remain in the lungs for a longer period (WHO 1997). In man, 80–90 % of NO_2 is taken up via the respiratory tract during normal breathing, and over 90 % at maximum breathing. Dosimetric model calculations showed that NO_2 is absorbed mainly in the lower respiratory tract, where NO_2 accumulates particularly in the area between the conductive and respiratory airways (pulmonary acinus), in which morphological changes are observed (WHO 1997).

3.2. Acute toxicity

3.2.1. Human data

In the majority of studies with healthy volunteers, no effects on lung function were observed after short-term exposure (a maximum of 4 hours) to NO_2 concentrations in the air of 0.1 to about 2 ppm (National Research Council of the National Academies 2012). Effects on lung function were described at even lower concentrations in two studies including subjects with asthma (Bylin *et al* 1985 and 1988, Kulle 1982).

Increased airway resistance was reported in several studies only at 2 ppm and above (DFG 2005). Evidence of increased bronchial reactivity was observed after NO_2 concentrations of 1.5 ppm and above (Frampton *et al* 1989 and 1991, Mohsenin 1987 and 1988).

In particular, a study by Frampton *et al* (2002) appears relevant and is therefore described in detail below.

In this study, 21 volunteers were exposed to NO_2 at 0, 0.6 and 1.5 ppm for 3 hours during physical exercise (10 minutes of physical exercise at a ventilation rate of 40 l/min alternating with 20 minutes without physical exercise) at one-week intervals. NO_2 gas (5 000 ppm) was mixed with pure air and introduced into an exposure chamber (45 m^3) to generate the desired NO_2 concentration. More than 90 % of the desired NO_2 concentrations were reached within 4 minutes. This publication includes no data on the NO_2 analysis. It is assumed that the method described in the earlier publications by Frampton *et al* (1989 and 1991) was used. There, continuous measurement was applied using an EPA reference standard NO/NO_x analyzer, which was calibrated with standardised NO_2 and NO gas and verified by means of a caloric method. The background concentrations of NO_x, ozone, dust and SO_x were recorded continuously, but the results were not reported. No significant changes of spirometric parameters were observed. No effect of *in vivo* exposure to NO_2 on the inactivation of influenza viruses *in vitro* in alveolar macrophages or in bronchial epithelial cells was found, as had been described in a previous study (Frampton *et al* 1989). After exposure, a slightly, but statistically significantly reduced haemoglobin concentration and a reduced haematocrit (no difference between men and women) resulting from a decreased erythrocyte count were found in the blood of the volunteers at both NO_2 concentrations. The authors pointed out that such effects had also been observed in another volunteer study at NO_2 concentrations of 1 and 2 ppm (Posin *et al* 1978). The leukocyte count (polymorphonuclear leukocytes, lymphocytes, monocytes and eosinophils) was also reduced in the blood, again at the low concentration and in both sexes in most cases. A differentiation of the blood lymphocyte populations revealed a lymphocyte $CD4^+/CD8^+$ ratio, which was increased in men but decreased in women. Total protein and albumin concentrations were not altered in the bronchial and alveolar lavage. In the bronchial lavage, clearly increased number of polymorphonuclear leukocytes (as a marker of inflammation) were found in men and, to a lesser degree, in women after exposure to 1.5 ppm; lymphocyte count increases were observed in both sexes, but they were more pronounced at 0.6 ppm than at 1.5 ppm. As in the bronchial lavage, an increased number of $CD4^+$ cells and an increased ratio of $CD4^+/CD8^+$ lymphocytes were found in the alveolar lavage particularly in men at both 0.6 and 1.5 ppm, whereas in women the number of $CD4^+$ cells was increased only at 1.5 ppm. The authors pointed out that other publications also described sex differences in the T lymphocyte concentrations. They concluded that NO_2 exposure reduced the number of circulating T lymphocytes. The authors also pointed out that all effects observed were slight and presumably of no clinical relevance to healthy persons (Frampton *et al* 2002).

The findings obtained in this study at 0.6 ppm appear not to be adverse, particularly as in other volunteer studies effects occurred only in ranges starting from 1.5 ppm. Thus, in the bronchoalveolar lavage fluid (BAL or BALF), initial signs of inflammatory reactions were observed at NO_2 concentrations of 1.5 and 2 ppm. These signs were, for example, a reduction of cytotoxic suppressor T cells and natural killer cells (1.5 ppm for 20 min every 2^{nd} day, 6 times; Sandström *et al* 1992), an increased number of $CD25^+$ lymphocytes and HLA-DR^+ macrophages in the BALF and neutrophils in the bronchial fraction (2 ppm NO_2 for 4 hours on 4 consecutive days; Blomberg *et al* 1999), a lower fraction of $CD4^+$ cells and an increased number of neutrophils in the bronchial fraction (Solomon *et al* 2000), a reduced capacity for phagocytosis of

alveolar macrophages and a decrease in superoxide production as well as an increased number of polymorphonuclear leukocytes in the bronchial lavage (2 ppm NO_2 for 4 hours, once; Devlin 1999; see DFG 2005 and 2010 for details).

Effects of NO_2 on lung function and airway responsiveness in healthy individuals are reported to occur at concentrations above 1 ppm (1.9 mg/m^3) (Folinsbee 1992, Bylin 1993). Exposure to 0.6 ppm (1.1 mg/m^3) NO_2 for 3 hours reduced the efficiency of macrophages to inactivate influenza virus in 4 out of 9 healthy volunteers. Nevertheless, the observed differences in virus inactivation after air and NO_2 exposure did not reach statistical significance at the $P < 0.05$ level, as it was stated by the authors (Frampton *et al* 1989). An increase in blood glutathione content was reported following exposure of volunteers to 0.2 ppm (0.4 mg/m^3) for 2 hours (Chaney *et al* 1981), but this effect is considered to be of less biological importance.

