Nitrogen dioxide

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

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Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen.

In dat kader bied ik u hierbij een advies aan over stikstofdioxide. Dit advies is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

De afhandeling van dit advies na de openbare commentaar fase heeft langer geduurd dan gebruikelijk is. Dit komt omdat de commissie veel commentaar heeft ontvangen.

Ook wil de commissie u erop attenderen dat de buitenluchtconcentraties van stikstofdioxide in sommige stedelijke gebieden zo hoog kunnen liggen dat deze dicht bij de gezondheidskundige advieswaarden voor stikstofdioxide komen.

Verder bespreekt de commissie in dit advies alleen de effecten van blootstelling aan stikstofdioxide, en gaat zij niet in op de gevolgen van mengselblootstelling met andere stikstofoxiden, gassen en deeltjes.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieu.

Hoogachtend,

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Nitrogen dioxide

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupatoinal Standards,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2004/01OSH, The Hague, 5 February 2004

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Inhoud

Samenvatting en advieswaarde

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie WGD van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht, waarbij beroepsmatige blootstelling kan plaatsvinden. Deze aanbevelingen vormen de eerste stap in een drietrapsprocedure die moet leiden tot wettelijke grenswaarden (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan stikstofdioxide en presenteert zij een gezondheidskundige advieswaarde voor die stof. De conclusies van de commissie zijn gebaseerd op gegevens uit wetenschappelijke publicaties die vóór juni 2002 zijn verschenen, waarbij de rapporten van de Scientific Committee on Occupational Exposure Limits (SCOEL) van de Europese Gemeenschap (SCO93, SCO97) als één van de bronnen van literatuur voor deze evaluatie dienden.

Fysische en chemische eigenschappen

Stikstofdioxide (NO_2; CAS nr. 10102-44-0) kan voorkomen als een kleurloze vaste stof, een gele vloeistof of als een roodbruin gas. In vaste of vloeibare vorm komt stikstofdioxide voornamelijk als een dimeer voor (distikstoftetroxide, N_2O_4). Stikstofdioxide heeft een irriterende geur met een reukgrens die ligt tussen de 0,2 en 0,8 mg/m^3 (0,10-0,41 ppm). De molaire massa is 46,01 g/mol, het smeltpunt -9,3 °C en het kookpunt 21,2 °C. Stikstofdioxide reageert met water, waardoor vrije nitraat- en

nitrietionen worden gevormd. Het is goed oplosbaar in onder andere alkalische oplosmiddelen en chloroform.

Stikstofdioxide wordt voornamelijk gebruikt als chemisch intermediair in allerlei chemische industriële processen. Daarnaast wordt de stof onder andere gebruikt voor het maken van geoxideerde cellulose verbindingen en dient het als oxidant in brandstof voor raketten.

Monitoring

Er bestaan verschillende methoden om stikstofdioxide in de lucht te kunnen bemonsteren en te analyseren. Zowel de 'National Institute for Occupational Safety and Health (NIOSH)' als de 'Occupational Safety and Health Asminsitration (OSHA)' bevelen aan luchtmonsters te nemen met behulp van grids, geïmpregneerd met triethanolamine. Deze luchtmonsters kunnen worden geanalyseerd door middel van absorptiespectrometrie, zoals NIOSH aanbeveelt, of met diverse andere beschikbare analyse methoden, zoals gaschromatografie en differentiële puls polarografie.

Een betrouwbare analyse van stikstofdioxide in bloed of in andere biologische monsters is niet beschikbaar.

Grenswaarden

In 1985 heeft de Commissie WGD een gezondheidskundige advieswaarde voor stikstofdioxide geadviseerd van 0,5 mg/m^3, gemiddeld over een achturige werkdag (tgg 8 uur), en van 1,0 mg/m^3 (tgg 15 minuten). Echter door sociaal-economische beperkingen is in Nederland een wettelijke grenswaarde van 4 mg/m^3 (tgg 8 uur) en geen grenswaarde tegen kortdurende blootstelling vastgesteld.

De SCOEL van de Europese Commissie heeft in 1997 een grenswaarde voorgesteld van 0,4 mg/m^3 bij langdurige blootstelling (tgg 8 uur) en van 1,0 mg/m^3 bij kortdurende blootstelling (tgg 15 min). In andere landen, zoals Zweden, Denemarken, het Verenigd Koninkrijk en de Amerikaanse organisatie ACGIH zijn grenswaarden vastgesteld tussen de 4 en 6 mg/m^3 (tgg 8 uur). In dezelfde landen en organisaties zijn grenswaarden voor kortdurende blootstelling (tgg 15 min) of 'ceiling'-waarden voor- of vastgesteld variërend van 9 tot 10 mg/m^3, met uitzondering van NIOSH die 1,8 mg/m^3 heeft geadviseerd.

