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

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8-hour TWA: 0.5 ppm (0.955 mg/m³)
STEL (15-min): 1 ppm (1.91 mg/m³)
BLV: -
Notation: -


1. Substance identification, physico-chemical properties

Chemical name: Nitrogen dioxide
Synonyms: Nitrogen peroxide
Molecular formula: NO₂
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/m³ = 1 ppm
(20 °C, 101.3 kPa) 1 mg/m³ = 0.524 ppm

EU 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 (NO₂) 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/m³) (US EPA 2008, DFG 2005).

2. Occurrence/Use

Nitrogen dioxide (NO₂) is found in ambient air as a product of natural as well as human activities. Production of NO₂ 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 NO₂ and nitric oxide (NO) is ubiquitous. Oxidation of NO to NO₂ may easily occur in some circumstances.

2.1. Analytical measurement

There are different methods for the measurement of airborne NO₂ (electrochemical cell, chemiluminescence). The lowest detection limit (LDL) of 0.002 ppm is reached by using chemiluminescence. Chemiluminescence measurements are considered to represent the “gold standard” of NO/NO₂ analysis (LDL: 0.002 ppm). However, there are restrictions to the applicability of this method in underground mining (especially coal mining) or at workplaces where heat is involved (Dahmann et al. 2007, 2009). By contrast, the “multiwarn” electrochemical device, which allows personal air sampling, has an LDL of only 1 ppm, but the “multiwarn” instrument is suitable for the measurements of even very short exposure peaks (1 min). In essence, with the presently available standard instrumentation the occupational exposure limit (OEL) of 0.5 ppm NO₂, as recommended here, may not be adequately controlled in underground coal mines. However, for other workplaces, this not the case.

3. Health significance

The critical effect of NO₂ is irritation of the deep compartment of the respiratory tract in both animals and man. NO₂ 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

NO₂ 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 NO₂ 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 NO₂ or its products can remain in the lungs for a longer period (WHO 1997). In man, 80–90 % NO₂ is taken up via the respiratory tract during normal breathing, and over 90 % at maximum breathing. Dosimetric model calculations showed that NO₂ is absorbed mainly in the lower respiratory tract, where it 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 NO₂ 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 (see DFG 2005). Evidence of increased bronchial reactivity was observed after NO₂ 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\textsubscript{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\textsubscript{2} gas (5 000 ppm) was mixed with pure air and introduced into an exposure chamber (45 m\textsuperscript{3}) to generate the desired NO\textsubscript{2} concentration. More than 90 % of the desired NO\textsubscript{2} concentrations were reached within 4 minutes. This publication includes no data on the NO\textsubscript{2} analysis. It is assumed that the method described in the earlier publications by Frampton \textit{et al} (1989, 1991) was used. There, continuous measurement was applied using an EPA reference standard NO/NO\textsubscript{x} analyzer, which was calibrated with standardised NO\textsubscript{2} and NO gas and verified by means of a colorimetric method. The background concentrations of NO\textsubscript{x}, ozone, dust and SO\textsubscript{x} were recorded continuously, but the results were not reported. No significant changes of spirometric parameters were observed. No effect of \textit{in vivo} exposure to NO\textsubscript{2} on the inactivation of influenza viruses \textit{in vitro} in alveolar macrophages or in bronchial epithelial cells was found, as had been described in a previous study (Frampton \textit{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\textsubscript{2} concentrations. The authors pointed out that such effects had also been observed in another volunteer study at NO\textsubscript{2} concentrations of 1 and 2 ppm (Posin \textit{et al} 1978) and in mice. 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\textsuperscript{+}/CD8\textsuperscript{+} 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 were more pronounced at 0.6 ppm than at 1.5 ppm. As in the bronchial lavage, an increased number of CD4\textsuperscript{+} cells and an increased ratio of CD4\textsuperscript{+}/CD8\textsuperscript{+} 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\textsuperscript{+} 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\textsubscript{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 \textit{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\textsubscript{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\textsuperscript{nd} day, 6 times; Sandström \textit{et al} 1992), an increased number of CD25\textsuperscript{+} lymphocytes and HLA-DR\textsuperscript{+} macrophages in the bronchoalveolar lavage and neutrophils in the bronchial fraction (2 ppm NO\textsubscript{2} for 4 hours on 4 consecutive days; Blomberg \textit{et al} 1999), a lower fraction of CD4\textsuperscript{+} cells and an increased number of neutrophils in the bronchial fraction (Solomon \textit{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\textsubscript{2} for 4 hours, once; Devlin 1999; see DFG 2005 and 2010 for details).