Studies on the effects of NO_2 on pulmonary function in asthmatics and in patients with chronic lung disease or bronchitis were reviewed *in extenso* by the US National Research Council of the National Academies (2012). Overall, these studies were found conflicting and inconclusive with regard to the derivation of limit values. It was concluded that some asthmatic subjects exposed to 0.3–0.5 ppm NO_2 may respond with either subjective symptoms or slight changes in pulmonary function, which were rated to be of no clinical significance. As a potential population subgroup with higher susceptibility to NO_2, children (5–12 years old) were identified. As children of this age are not exposed occupationally, such studies are not considered here.

3.2.2. Animal data

For rats, the LC_{50} after exposure for 15 minutes was given as about 200 ppm and after 60 minutes as 115 ppm (Carson *et al* 1962). The LC_{50} values for various strains of rat after exposure to NO_2 for 16 hours were given as 39–56 ppm. Mice, depending on the strain, were found to have LC_{50} values between 33 and 67 ppm after exposure for 16 hours, golden hamsters values of 22 ppm (females) and 28 ppm (males) and Hartley Guinea pigs values of 50 ppm (females) and 62 ppm (males) (Sagai and Ichinose 1987). For rabbits, the LC_{50} after exposure for 15 minutes was found to be 315 ppm. For dogs, 53 ppm was given as the 50 % value of the LC_{50} (corresponding to an LC_{50} of about 105 ppm) after exposure to NO_2 for 60 minutes. In rats and rabbits, severely impaired breathing, eye irritation and reduced body weights were observed (Carson *et al* 1962).

Other animal experiments with single exposures are described in WHO (1997).

3.3. Sensory threshold and irritation

NO_2 has a penetrating odour. Depending on the study conditions, the perception threshold is between 0.1 and 0.2 ppm (Feldman 1974, Shalamberidze 1971). With slowly increasing concentrations the odour is not perceived until much higher concentrations have been reached (Henschler *et al* 1960), so the warning effect of the gas is poor under this condition.

The irritation threshold for NO_2 in air was given as 20–30 ppm (Henschler *et al* 1960). At the workplace, exposure to 25–75 ppm led to bronchitis or bronchopneumonia with complete restitution, 50–100 ppm to reversible bronchiolitis and focal pneumonitis, 150–200 ppm to lethal *bronchiolitis fibrosa obliterans* and more than 300 ppm to lethal pulmonary oedema and asphyxia (combined with methaemoglobinaemia) (Grayson 1956).

3.4. Sensitisation

There were no data pointing to a sensitisation potential.

3.5. Repeated dose toxicity

3.5.1. Human data

In studies with occupational exposure to NO_2, the exposure is always to a mixture of substances e.g. with Diesel motor emissions, NO, sulphur dioxide, smoke or mineral dust, which also impair the respiratory tract. Diesel motors are an important source of exposure to NO_2. As a result of the exposure to a mixture of substances these studies are, however, unsuitable for the adequate evaluation of NO_2-related effects.

In a questionnaire, 232 workers from 4 Diesel bus garages were asked about acute respiratory diseases. Lung function tests were carried out before and after the shift, and the NO_2 concentration was determined during the shift. Concentrations of other irritant gases were below the US national standards. The highest NO_2 concentrations in the air were 0.56 ± 0.38 ppm, the lowest 0.13 ± 0.06 ppm. Short-term personal air sampling often yielded exposure concentrations of over 1 ppm. The authors reported that the prevalence of acute respiratory symptoms was higher than expected only in the high exposure group (> 0.3 ppm) (Gamble *et al* 1987).

In 259 workers of a salt mine who were exposed to Diesel motor emissions, chronic respiratory effects were reported in a questionnaire and X-rays and lung function tests were carried out. The NO_2 exposure was determined by personal air sampling and was on average between 0.2 ± 0.1 ppm and 2.5 ± 1.3 ppm. The authors reported that coughing was associated with age and smoking, and dyspnoea with age. There was no association, however, with the NO_2 exposure (Gamble *et al* 1983).

In a study with 20 000 coal mining workers exposed on average to NO_2 concentrations of 0.03 ppm and NO concentrations of 0.2 ppm, workers were not absent due to illness more frequently than unexposed persons because of airway infections. There were, however, problems in this study as regards the classification of exposure (Jacobsen *et al* 1988, cited in WHO 1997).

Robertson *et al* (1984) measured the levels of NO_2 and NO in nine British collieries between 1974 and 1979, which ranged, respectively, from 0.02 and 0.08 ppm and 0.11 and 1.23 ppm depending on colliery and type of work. No relationship was found between exposure and respiratory symptoms or decline in FEV_1, nor was there any differences in symptoms or lung function between 44 pairs of men matched for age, dust exposure, smoking habit, coal rank and type of work but differing in respect of exposure to oxides of nitrogen.

In principle, reports on the effects of long-term occupational exposure relating to mixed exposures are difficult to assess with respect to deriving a recommendation for an occupation exposure limit (OEL), when a discrimination of the effects of nitrogen oxides from those of other exposure components (particulates, gases, basic variables) has not been made. In such studies, attention has been drawn to effects on lung functions and immune functions in miners (salt and iron) exposed to nitrogen oxides, together with other pollutants like Diesel exhausts, dust and CO, with NO_2 levels ranging from 0.14 to 0.5 ppm (Backé *et al* 2004, Adelroth *et al* 2006, Latza *et al* 2009).