Kinetiek

Stikstofdioxide bereikt de diepere ademhalingswegen en longen. Daar lost het vervolgens gemakkelijk op in het waterrijke slijm dat het longweefsel bedekt. Dit

resulteert in de vorming van nitriet- en nitraatzouten of in de overeenkomende salpeter- of salpeterigzuren. Aangenomen wordt dat juist deze zouten en niet zozeer de stikstofdioxidemoleculen zelf, worden opgenomen in en verspreid door het lichaam via de bloedbaan. Het nitriet wordt in het lichaam vrij eenvoudig omgezet in nitraat, dat vervolgens snel door het lichaam wordt uitgescheiden via de urine.

Stikstofdioxide is een sterk oxiderende stof. Dit betekent dat het schade kan toebrengen aan de vetzuren aanwezig in celmembranen en aan eiwitten.

Effecten

De vele wetenschappelijke publicaties over de schadelijke gezondheidseffecten van stikstofdioxide bij zowel mensen als dieren kenmerken zich door een brede variatie aan blootstellingsconcentraties en -perioden en een grote variatie in onderzochte effecten. Omdat deze evaluatie bedoeld is om een gezondheidskundige advieswaarde te adviseren, heeft de commissie zich beperkt tot die gegevens waarin sprake was van een lage blootstelling met een voor de arbeidssituatie relevant biologisch effect.

Mensgegevens

In verschillende onderzoeken veroorzaakte blootstelling aan stikstofdioxide irritatie aan de ogen en bovenste luchtwegen (neus, keel). Uit epidemiologisch onderzoek en uit praktijksituaties blijkt verder dat stikstofdioxide het functioneren van de longen aan kan tasten. In de ergste gevallen was sprake van longoedeem en ontstekingen in de bronchiën en de longen. Naar aanleiding van bovenstaande bevindingen zijn veel klinische onderzoeken uitgevoerd naar de relatie tussen stikstofdioxideblootstelling en longeffecten.

Deze klinische onderzoeken betroffen echter vaak een eenmalige blootstelling van niet meer dan 15 minuten tot een paar uur, soms gecombineerd met lichamelijke inspanning, bijvoorbeeld op een fietsergometer. Dergelijke blootstellingen leidden vrijwel direct na de start van de blootstelling tot een verhoogde luchtwegreactiviteit en -weerstand, waardoor ademhaling moeilijker wordt. In het algemeen traden deze effecten op vanaf een blootstellingsconcentratie van 3,8 mg/m^3 (2 ppm). Onder deze concentratie waren de gegevens minder eenduidig. De laagste concentratie waarbij significante effecten optraden (toegenomen luchtwegreactiviteit tegen carbachol) was 2,9 mg/m^3. De ondergrens waarbij geen duidelijke luchtwegeffecten meer zijn geconstateerd lag bij ongeveer 1,0 mg/m^3.

In één klinisch onderzoek is het effect van piekblootstelling bestudeerd. In dat onderzoek werden vrijwilligers drie uur lang blootgesteld aan 0,1 mg/m^3 stikstofdioxide; tevens werden zij tijdens die drie uur drie keer blootgesteld aan 3,8

mg/m^3 gedurende telkens 15 minuten. Deze piekblootstellingen leidden echter niet tot noemenswaardige effecten op longfunctie en afweer.

In andere klinische onderzoeken is onderzocht in hoeverre herhaalde blootstelling een effect op de luchtwegen en de longen had. De gegevens van deze onderzoeken vindt de commissie echter te beperkt om een duidelijke conclusie te kunnen trekken of en in welke mate herhaalde blootstelling gerelateerd is aan de bepaalde effecten.

Epidemiologisch onderzoek onder de algemene en beroepsbevolking toonde een duidelijke relatie aan tussen stikstofdioxideblootstelling en ziekten aan de luchtwegen. Echter, voorzichtigheid is geboden bij de interpretatie van deze epidemiologische gegevens. In veel van deze epidemiologische onderzoeken spelen namelijk andere factoren mee die de resultaten kunnen hebben beïnvloed. Het gaat hierbij onder andere om de participatie van rokers en gevoelige mensen (ouderen, kinderen) en om mengselblootstelling met andere toxische stoffen.

Diergegevens

De effecten die zijn gezien bij mensen na een eenmalige blootstelling, zijn ook waargenomen bij dieren. Daarnaast zijn bij dieren ook effecten op het longweefsel zelf en op biochemische parameters beschreven. Behalve eenmalige blootstellingen zijn veel dieronderzoeken uitgevoerd naar de schadelijke effecten van stikstofdioxide bij langdurige blootstelling.