Effects of NO\textsubscript{2} on lung function and airway responsiveness in healthy individuals were reported to occur at concentrations above 1 ppm (1.9 mg/m\textsuperscript{3}) (Folinsbee 1992, Bylin 1993). Exposure to 0.6 ppm (1.1 mg/m\textsuperscript{3}) NO\textsubscript{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 NO₂ 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/m³) for 2 hours (Chaney et al 1981), but this effect is considered to be of less biological importance.

Studies on the effects of NO₂ on pulmonary function in asthmatics and in patients with chronic lung disease or bronchitis were reviewed in extenso by the 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 NO₂ may respond with either subjective symptoms or slight changes in pulmonary function, which were rated to be of no clinical significance. Children (5–12 years old) were identified as a potential population subgroup with higher susceptibility to NO₂. As children of this age are not exposed occupationally, such studies are not considered here.

3.2.2. Animal data

For rats, the LC₅₀ after exposure for 15 minutes was given as about 200 ppm and after 60 minutes as 115 ppm (Carson et al 1962). The LC₅₀ values for various strains of rat after exposure to NO₂ for 16 hours were given as 39–56 ppm. Mice, depending on the strain, were found to have LC₅₀ values of 33–67 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 LC₅₀ after exposure for 15 minutes was found to be 315 ppm. For dogs, 53 ppm was given as the 50 % value of the LC₅₀ (corresponding to an LC₅₀ of about 105 ppm) after exposure to NO₂ for 60 minutes. In rats and rabbits, severely impaired breathing, eye irritation and reduced body weights were observed (Carson et al 1962). For a description of other animal experiments with single exposures, see WHO (1997).

3.3. Sensory threshold and irritation

NO₂ has a penetrating odour. Depending on the study conditions, the perception threshold is between 0.1 and 0.2 ppm (Feldman 1974, Shalamberidze 1967). 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 NO₂ 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 led 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 NO₂, 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 NO₂. However, as a result of the exposure to a mixture of substances these studies are, unsuitable for the adequate evaluation of NO₂-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 NO₂ concentration was determined during the shift. Concentrations of other irritant gases were below the US national standards. The highest NO₂ 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 NO₂ 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 NO₂ exposure (Gamble et al 1983).

In a 4-year study with 560 coal mining workers exposed to Diesel soot, no impairment in lung function (FEV₁) and no respiratory symptoms were observed. The concentrations of NO₂ were in the range between 0.02 and 0.06 ppm in air, the concentrations of NO were much higher and between 0.13 and 1.19 ppm (Robertson et al 1984).

In a study with 20 000 coal mining workers exposed on average to NO₂ concentrations of 0.03 ppm and NO concentrations of 0.2 ppm, workers were not absent due to illness more frequently because of airway infections. There were, however, problems in this study as regards the classification of exposure (Jacobsen et al 1988).

Robertson et al (1984) measured the levels of NO₂ and NO in nine British collieries between 1974 and 1979, which ranged, respectively, from 0.02 to 0.08 ppm and from 0.11 to 1.23 ppm depending on colliery and type of work. No relationship was found between exposure and respiratory symptoms or decline in FEV₁, nor were 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 derivation of a recommended 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 NO₂ levels ranging from 0.14 to 0.5 ppm (Backe et al 2004, Adelroth et al 2006, Latza et al 2009).