Recently, two larger epidemiological studies have been performed in miners: by Lotz *et al* (2008) in German salt mines, and by Morfeld *et al* (2010) in German hard coal mines. The analytical part of the first was published by Dahmann *et al* (2007) and that

of the second study by Dahmann *et al* (2009). The first study (Lotz *et al* 2008) in salt mines was accompanied by a multitude of analytical measurements, covering 600–700 shift mean values in total. This data base allowed a very precise statistical analysis of the distribution of shift exposures: the 95th percentile of 8-hour shift data was slightly higher than the two-fold of the mean, both for NO and for NO_2.

The generation of a comparable body of analytical data was not possible in the coal mine study (Morfeld *et al* 2010). The main reason for this deficit of the coal mine study was that the chemoluminescence instruments did not fulfil the very strict explosion safety requirements for underground coal mines. This led to relatively few analytical measurements reported in the coal mine study (e.g. 21 in Diesel locomotive drivers, 5 in blasting workers). However, on the basis of the pre-existing knowledge (Dahmann and Monz 2000, Dahmann *et al* 2007) it was anticipated that the general data distribution pattern of analytical data for NO/NO_2 in the coal miners would be similar to those found in the salt miners.

Lotz *et al* (2008) examined 410 and 463 miners (salt mines A and B) cross-sectionally; 75 and 64 % of the first cohort were again examined after a 5-year period. Exposure was measured by personal sampling. Personal lifetime exposure doses of salt dust, Diesel exhaust, NO_2 and NO were calculated for all miners. Dose-response relationships were calculated by multiple regression analysis. In the 5-year period, the adjusted (age, smoking etc.) effect of the exposure indicators resulted in a mean decrease of FEV_1 between -18 ml/year (mine A) and -10 ml/year (mine B). The personal concentrations related to this effect were 12.6 and 7.1 mg/m^3 inhalable dust, 2.4 and 0.8 mg/m^3 respirable dust, 0.09 and 0.09 mg/m^3 Diesel exhaust, 0.4 and 0.5 ppm NO_2, and 1.7 and 1.4 ppm NO (mines A and B). Exposure was related to symptoms of chronic bronchitis only in mine B. The authors concluded that the effects found in both mines indicated that the prevailing mixed exposure may cause lung function impairment in salt miners exposed over a long period of time. In this study, it was not possible to determine the effects of a single exposure component (nitrogen oxides vs. dust, Diesel exhaust etc.) separately.

This limitation was avoided in the study of hard coal miners by Morfeld *et al* (2010) by use of General Estimation Equation (GEE) models. This allowed the discrimination of effects of nitrogen oxides from those of other exposure component variables. A longitudinal inception cohort study (1974–1998) was conducted on miners who started working underground at two coal mines between 1974 and 1979. The authors determined the number of shifts underground, the exposure to coal mine dust, quartz dust, NO, NO_2, smoking behaviours, and the lung function parameters FVC, FEV_1 and FEV_1/FVC. In total, 1 369 miners worked on average 3 017 shifts underground per person. The total mean respirable coal mine dust concentration was 1.89 mg/m^3 (quartz: 0.067 mg/m^3), and nitrogen oxide concentrations were 0.58 ppm NO and 0.007 ppm NO_2. However, the exposure in defined subgroups was consistently higher (mean 8-hour shift concentrations: Diesel engine drivers, 1.35 ppm NO and 0.21 ppm NO_2; Diesel train drivers, 1.35 ppm NO and 0.52 ppm NO_2; blasting specialists, 0.84 ppm NO and 0.014 ppm NO_2; see Dahmann *et al* 2009). The GEE-regression models did not reveal clear adverse dust exposure effects. Nitrogen oxides combined (NO + NO_2) showed small, statistically insignificant, effects on lung function, which were not considered as being adverse. It was concluded that nitrogen oxide exposures, including those in the subgroups, showed no adverse influence on lung function in this long-term longitudinal study.

Regarding the distribution of exposure data, the 95th percentiles of 8-hour shift data (Dahmann *et al* 2009, Morfeld *et al* 2010) were slightly higher than the two-fold of the means, which was also supported by the data of the preceding study in salt miners (Dahmann *et al* 2007, Lotz *et al* 2008).

3.5.2. Animal data

Numerous animal studies with repeated exposure are described in WHO (1997). Many animal studies have shown that long-term (continuous) exposure to NO_2 concentrations of 5 ppm in air can cause emphysema, which has also been observed in man (WHO 1997, DFG 2005).

Several studies were conducted, in which Wistar rats were continuously exposed (24 hours/day) to NO_2 at 0, 0.4, 1.2 and 4 ppm for 1, 2, 4, 8, 12 and 16 weeks (Ichinose and Sagai 1982) and to NO_2 at 0, 0.04, 0.4 and 4 ppm for 4, 9, 18 and 27 months (Kubota *et al* 1987, Sagai and Ichinose 1987, Sagai *et al* 1984). The NO_2 concentrations were measured continuously using the Monitor Labs 8440-L Nitrogen Oxide Analyzer (Ichinose and Sagai 1982), but the measurement accuracy is not known, particularly at the very low concentrations of 0.4 and 0.04 ppm (DFG 2005).