Aangaande eenmalige en kortdurende blootstellingen, zijn effecten als verminderde afweer tegen infecties, activatie van het antioxidant afweermechanisme en beschadigingen van het longweefsel gevonden bij concentraties vanaf 1,0 mg/m^3 en hoger. Bij lagere blootstelling worden de gegevens minder duidelijk. De laagste concentratie waarbij nog schadelijke effecten zijn waargenomen is 0,65 mg/m^3. Bij deze concentratie vertoonden muizen, die 6 weken lang werden blootgesteld, schade aan longcellen.

Een aantal van bovengenoemde effecten verergerde naarmate de blootstelling langer duurde. Zo leidde bijvoorbeeld langdurige blootstelling tot een hogere sterfte en een afgenomen overlevingstijd in dieren die werden besmet met bacteriën of virussen. Verder nam de weefselschade aan de longen dermate toe dat deze onherstelbaar was (longfibrose of -emfyseem). Dergelijke ernstige effecten zijn beschreven bij blootstelling aan 0,96 mg/m^3 stikstofdioxide en hoger. Ook onder de 0,96 mg/m^3 zijn dergelijke effecten beschreven, maar bijna al het onderzoek bij deze lage concentraties vertoonde tekortkomingen of betroffen slechte rapportages. In één van die onderzoeken werden ratten continu blootgesteld aan 0,08, 0,76 of 7,6 mg/m^3 stikstofdioxide gedurende 27 maanden. Als gevolg van deze blootstelling, vertoonden de longen lichte (0,76 mg/m^3) tot zware (7,6 mg/m^3) weefselschade in de vorm van hyperplasie en

fibrose. De laagst-blootgesteld groep vertoonde geen noemenswaardige longschade. In een ander onderzoek werden ook geen noemenswaardige morfologische veranderingen geconstateerd in de longen van honden die waren blootgesteld aan 0,26 mg/m^3 (plus een hoge concentratie stikstofmonoxide (2,05 mg/m^3)) gedurende 86 maanden (gevolgd door een herstelperiode van 36 maanden). Verder zijn geen noemenswaardige afwijkingen gevonden in de longen van muizen die een jaar lang waren blootgesteld aan 0,38 mg/m^3. In hetzelfde muizenonderzoek zijn ook geen veranderingen in longfunctie en afweer gevonden.

Naast de kort- en langdurende onderzoeken, zijn ook onderzoeken uitgevoerd naar de effecten van piekblootstelling. Deze onderzoeken beperkten zich voornamelijk tot effecten van stikstofdioxide op de afweer tegen infecties. Hoewel de commissie zich realiseert dat piekblootstellingen een dagelijkse praktijk is, is zij van mening dat piekblootstellingen moeilijk te onderscheiden zijn van de continue blootstelling.

Tot slot is in een enkel dieronderzoek aandacht besteed aan immunologische effecten. Het lijkt erop dat stikstofdioxide het immuunsysteem kan beïnvloeden. Voor de commissie is echter niet duidelijk of deze effecten normale biologische reacties zijn of dat het om schadelijke effecten gaat.

Kankerverwekkende eigenschappen

Onderzoek naar de kankerverwekkende eigenschappen van stikstofdioxide is beperkt en geeft geen betrouwbare informatie. Wel was stikstofdioxide genotoxisch in enkele genotoxiciteitstesten, maar ook hier zijn de gegevens beperkt en is de betekenis van de positieve resultaten niet altijd duidelijk. De commissie is daarom van mening dat stikstofdioxide onvoldoende is onderzocht op mogelijke kankerverwekkende en genotoxische eigenschappen. Volgens de EU richtlijnen, adviseert zij daarom stikstofdioxide niet te classificeren.

Effecten op de voortplanting

De uitkomsten van enkele dierexperimentele onderzoeken naar effecten op de vruchtbaarheid en op eventuele ontwikkelingsstoornissen zijn niet eenduidig en van onvoldoende kwaliteit om een duidelijke conclusie te kunnen trekken of stikstofdioxide schadelijk is voor de vruchtbaarheid en het nageslacht.

Evaluatie en advies

De commissie beschouwt de effecten op de longen en diepere luchtwegen opgewekt door inademing van stikstofdioxide, als de meest gevoelige waarop de

gezondheidskundige advieswaarde moet worden gebaseerd. Deze effecten omvatten longfunctieveranderingen, verhoogde gevoeligheid voor infecties en op de lange termijn ernstige weefselschade aan de longen. Deze effecten zijn voornamelijk lokaal van aard en treden niet alleen op na langdurige blootstelling, maar ook na kortdurende blootstelling. Om de werknemers te beschermen tegen deze schadelijke effecten is een gezondheidskundige advieswaarde nodig voor zowel korte (tgg 15 minuten) als langdurige (tgg 8 uur) blootstellingen.