Recently, two larger epidemiological studies were 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 study was published by Dahmann et al (2007), 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 twofold of the mean, both for NO and for NO₂.

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 chemiluminescence 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/NO₂ in 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, NO₂ 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 FEV₁ 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/m³ inhalable dust, 2.4 and 0.8 mg/m³ respirable dust, 0.09 and 0.09 mg/m³ Diesel exhaust, 0.4 and 0.5 ppm NO₂ and 1.7 and 1.4 ppm NO (mine 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 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, NO₂, smoking behaviours, and the lung function parameters FVC, FEV₁ and FEV₁/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/m³ (quartz: 0.067 mg/m³), and nitrogen oxide concentrations were 0.58 ppm NO and 0.007 ppm NO₂. However, the exposure in defined subgroups was consistently higher (mean 8-hour shift concentrations: Diesel engine drivers, 1.35 ppm NO, 0.21 ppm NO₂; Diesel train drivers, 1.35 ppm NO, 0.52 ppm NO₂; blasting specialists, 0.84 ppm NO, 0.014 ppm NO₂; see Dahmann *et al* 2009). The GEE regression models did not reveal clear adverse dust exposure effects. Nitrogen oxides combined (NO + NO₂) 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 twofold 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 by WHO (1997). Many animal studies have shown that long-term (continuous) exposure to NO₂ 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 per day) to NO₂ at 0, 0.4, 1.2 and 4 ppm for 1, 2, 4, 8, 12 and 16 weeks (Ichinose and Sagai 1982) and to NO₂ 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 NO₂ 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 NO₂, 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 GSH-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 no observed adverse effect concentration (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 to a maximum of 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 sulphhydril 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 lowest observed adverse effect concentration (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 NO₂, 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 NO₂ exposure and to investigate the activity of alveolar macrophages and the production of cytokines. Males and females were exposed to
NO\textsubscript{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\textsubscript{2} in whole-body exposure chambers on 5 days for 6 hours per day. The desired NO\textsubscript{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 IR spectrophotometer with NO\textsubscript{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\textsubscript{2} at the apparatus and problems with the correct regulation of the supply of air and NO\textsubscript{2} as possible causes of the low NO\textsubscript{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 bronchoalveolar lavage fluid (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 and an increased activity of \(\gamma\)-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\textsubscript{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\textsubscript{2} in whole-body exposure chambers at 0, 0.1, 0.5 and 1 ppm on 5 days per week for 6 hours per 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\textsubscript{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 genotoxic effect 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\textsubscript{2}; Ritz et al 2002).

### 3.8.2. Animal data

The reproductive toxicology of NO\textsubscript{2} has not been adequately investigated so far.

### 4. Recommendation

SCOEL considers that available human data obtained in working populations are reliable for deriving a recommended 8-hour OEL, as these are seconded by both experimental human studies of shorter duration of NO\textsubscript{2} exposure 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\textsubscript{2}, respectively (Dahmann et al 2009). Regarding the distribution of exposures, the 95\textsuperscript{th} percentile of the 8-hour shift data was about 2-fold of the mean. Considering these data, the conclusion of a human NOAEC of 0.5 ppm NO\textsubscript{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\textsubscript{2}, are consistent with this human NOAEC. This argues in favour of a recommended OEL for NO\textsubscript{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 cannot 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\textsubscript{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. But 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 studies are in full support of a recommended OEL of 0.5 ppm for NO\textsubscript{2}.

Therefore, **SCOEL proposes an OEL (8-hour TWA) for NO\textsubscript{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 bronchoalveolar lavage, 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, 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.

With regard to possible measurement difficulties in certain situations, see Section 2.1.

The present Recommendation was adopted by SCOEL on Date Month Year.
5. References