After up to 16 weeks of continuous exposure of 6 rats per group to NO_2, no significant alterations were observed at 0.4 ppm. At 1.2 ppm and above, the level of sulphhydryl (SH) groups not associated with proteins was significantly increased in the lungs (as evidence of increased glutathione dependent enzyme activities); glutathione reductase activity was significantly increased in week 4 and ethane exhalation was slightly increased in the first 4 weeks. At 4 ppm, ethane exhalation was considerably increased in the first 4 weeks, the increase still being significant at the end of exposure. The following significant lung changes were also observed at this concentration: increased thiobarbituric acid reactants and significantly increased glutathione reductase, glucose-6-phosphate dehydrogenase and superoxide dismutase activities (Ichinose and Sagai 1982). When the NOAEC of 0.4 ppm is converted from continuous 24-hour exposure to 8-hour daily exposure, it would theoretically correspond to a concentration of about 1.2 ppm.

After up to 27 months of exposure of 3–6 rats per group, ethane exhalation was significantly increased at 0.04 ppm and above. From 0.4 ppm, the mean thickness of the air-blood barrier was increased slightly after 18 months and significantly after 27 months. Moreover, there was some interstitial oedema and slight bronchiolar and alveolar epithelium changes. In the lungs, the non-protein SH group and thiobarbituric acid reactant levels were significantly increased and glutathione peroxidase activity was reduced. Hypertrophy and hyperplasia of the bronchiolar epithelium, Clara cell hyperplasia, interstitial fibrosis and type I and type II cell hypertrophy were observed at 4 ppm. Clear increases in lipid peroxidation and alterations in protective enzyme activities were also found (Kubota *et al* 1987, Sagai and Ichinose 1987, Sagai *et al* 1984). Ethane exhalation is also used as a sensitive and non-invasive test to detect lipid peroxidation in humans (Kneepkens *et al* 1994); however, ethane exhalation is a marker of effects that are not of adverse nature. Therefore, 0.04 ppm could be assessed under these conditions as the NOAEC and 0.4 ppm as the LOAEC. When the NOAEC of 0.04 ppm and the LOAEC of 0.4 ppm are converted from 24-hour exposure to 8-hour daily exposure, these may correspond to concentrations of about 0.12 ppm and 1.2 ppm, respectively. However, because of the continuous exposure, this study is not considered to be a suitable basis for recommending an OEL.

After a 15-day exposure of mice to NO_2, changes in the distribution of leukocyte subpopulations in the BALF were observed at 20 ppm, but not at 10 ppm (Wegmann *et al* 2002/2003).

Brown Norway rats were used as a sensitive model of allergic diseases to detect immunological effects of NO_2 exposure and to investigate the activity of alveolar macrophages and the production of cytokines. Males and females were exposed to NO_2 at 0.2, 0.5 and 2 ppm during mating. In the case of the pups, which were also exposed, there are no data on the exact exposure period or duration. At 0.5 and

2 ppm, changes in the BALF were observed in the 8- and 12-week-old pups – in most cases in the animals which were exposed directly. No alterations were observed at 0.2 ppm (Kumae and Arakawa 2006).

In an inhalation study, which was conducted according to modern standards (BASF 2006a), 18 male Wistar rats per group were exposed to NO_2 in whole-body exposure chambers on 5 days for 6 hours/day. The desired NO_2 concentrations, which were calculated by means of the gas supply and the pump rate, were 0, 0.5, 5 and 20 ppm. The analytical concentrations, which were calculated by means of an infrared (IR) spectrophotometer with NO_2 calibration, were 0, 0.1, 4.9 and 19.2 ppm. The authors discussed the high relative humidity of 50 % with possible condensation of water and NO_2 at the apparatus and problems with the correct regulation of the supply of air and NO_2 as possible causes of the low NO_2 concentration measured in the low concentration group. In addition to the usual examinations (mortality, body weight gain, feed consumption, haematology, clinical chemistry, organ weight determinations, gross-pathological and histopathological organ examinations), lipid peroxidation (malondialdehyde formation) and 8-hydroxy-2-deoxyguanosine formation were investigated in lung homogenates, the BALF was examined for cellular components, and enzyme activities and cell proliferation and apoptosis were measured in the bronchi, bronchioles and alveoli at the end of the 5-day exposure period. No alterations were observed in the low exposure group. Histopathological alterations in the lungs (bronchoalveolar hyperplasia, mononuclear cell infiltration and alveolar histiocytes) and in the trachea (diffuse hyperplasia of the tracheal epithelium) and cell proliferation in the large and medium bronchi, terminal bronchioles and in the alveoli occurred at 5 ppm and above. Significantly increased lung weights, alveolar oedema, increased apoptosis rates in the medium and large bronchi as well as increased cell numbers, an increased number of macrophages and polymorphonuclear neutrophils, increased total protein levels and an increased activity of γ-glutamyltransferase and lactate dehydrogenase in the BALF were additionally found at 20 ppm. The authors specified a NOAEC of 0.5 ppm (BASF 2006a), but the accuracy of this concentration is unclear and it rather seems to be the detection limit of the method used. The LOAEC was 5 ppm. Since no increased malondialdehyde concentration was measured even at the high NO_2 concentration of about 20 ppm, the method used (total lung homogenates) cannot be regarded as sensitive enough to detect lipid peroxidation. Nor was a formation of 8-hydroxy-2-deoxyguanosine in lung homogenates observed, which means that this measurement may not be sensitive enough either.