Gezondheidskundige advieswaarde tegen korte termijn effecten (tgg 15 minuten)

De gezondheidskundige advieswaarde tegen korte termijn effecten (tgg 15 min) is gebaseerd op de complete set aan humane onderzoeken met kortdurende eenmalige blootstellingen. Uit de gegevens afkomstig van deze onderzoeken valt af te leiden dat duidelijke effecten op de longfunctie en op de respiratoire weerstand optreden vanaf 3,8 mg/m^3 en hoger. Het laagste significante effect, te weten een toegenomen luchtweg reactiviteit tegen carbachol, is gevonden bij 2,9 mg/m^3. Tussen de 1,1 en 2,9 mg/m^3 zijn slechts een paar onderzoeken uitgevoerd. De meeste van deze onderzoeken vertoonden beperkingen, zoals het lage aantal vrijwilligers, deelname van rokers en de afwezigheid van een gedetailleerd rapport. Dit in tegenstelling tot onderzoeken waarbij vrijwilligers aan 1,0 mg/m^3 stikstofdioxide zijn blootgesteld. Bij deze blootstelling zijn niet alleen meer onderzoeken beschikbaar, maar namen ook veel meer vrijwilligers deel. In geen van de aan 1,0 mg/m^3 blootsgestelde vrijwilligers zijn significante respiratoire effecten gevonden. Dit alles in overweging nemende beschouwt de commissie 1,0 mg/m^3 als de NOAEL. Correctie van deze concentratie voor mogelijke interindividuele verschillen is niet nodig, omdat er een grote en consistente gegevensbestand beschikbaar is.

De commissie beschouwt daarom 1,0 mg/m^3 als de gezondheidskundige advieswaarde voor stikstofdioxide, gemiddeld over een 15 minuten durende werkperiode (tgg 15 minuten).

Gezondheidskundige advieswaarde tegen lange termijn effecten (tgg 8 uur)

De commissie baseert de achturige gezondheidskundige advieswaarde op diergegevens, omdat het aantal mensgegevens over langdurige blootstelling beperkt is en voor een deel onvoldoende van kwaliteit. Het meest zorgwekkende effect dat na langdurige blootstelling is waargenomen zijn de veranderingen in het longweefsel, die wijzen op het ontstaan van longfibrose. Longfibrose is een progressieve ziekte, die afhankelijk van de ernst tot levensbedreigende hartklachten kan leiden. De achtuurswaarde is er dan ook op gericht dit te voorkomen.

Er is één chronisch dieronderzoek uitgevoerd naar de relatie tussen de blootstellingsconcentratie en morfologische longafwijkingen bij relatief zeer lage concentraties (0,08, 0,76 en 7,6 mg/m^3). Daaruit kwam een niet-waargenomen-nadelig-effect-niveau (NOAEL) van 0,08 mg/m^3 naar voren en een laagst-waargenomen-nadelig-effect-niveau (LOAEL) van 0,76 mg/m^3. Het verschil tussen de NOAEL en LOAEL is echter erg groot (factor 10); de werkelijke NOAEL kan dus een stuk hoger liggen. Andere dieronderzoeken bevestigen dit vermoeden en geven aan dat onder 0,38 mg/m^3 geen noemenswaardige chronische effecten te verwachten zijn. Deze onderzoeken in overweging nemende, heeft de commissie besloten 0,38 mg/m^3 als de NOAEL te beschouwen.

Voor de vaststelling van een gezondheidskundige advieswaarde wordt rekening gehouden met verschillende onzekerheden. Eén van die onzekerheden is het verschil tussen diersoorten. De NOAEL is echter gebaseerd op dieronderzoeken waarin drie verschillende diersoorten zijn gebruikt. Al deze diersoorten vertoonden effecten binnen een nauw concentratiebereik. Vanwege deze redenen is de commissie van mening dat compensatie voor soortverschillen niet nodig is. Een andere onzekerheid is dat mensen onderling, door bijvoorbeeld genetische factoren en verschillen in levensstijl, verschillend kunnen reageren op stikstofdioxide blootstelling. Daarvoor wordt de NOAEL gecompenseerd met een factor van 3. Tenslotte is er het gegeven dat de dieren waarop de NOAEL is gebaseerd bijna altijd continu zijn blootgesteld. Dit betekent dat zij geen of nauwelijks tijd hadden om zich te herstellen van de door de blootstelling ontstane longweefselschade en dus deze schade makkelijker kon verergeren in longfibrose. Dit in tegenstelling tot de arbeidssituatie, waarin mensen slechts voor een deel van de dag zijn blootgesteld en er dus na de blootstelling tijd is om van de eventuele weefselschade te herstellen. In hoeverre de hersteltijd het proces van longfibrose exact vertraagd of zelfs voorkomt is moeilijk vast te stellen. Echter, zich baserend op de wet van Haber, gaat de commissie ervan uit dat de hoogte van de onzekerheidsfactor voor de hersteltijd de onzekerheidsfactor voor interindividuele verschillen opheft.