In the subsequent subchronic (13-week) inhalation study (BASF 2006b), groups of 15 male and 10 female Wistar rats were exposed to NO_2 in whole-body exposure chambers at 0, 0.1, 0.5 and 1 ppm on 5 days/week for 6 hours/day. The analytical concentrations of 0.008, 0.25, 0.82 and 2.15 ppm, which were measured by means of an IR spectrophotometer with NO_2 calibration, were higher than the desired concentrations by about a factor of 2 since calibration was based on an incorrect correlation equation. At the end of the 13-week exposure period, the BALF was examined again and cell proliferation and apoptosis were measured in the lungs in addition to the usual examinations (mortality, body weight gain, feed consumption, haematology, clinical chemistry, organ weight determinations, gross-pathological and histopathological organ examinations). However, no substance-induced effects were observed up to 2.15 ppm. Therefore, the authors specified a NOAEC of 2.15 ppm for this study. No major measurement inaccuracies are expected at this concentration.

3.6. Genotoxicity

NO_2 is mutagenic in bacteria (Biggart and Rinehart 1987) and clastogenic in mammalian cells *in vitro* (Görsdorf *et al* 1990, Tsuda *et al* 1981). *In vivo*, no induction

of chromosome aberrations was observed in leukocytes and spermatocytes of mice exposed to NO_2 (Gooch *et al* 1977). Dose-dependent increases in mutations and in chromosome aberrations were seen in lung cells from rats exposed to NO_2 at 8, 15, 21 and 28 ppm (15, 29, 40 and 53 mg/m^3) (Isomura *et al* 1984), but because of the low survival of the lung cells (10–15 %), this study is not considered to be conclusive evidence of *in vivo* genotoxicity. The available data therefore give no indication of a systemic genotoxic effect of NO_2, but further studies may be required to evaluate a possible local genotoxicity on epithelia of the airways.

3.7. Carcinogenicity

The possibility that inhaled nitrous gases could react with amines of the mucous membranes of the respiratory tract to form carcinogenic nitrosamines was noted early on (Druckrey and Preussmann 1962). Valid long-term studies of the carcinogenicity of NO_2 are, however, not available. Long-term studies with limited validity did not yield evidence of carcinogenic effects of NO_2. Initiation promotion studies yielded evidence of tumour-promoting effects of NO_2 in the rat lung.

3.8. Reproductive toxicity

3.8.1. Human data

Pregnant women (n = 51) who were exposed to nitrogen oxides in the air (average NO_2 concentrations of 0.023 mg/m^3 with peak levels up to 0.239 mg/m^3), drinking water (nitrate concentrations in well water of up to 400 mg/l) and food, were examined during the birth. Methaemoglobin (MetHb) in the blood of the mother and in the umbilical cord blood was determined as the effect marker, and blood lipids and glutathione as markers for oxidative stress. In the newborn babies, the birth weight, the APGAR index (evaluating the most important body functions of the baby) and clinical diagnoses at birth were recorded. MetHb concentrations of a maximum of 2 % in maternal blood and of a maximum of 2.8 % in cord blood were regarded as normal. Around 55 % of the maternal blood samples were above 2 % MetHb and about 20 % above 5 %. Around 80 % of the cord blood samples were above 2 % MetHb and about 45 % above 5 %. In the case of increased MetHb concentrations in maternal or cord blood, the values for glutathione (total and reduced) were decreased and those for lipid peroxides increased. A strong association was found between increased lipid peroxides in cord blood and adverse birth outcome. With premature births in particular, the levels of MetHb in cord blood were increased (Tabacova *et al* 1998). The study suggests that increased concentrations of MetHb and lipid peroxides in cord blood can lead to premature births and other impairments at birth. A correlation of the findings with NO_2 concentrations in the air is, however, not possible from this study. As nitrite in particular (formed from nitrate) is responsible for the formation of MetHb, the oral uptake of nitrate and nitrite via water and food is regarded in the study of Tabacova *et al* (1998) as having a decisive influence.

In an earlier study, MetHb levels in blood were increased by 1 % after exposure of volunteers to NO_2 concentrations of 20 ppm for 2 hours (Henschler and Lüdtke 1963). A significant increase in the MetHb concentration is therefore not to be expected when NO_2 concentrations are below 1 ppm in air. The possibility of overestimating the MetHb in cord blood due to the presence of foetal haemoglobin (Lynch *et al* 1998) must also be considered.

The influence of air pollution with regard to birth defects was investigated in a study. Associations between individual defects and carbon monoxide and ozone were found;

no such associations were found with the other substances (including NO_2; Ritz *et al* 2002).

3.8.2. Animal data

The reproductive toxicology of NO_2 has not been adequately investigated so far.

4. Recommendation

SCOEL considers that available human data on NO_2 obtained in working populations are reliable for recommending an 8-hour OEL, as these are seconded by both experimental human studies of shorter duration and experimental animal studies.

The primary target to be considered is the deep respiratory tract. The long-term longitudinal study in hard-coal miners by Morfeld *et al* (2010) did not reveal clear adverse effects on lung function, under the conditions given in this study (Section 3.5.1). This study included sub-collectives of higher exposures, namely Diesel engine drivers and Diesel train drivers, with average shift exposures of 0.21 and 0.52 ppm NO_2, respectively (Dahmann *et al* 2009). Regarding the distribution of exposures, the 95th percentile of the 8-hour shift data was about two-fold of the mean. Considering these data, the conclusion of a human NOAEC of 0.5 ppm NO_2 regarding lung function, under conditions of chronic occupational exposure, appears to be safe. This NOAEC is not contradicted by earlier studies in salt miners (Lotz *et al* 2008) and in British collieries (Robertson *et al* 1984), which could not discriminate effects of other components (dust and chemicals). Moreover, short-term experimental human studies (see below), showing first effects at about 1.5 ppm NO_2, are consistent with this human NOAEC. This argues in favour of a recommended OEL for NO_2 at 0.5 ppm, which is primarily derived from human data.

A proof of plausibility of this derivation is provided by the available data in experimental animals. In this respect, most older experimental studies can not be considered as a reliable assessment basis, because exposures in these studies were continuous. However, there are recent inhalation studies in rats, performed according to modern standards, with exposures 6 hours/day for 5 days (Section 3.5.2). In these studies, a LOAEC of 5 ppm was obtained after 5 days of exposure (BASF 2006a) and a NOAEC of 2.15 ppm after 13 weeks of exposure (BASF 2006b). In interpreting the experimental NOAEC of 2.15 ppm the following must be considered: (i) There were problems in the analytical measurement of the NO_2 concentrations; the actual experimental exposure levels might have been overestimated. (ii) Uncertainties must be considered with regard to the only subchronic experimental exposure period. On the one hand, from comparison of the results of the subacute (5 days) range finding study with those of the subchronic (90 days) study, it might be concluded that the length of exposure did not play a major role. However, inhalation studies with continuous exposure suggest that effects occur at lower concentrations with an increase in the duration of exposure (from 16 weeks to 27 months; Ichinose and Sagai 1982, Kubuta *et al* 1987, Sagai and Ichinose 1987). Given these prevailing uncertainties, the experimental animal studies are in full support of an OEL of 0.5 ppm for NO_2.

Therefore, SCOEL recommends an OEL (8-hour TWA) for NO_2 of 0.5 ppm.

For limiting short-term exposures (STEL), it must be noted that the critical effects are local, on the respiratory system (Section 3.2.1). Studies with volunteers show that increased bronchial reactivity can be observed after exposure to NO_2 concentrations of 1.5 ppm and more for 3 hours (Frampton *et al* 1989), and increased airway resistance

after exposure to 2 ppm for 4 hours for 4 days (Blomberg *et al* 1999). Marked changes in the BALF, a relevant method for determining toxic effects of NO_2 in the alveolar fluid, were evident after NO_2 concentrations of 1.5 ppm and more (Sandström *et al* 1992) and 2 ppm and more (Blomberg *et al* 1999, Devlin *et al* 1999, Solomon *et al* 2000). After exposure to NO_2 concentrations of 0.6 ppm, evidence of inflammation was inconsistent (Frampton *et al* 1989, 1991 and 2002). Since changes in the BALF were observed in volunteers after a 3-hour exposure to NO_2 at 1.5 ppm and above, *a STEL (15 min) of 1.0 ppm is proposed with a sufficient intrinsic margin of safety.*

There is no indication of relevant skin absorption. Therefore, no "skin" notation is considered necessary. The available data give no indication of systemic genotoxic effects. However, as pointed out above, further studies should evaluate a possible local genotoxicity on airway epithelia.

There are no human studies on effects of low concentrations of NO_2 at workplaces in asthmatics. The studies in miners do not answer this question, as asthmatics are not among the workforce in underground mines. However, the absence of irritative and inflammatory effects at the recommended OEL suggests that this should also be protective in asthmatics.

With regard to analytical measurement (see Section 2.1), no major difficulties are foreseen, based on new technological development.

The present Recommendation was adopted by SCOEL on 11 June 2014.

5. References

ACGIH, American Conference of Governmental Industrial Hygienists (2012). Guide to occupational exposure values 2012. ACGIH, Cincinnati, Ohio.

Adelroth E, Hedlund U, Blomberg A, Helleday R, Ledin MC, Levin JO, Pourazar J, Sandström T, Järvholm B (2006). Airway inflammation in iron ore miners exposed to dust and diesel exhaust. Eur Resp J 27:714-719.

Backé E, Lotz J, Tittelbach U, Plitzko S, Gierke E, Schneider WD (2004). Immunological biomarkers in salt miners exposed to salt dust. Int Arch Occup Environ Health 77:319-327.

BASF (2006a). NO_2 – Subacute 5-day range finding inhalation study in male Wistar rats – gas exposure. Project No 99I0375/03027, BASF AG Ludwigshafen, unpublished (for a data summary, see DFG 2010).

BASF (2006b). Nitrogen dioxide – Subchronic 90-day inhalation study in Wistar rats – gas exposure. Project No 99I0375/03055, BASF AG Ludwigshafen, unpublished (for a data summary, see DFG 2010).

Biggart NW, Rinehart RR (1987). Comparison between aqueous-phase and gas-phase exposure protocols for determining the mutagenic potential of nitrogen dioxide and the gas fraction of welding fumes. Mutat Res 188:175-184.

Blomberg A, Krishna MT, Helleday R, Söderberg M, Ledin MC, Kelly FJ, Frew AJ, Holgate ST, Sandström T (1999). Persistent airway inflammation but accommodated antioxidant and lung function responses after repeated daily exposure to nitrogen dioxide. Am J Respir Crit Care Med 159:536–543.

Bylin G, Lindvall T, Rehn T, Sundin B (1985). Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Eur J Respir Dis 66:205-217.

Bylin G, Hedenstierna G, Lindvall T, Sundin B (1988). Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. Eur Respir J 1:606-612.

Bylin G (1993). Health risk evaluation of nitrogen oxide. Controlled studies on humans. Scand J Work Environ Health 19 (Suppl. 2):37-43.