Alles overwegende, beveelt de commissie een gezondheidskundige advieswaarde voor stikstofdioxide aan van 0,4 mg/m^3 (afgerond), ter voorkoming van lange termijn effecten van stikstofdioxide.

Gezondheidskundige advieswaarde

De Commissie WGD van de Gezondheidsraad beveelt een gezondheidskundige advieswaarde voor beroepsmatige blootstelling aan stikstofdioxide aan van 0,4 mg/m^3, gemiddeld over een achturige werkdag (tgg 8 uur) en van 1,0 mg/m^3, gemiddeld over een 15 minuten durende werkperiode (tgg 15 min).

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in air in the workplace. The recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). They constitute the first step in a three-step procedure that leads to legally binding limit values.

In this report, the committee discusses the consequences of occupational exposure to nitrogen dioxide and recommends a health-based occupational exposure limit (HBR-OEL). The committee's conclusions are made on literature retrieved from the documents produced by the Scientific Committee on Occupational Exposure Limits of the European Union (SCOEL; SCO93, SCO97) and on additional scientific papers and reviews that have been published prior to June 2002.

Physical and chemical properties

Nitrogen dioxide (NO_2; CAS no. 10102-44-0) exists as a colourless solid, a yellow liquid, or as a reddish-brown gas. Solid and liquid nitrogen dioxide is present in the form of the dimer dinitrogen tetroxide (N_2O_4). The substance has an irritating odour; its odour threshold ranges between 0.2 and 0.8 mg/m^3 (0.10-0.41 ppm). The molar mass of nitrogen dioxide is 46.01, its melting point -9.3 °C, and its boiling point 21.15 °C.

Nitrogen dioxide dissociates in water into free nitrate and nitrite ions or into the corresponding acids. Furthermore, the compound is soluble in alkalic solvents and chloroform.

Regarding its use, nitrogen dioxide is primarily used as a chemical intermediate. In addition, the substance is also used to manufacture oxidized cellulose compounds and as an oxidising agent in rocket fuels.

Monitoring

Various sampling and analysis techniques are available for determining ambient concentrations of nitrogen dioxide in an occupational setting.

Both the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) recommend using samplers containing a triethanolamine-impregnated molecular sieve.

Air samples can be analysed using various methods, such as: infrared absorption spectroscopy; gas chromatography; differential pulse polarography; and, colorimetric spectrophotography. In particular, NIOSH recommends using visible absorption spectrophotometry (working range for a 3-L air sample: 1 to 47 mg/m^3 (0.5 to 25 ppm)).

Concerning biological monitoring, no validated method is available.

Limit values

In 1985, the DECOS recommended an HBR-OEL for nitrogen dioxide of 0.5 mg/m^3 (8-h TWA) and of 1.0 mg/m^3 (15-min TWA). However, due to socio-economic constraints, the Netherlands has set a legal occupational exposure limit of 4 mg/m^3 (8-h TWA). More recently, the SCOEL has recommended OELs of 0.4 mg/m^3 (8-h TWA) and of 1.0 mg/m^3 (15-min TWA; STEL). Sweden, Denmark, the United Kingdom and the ACGIH of the United States have set OELs for nitrogen dioxide ranging from 4 to 6 mg/m^3 (8-h TWA). In addition, Sweden, Denmark, the United Kingdom, and the American organisations ACGIH and OSHA have set or proposed short-term OELs (15-min TWA) or ceiling values that ranged from 9 to 10 mg/m^3. Only NIOSH has recommended a lower short-term OEL, namely of 1.8 mg/m^3.

Kinetics

Nitrogen dioxide reaches easily the lower respiratory tract. On the surface of the epithelial lung cells, it dissolves in lung fluids, forming products, such as nitrous and nitric acid and their respective nitrite and nitrate salts. It is assumed that mainly these products, and not the nitrogen dioxide molecules, are absorbed by the lung epithelium

and spread through the body via the bloodstream. In the body, nitrite is easily converted into nitrate, which is quickly released from the body as urinary nitrate.

Nitrogen dioxide is an oxidant. As such, it may damage cellular membrane lipids and proteins.

Effects

A large number of human and animal studies have been published on the adverse health effects of nitrogen dioxide inhalation. In these studies, a broad range of exposure levels, exposure periods and effect parameters have been used. Since the purpose of this evaluation is to set an HBR-OEL, the committee restricted its evaluation to low exposure data with biological consequences.

Human data

In numerous human studies, irritative symptoms of the eyes and upper respiratory tract have been observed. In addition, case reports and epidemiological studies indicate that nitrogen dioxide affects the lower respiratory tract. In the worst case this led to lung oedema, bronchiolitis obliterans and pneumonia. For this reason, a lot of controlled laboratory studies have been directed on the adverse effects of nitrogen dioxide in the lungs.