Carson TR, Rosenholtz MS, Wilinski FT, Weeks MH (1962). The response of animals inhaling nitrogen dioxide for single, short-term exposures. Ind Hyg J 23:257-462.

Chaney S, Blomquist W, DeWitt P, Muller K (1981). Biochemical changes in humans upon exposure to nitrogen dioxide while at rest. Arch Environ Health 36:53-58.

Dahmann D, Monz C (2000). Arbeitsplatzexpositionsprofile (AEP). Ein neues Werkzeug zur Beurteilung von Kurzzeitexpositionen an Arbeitsplätzen. Gefahrstoffe – Reinhaltung der Luft 60(10):397-401.

Dahmann D, Monz C, Sönksen H (2007). Exposure assessment in German potash mining. Int Arch Occup Environ Health 81:95-107.

Dahmann D, Morfeld P, Monz C, Noll B, Gast F (2009). Exposure assessment for nitrogen oxides and carbon monoxide in German hard coal mining. Int Arch Occup Environ Health 82:1267-1279.

DECOS, Dutch Expert Committee on Occupational Standards (2004). Nitrogen dioxide: Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, publication no. 2004/01OSH.

Devlin RB (1999). Inflammatory response in humans exposed to 2.0 ppm nitrogen dioxide. Inhal Toxicol 11:89-109.

DFG, Deutsche Forschungsgemeinschaft (2005). Nitrogen dioxide. In: The MAK-Collection Part I: MAK value documentations (Greim H, ed.), Vol. 21, pp. 205-260. Wiley-VCH GmbH & Co. KGaA, Weinheim. Available online via: http://onlinelibrary.wiley.com/book/10.1002/3527600418/topics.

DFG, Deutsche Forschungsgemeinschaft (2010). Stickstoffdioxid. Nachtrag 2010. In: The MAK-Collection Part I: MAK value documentations (Greim H, ed.). Wiley-VCH GmbH & Co. KGaA, Weinheim. Available online via: http://onlinelibrary.wiley.com/book/10.1002/3527600418/topics.

Druckrey H, Preussmann R (1962). Zur Entstehung carcinogener Nitrosamine am Beispiel des Tabakrauches. Naturwissenschaften 49:498-499.

Feldman JG (1974). The combined action on a human body of a mixture of the main components of motor traffic exhaust gases (carbon monoxide, nitrogen dioxide, formaldehyde and hexane). Gig Sanit 10:7-10.

Folinsbee LJ (1992). Does nitrogen dioxide exposure increase airways responsiveness? Toxicol Ind Health 8:273-283.

Frampton MW, Smeglin AM, Roberts NJ Jr, Finkelstein JN, Morrow PE, Utell MJ (1989). Nitrogen dioxide exposure in vivo and human alveolar macrophage inactivation of influenza virus in vitro. Environ Res 48:179-192.

Frampton MW, Morrow PE, Cox C, Gibb FR, Speers DM, Utell MJ (1991). Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. Am Rev Respir Dis 143:522-527.

Frampton MW, Boscia J, Roberts NJ Jr, Azadniv M, Torres A, Cox C, Morrow PE, Nichols J, Chalupa D, Frasier LM, Gibb FR, Speers DM, Tsai Y, Utell MJ (2002). Nitrogen dioxide exposure: effects on airway and blood cells. Am J Physiol Lung Cell Mol Physiol 282:L 155-165.

Gamble J, Jones W, Hudak J (1983). An epidemiological study of salt miners in diesel and nondiesel mines. Am J Ind Med 4:435-458.

Gamble J, Jones W, Minshall S (1987). Epidemiological-environmental study of diesel bus garage workers: acute effects of NO_2 and respirable particulate on the respiratory system. Environ Res 42:201-214.

Gooch PC, Luippold HE, Creasia DA, Brewen HG (1977). Observations on mouse chromosomes following nitrogen dioxide inhalation. Mutat Res 48:117-120.

Görsdorf S, Appel KE, Engeholm C, Obe G (1990). Nitrogen dioxide induces DNA single-strand breaks in cultured Chinese hamster cells. Carcinogenesis 11:37-41.

Grayson RR (1956). Silage gas poisoning: nitrogen dioxide pneumonia, a new disease in agricultural workers. Ann Intern Med 45:393-408.

Henschler D, Lüdtke W (1963). Methämoglobinbildung durch Einatmung niederer Konzentrationen nitroser Gase. Int Arch Gewerbepathol Gewerbehyg 20:362-370.

Henschler D, Stier A, Beck H, Neumann W (1960). Geruchsschwellen einiger wichtiger Reizgase (Schwefeldioxid, Ozon, Stickstoffdioxid) und Erscheinungen bei der Einwirkung geringer Konzentrationen auf den Menschen. Arch Gewerbepath Gewerbehyg 17:547-570.

Ichinose T, Sagai M (1982). Studies on biochemical effects of nitrogen dioxide. III. Changes of the antioxidative protective systems in rat lungs and of lipid peroxidation by chronic exposure. Toxicol Appl Pharmacol 66:1-8.

Isomura K, Chikajira M, Teranishi K, Hamada K (1984). Induction of mutations and chromosme aberrations in lung cells following in vivo exposure of rats to nitrogen oxides. Mutat Res 136:119-125.

Jacobsen M, Smith TA, Hurley JP, Robertson A, Roscow R (1988). Respiratory infections in coal miners exposed to nitrogen oxides. Institute of Health Effects, Research Report No. 18, Cambridge, Massachusetts, cited in WHO 1997.