These laboratory studies have been mainly performed to study pulmonary effects of nitrogen dioxide after a single exposure. In addition, they lasted from 15 minutes up to several hours. Additionally, in some of these studies, volunteers (healthy and non-smoking) exercised on bicycle ergometers during the exposure. Single exposure increased the airway resistance and reactivity almost immediately after starting the exposure. Overall, these effects were consistently observed when volunteers were exposed to 3.8 mg/m^3 or higher. The lowest significant effect, namely increased airway reactivity against carbachol, was found at 2.9 mg/m^3. In many studies in which volunteers were exposed to around 1.0 mg/m^3, no significant effects were found.

Among the controlled studies that have been performed at low exposure levels, one concerned peak exposures and a few repeated short-term exposures. In the peak-exposure study, healthy volunteers were exposed to 0.1 mg/m^3 for three hours. During these three hours, they were also exposed three times to 3.8 mg/m^3 for 15 minutes each. However, peak exposure did not affect lung function or pulmonary host defense. Regarding repeated short-term exposure, the studies were too limited to allow a conclusion about the concentration-response relationship between subchronic nitrogen dioxide exposure and pulmonary effects.

Also, no clear concentration-response relationship could be established from epidemiological studies. Although these types of studies give some evidence that nitrogen dioxide may induce respiratory illness, the results should be interpreted with caution, because of the presence of confounders. These include: the inclusion of vulnerable people; absence of job history; missing data concerning smoking habits; and co-exposure to additional chemical substances.

Animal data

The observations made in humans after a single exposure are supported by the observations made in animals, concerning effects on lung function. Furthermore, effects on pulmonary host defense, lung morphology and lung biochemistry (anti-oxidant defense system) have been reported. To get a better insight in the development of chronic (respiratory) diseases, numerous long-term animal studies have been performed.

In more detail, single or short-term exposure revealed, for instance, a decrease in host defense activity, activation of the anti-oxidant defense system and a changed lung morphology at 1.0 mg/m^3 and higher. Below this concentration the observations became less consistent. The lowest relevant effect was observed at 0.65 mg/m^3. At this level, morphological changes in the lungs of mice exposed for 6 weeks were observed by quantitative image analysis. Additional data suggest that adaptation did occur.

Some of the effects worsened with extended exposure. For instance, (sub)chronic exposure led to increased mortality and decreased survival time after challenge with infectious agents. Furthermore, morphological changes in the lungs revealed more severe defects resembling hyperplasia, fibrosis or emphysema, of which the last two are known to be non-reversible. These and other effects, such as lung malfunction and lowered pulmonary host defense, were consistently found at 0.96 mg/m^3 and higher. Some studies included also effects at lower concentrations, but all these showed some shortcomings or insufficient reporting. For instance, in one comprehensive study, a limited number of rats was continuously exposed to 0.08, 0.76 or 7.6 mg/m^3 (0.04, 0.4 or 4.0 ppm) for up to 27 months. As a result of the exposure, the lungs of exposed animals showed slight (0.76 mg/m^3) to severe (7.6 mg/m^3) signs of hyperplasia and fibrosis, whereas no remarkable morphological change was observed in animals exposed to 0.08 mg/m^3. In another low-exposure study, beagle dogs did not develop emphysema or other morphological changes in the lungs, after being exposed to 0.26 mg/m^3 for 86 months; however, these dogs were simultaneously exposed to non-toxic levels of nitrogen monoxide (concentration NO: 2.05 mg/m^3) and were allowed to recover for up to 36 months, before morphological examinations took place. Additionally, in mice continuously exposed to 0.38 mg/m^3 for up to 12 months, no remarkable pathological

lesions were found, nor any relevant changes in lung function or pulmonary host defense.

As for peak exposure, several short-term and long-term animal studies have been carried out in mice and rats to find out whether peak exposure affected health. Following peak exposure for several days to several months, nitrogen dioxide clearly affected the pulmonary host defense to infectious agents. However, although peak exposure may reflect the occupational situation, it is difficult to separate effects of peak exposure from the 'background' exposure.

Finally, few animal studies on low nitrogen dioxide exposure reported changes in the systemic immune system. However, the committee considers these results inconclusive, because the effects could be considered as adaptive physiological reactions.

Carcinogenesis and genotoxicity

Studies on the carcinogenic or tumour promoting potential of nitrogen dioxide are limited and give no conclusive information. On the other hand, additional *in vivo* and *in vitro* data have provided some evidence of genotoxicity. However, the committee noted the insufficient reporting of particularly the *in vivo* data. Overall, the committee concludes that the carcinogenic and genotoxic properties of nitrogen dioxide were insufficiently investigated. Therefore, according to the EU guidelines, the committee recommends not classifying nitrogen dioxide.

Reproduction toxicity

Animal studies on the fertility and developmental toxicity by inhalation of nitrogen dioxide are of insufficient quality. For this reason, no firm conclusions can be drawn.

Evaluation and recommendation

Hazard identification

Taking the whole set of available data into account, the committee considers the nitrogen dioxide effects on the deeper parts of the respiratory tract as the most critical effects. These include increased airway resistance, enhanced susceptibility to bacterial or viral airway infections, and, on long-term, irreversible damage of the lung tissue. It is evident that the respiratory effects are primarily local effects, and that they are not only observed after repeated exposure, but also immediately or during a brief exposure. To

prevent workers from these harmful acute and long-term effects, a 15-minute as well as an 8-hour HBR-OEL is warranted.

Recommendation of an HBR-OEL, 15-min TWA (STEL)

The committee proposes an HBR-OEL (15-min TWA) based on the complete set of human single-exposure studies instead of a single study, because of the large and consistent set of data that is available. In the clinical studies on healthy volunteers toxicological significant effects on lung function or respiratory resistance were observed from 3.8 mg/m^3 upwards. The lowest significant effect, namely increased airway reactivity against carbachol, was found at 2.9 mg/m^3. Between 1.1 and 2.9 mg/m^3 only a few human laboratory studies have been performed. However, all these showed some limitations, such as a low number of volunteers, inclusion of smokers and the absence of a detailed report. On the other hand, at around 1.0 mg/m^3 not only many more studies were performed, but also more volunteers participated in those studies; in none of them, significant respiratory effects were found. Taking into account all these facts, the committee considers 1.0 mg/m^3 as the NOAEL. Compensation for differences between individuals is not necessary, because of the very large and consistent set of data. Taking these considerations into account, the committee recommends an HBR-OEL for nitrogen dioxide of 1.0 mg/m^3 (15-min TWA).

Recommendation of an HBR-OEL, 8-hour TWA

The committee decided to derive an HBR-OEL from animal data, because human, long-term exposure data are limited in number and inadequate. Overall, the most serious long-term effects are changes in lung morphology, resembling lung fibrosis. Lung fibrosis is a slowly progressive disease that ultimately ends in life-threatening cardial diseases. Therefore, the committee is of the opinion that lung fibrosis should be prevented.

In assessing an HBR-OEL, one long-term animal study included a concentration-response relationship at very low nitrogen dioxide concentrations (0.08, 0.76 and 7.6 mg/m^3); with a NOAEL of 0.08 mg/m^3 and a LOAEL of 0.76 mg/m^3. However, between the NOAEL and the LOAEL is a large gap (factor of 10). In addition, additional long-term animal studies suggest that up to 0.38 mg/m^3 no remarkable respiratory tract effects are to be expected. Hence, the committee has taken the complete set of long-term animal data into account, and set the NOAEL at 0.38 mg/m^3.

For the assessment of the HBR-OEL, several aspects and uncertainties were considered. For instance, interspecies differences should be taken into account. However, the committee based its NOAEL on different studies with three different

animal species. In these different species comparable effects were observed within a narrow concentration range. For this reason no additional extrapolation is needed for interspecies differences. Furthermore, differences among people should be taken into account. This requires an additional uncertainty factor of three for inter-individual differences. Another aspect is the experimental condition: the animals, in the previously mentioned studies, were mainly continuously (24 hours/day, 7 days/week) exposed for a long period. This means that they did not have time to recover from initial lung tissue injuries and that this may have facilitated the development of lung fibrosis. However, the exposure pattern of workers allows a period of recovery. This aspect should be taken into account. However, it is difficult to assess the exact height of the uncertainty factor as to the effect of exposure time on the human NOAEL. Nevertheless, taking the law of Haber into account, the committee is of the opinion that this uncertainty counterbalances the uncertainty of intraspecies differences.

Considering all these aspects, the committee recommends an HBR-OEL for nitrogen dioxide of 0.4 mg/m^3 (rounded up), as an 8-hour TWA.

Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards proposes a health-based recommended occupational exposure limit for nitrogen dioxide of 0.4 mg/m^3, as an eight-hour time weighted averaged concentration (8-h TWA), and of 1.0 mg/m^3 as a 15-minute TWA (STEL).

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, at the request of the Minister of Social Affairs and Employment (annex A). The purpose of the committee's evaluation is to set a Health-Based Recommended Occupational Exposure Limit (HBR-OEL) for the atmospheric concentration of the substance, provided the database allows the derivation of such a value.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister on the feasibility of using the health-based limit as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the legally binding OEL.

1.2 Committee and procedure

The present document contains the assessment of the DECOS, hereafter called the committee, of the health hazard of nitrogen dioxide. The document is an update of a criteria document on the occupational exposure limit of nitrogen dioxide, published by the committee in 1985 (DEC85).