Kneepkens CM, Lepage G, Roy CC (1994). The potential of the hydrocarbon breath test as a measure of lipid peroxidation. Free Radical Biol Med 17:127–160; Erratum in: Free Radical Biol Med 17:609.

Kubota K, Murakami M, Takenaka S, Kawai K, Kyono H (1987). Effects of long term nitrogen dioxide exposure on rat lung: morphological observations. Environ Health Perspect 73:157-169.

Kulle TJ (1982). Effects of nitrogen dioxide on pulmonary function in normal healthy humans and subjects with asthma and chronic disease. Stud Environ Sci 21:477-486.

Kumae T, Arakawa H (2006). Comparison of effects of in vivo nitrogen dioxide exposure starting from different periods on alveolar macrophage activity, assessed by a chemiluminescence technique in Brown-Norway rats. Luminescence 21:226–232.

Latza U, Gerdes S, Baur X (2009). Effects of nitrogen dioxide on human health: systematic review of experimental and epidemiologic studies conducted between 2002-2006. Int J Hyg Environ Health 212:271-287.

Lotz J, Plitzko S, Gierke E, Tittelbach U, Kersten N, Schneider WD (2008). Dose-response relationships between occupational exposure to potash, diesel exhaust and nitrogen oxides and lung function: cross sectional and longitudinal study in two salt mines. Int Arch Occup Environ Health 81:1003-1019.

Lynch PL, Bruns DE, Boyd JC, Savory J (1998). Chiron 800 system CO-oximeter module overestimates methemoglobin concentrations in neonatal samples containing fetal hemoglobin. Clin Chem 44:1569-1570.

Mohsenin V (1987). Effect of vitamin C on NO_2-induced airway hyperresponsiveness in normal subjects. Am Rev Respir Dis 136:1408-1411.

Mohsenin V (1988). Airway responses to 2.0 ppm nitrogen dioxide in normal subjects. Arch Environ Health 43:242-246.

Morfeld P, Noll B, Büchte SF, Derwall R, Schenk V, Bicker HJ, Lenaerts H, Schrader N, Dahlmann D (2010). Effects of dust exposure and nitrogen oxides on lung function parameters of German coal miners: a longitudinal study applying GEE regression 1974-1998. Int Arch Occup Environ Health 83:357-371.

Mücke H-G, Wagner HM (1998) VI-1. Anorganische Gase/Stickstoffdioxid (inorganic gases/nitrogen dioxide) (German). In: Wichmann HE, Schlipköter HW, Fülgraff G (Eds). Handbuch Umweltmedizin, 14th Supplement 10/98, Ecomed-Verlag, Landsberg/Lech.

National Research Council of the National Academies: Committee on Acute Exposure Guideline Levels, Committee on Toxicology, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies (2012). Nitrogen oxides. In: Acute exposure guideline levels for selected chemicals, Volume 11, p. 167-256. National Academies Press, Washington, DC.

Posin C, Clarks K, Jones MP, Patterson JY, Buckley RD, Hackney JD (1978). Nitrogen dioxide inhalation and human blood biochemistry. Arch Environ Health 33:318-324.

Ritz B, Yu F, Fruin S, Chapa G, Shaw GM, Harris JA (2002). Ambient air pollution and risk of birth defects in Southern California. Am J Epidemiol 155:17-25.

Robertson A, Dodgson J, Collings P, Seaton S (1984). Exposure to oxides of nitrogen: respiratoy symptoms and lung function in British coal miners. British J Ind Med 41:214-219.

Sagai M, Ichinose T, Kubota K (1984). Studies on the biochemical effects of nitrogen dioxide. Toxicol Appl Pharmacol 73:444-456.

Sagai M, Ichinose T (1987). Lipid peroxidation and antioxidative protection mechanism in rat lungs upon acute and chronic exposure to nitrogen dioxide. Environ Health Perspect 73:179-189.

Sandström T, Ledin MC, Thomasson L, Helleday R, Stjernberg N (1992). Reductions in lymphocyte subpopulations after repeated exposure to 1.5 ppm nitrogen dioxide. Br J Ind Med 49:850-854.

Shalamberidze OP, Tsereteli NT (1971). Effect of small concentrations of sulfurous gas and nitrogen dioxide on the estrual cycle and the genital function of animals in experiments (Russian). Gig Sanit 8:13-17.

Solomon C, Christian DL, Chen LL, Welch BS, Kleinman MT, Dunham E, Erle DJ, Balmes JR (2000). Effect of serial-day exposure to nitrogen dioxide on airway and blood leukocytes and lymphocyte subsets. Eur Respir J 15:922-928.

Tabacova S, Baird DD, Bablabaeva L (1998). Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. Arch Environ Health 53:214-221.

Tsuda H, Kushi A, Yoshida D, Goto F (1981). Chromosomal aberrations and sister-chromatid exchanges induced by gaseous nitrogen dioxide in cultured Chinese hamster cells. Mutat Res 89:303-309.

US EPA (2008). Integrated science assessment for oxide of nitrogen EPA/600/R-08/072.

Wegmann M, Renz H, Herz U (2002/2003). Long-term NO_2 exposure induces pulmonary inflammation and progressive development of airflow obstruction in C57BL/6 mice: a mouse model for chronic obstructive pulmonary disease? Pathobiology 70:284–286.

WHO, World Health Organization (1997). Nitrogen oxides (second edition). Environmental Health Criteria 188, 550 pp. WHO, Geneva.