The members of the committee are listed in annex B. AAE Wibowo, PhD, of the Coronel Institute, Academic Medical Center of Amsterdam, prepared the first draft of this report for the Ministry of Social Affairs and Employment.

In 2002 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on that draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of this report.

1.3 Data

In 1993, the SCOEL published a draft report called 'Recommendation of the Scientific Committee for Occupational Exposure Limits for nitrogen dioxide' (SCO93), of which a final summary report was published in 1997 (SCO97, included in annex D). These two documents served as starting point and source for the search of relevant literature. In addition, more recent literature was retrieved from on-line databases Toxline and Medline. The final search was carried out in June 2002. The searches were performed using nitrogen dioxide and CAS no. 10102-44-0, as search key words. Furthermore, in preparing the present report the following reviews have been consulted:

- World Health Organisation (WHO). Nitrogen dioxide (second edition). International Programme on Chemical Safety. Geneva, 1997. Environmental Health Criteria 188 (WHO97);
- Gezondheidsraad. Advies inzake stikstofdioxide. Advieswaarden voor de kwaliteit van de buitenlucht. Gezondheidsraad, Rijswijk, mei 1979. 1979/3 (Gez79) [Dutch].

A list of abbreviations and symbols used in this report can be found in annex G.

Chapter 2

Identity, properties and monitoring

2.1 Identity

CAS name	Nitrogen dioxide
Synonyms	Nitrogen peroxide, nitrogen tetroxide, dinitrogen tetroxide, liquid dioxide
CAS number	10102-44-0
EC number	007-002-00-0
RTECS number	QW 9800000
EINECS number	233-272-6

2.2 Physical and chemical properties

Molecular formula	NO_2
Molar mass	46.01 g/mol
Melting point	-9.3 °C
Boiling point	21.2 °C
Relative density (water=1, 20 °C)	1.45 (liquid), 158 (gas)
Vapour pressure (20 °C)	96 kPa
Relative vapour density (air=1)	1.58
Solubility	Decomposes in water; soluble in alkalies, chloroform, carbon disulphide, concentrated sulphuric acid and nitric acid.
Odour threshold	0.2-0.8 mg/m^3
Conversion factor (20 °C, 101 kPa)	1 mg/m^3= 0.52 ppm 1 ppm = 1.91 mg/m^3

Nitrogen dioxide exists as a colourless solid, a yellow liquid or as a reddish-brown gas. It has a sweetish rancid odour. Solid and liquid nitrogen dioxide is present in the form of the dimer dinitrogen tetroxide (N_2O_4).

When heated above 160 °C, nitrogen dioxide decomposes into nitrogen monoxide and free oxygen. The compound is a strong oxidizing agent and reacts heavily with many other compounds; with water it forms, for instance, nitric acid.

2.3 EU Classification and labelling

	T+	Very toxic
Risk phrases	R26	Very toxic by inhalation.
	R37	Irritating to respiratory system.
Safety phrases	S1/2	Keep locked up and out of reach of children.
	S7/9	Keep container tightly closed and in a well-ventilated place.
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S45	In case of accident or if you feel unwell, seek medical advice immediately (*show the label where possible*).

2.4 Validated analytical methods

2.4.1 Environmental monitoring

Air samples of nitrogen dioxide can be analysed using various methods, such as: infrared absorption spectrometry; gas chromatography; differential pulse polarography; and, colorimetric methods (SCO93).

NIOSH recommends Method 6400. This method describes air sampling with passive sampler sorbent tubes, which contains a triethanolamine-impregnated molecular sieve. The samples obtained in this way are then analysed by visible absorption spectrophotometry (working range for a 3-L air sample: 1 to 47 mg/m^3 (0.5 to 25 ppm)).

OSHA describes a validated method (no. ID-182). Air samples are taken in the breathing zone of workers by a sampling device. This device contains three separate tubes, including a triethanolamine-impregnated molecular sieve. The analysis is performed by ion chromatography (detection limit for a 3-L sample (sample rate 0.20 L/min): qualitative, 0.13 mg/m^3 (0.07 ppm); quantitative, 0.36 mg/m^3 (0.19 ppm)).

2.4.2 *Biological monitoring*

For biological monitoring of nitrogen dioxide, no validated method is available.

Urinary nitrate excretion may correlate with respiratory exposure to nitrogen dioxide. However, this parameter is probably not useful for biological monitoring, because considerable amounts of excreted nitrate may originate from food intake (SCO93).

Chapter 3

Sources

3.1 Natural occurrence

Nitrogen dioxides are produced naturally by bacteria, volcanic activity, lightning and by oxidation of NO in the atmosphere.

3.2 Man-made sources

Nitrogen dioxide is released into the environment as a constituent of diesel exhaust, and as a result of cooking or heating with unvented gas appliances. These activities are the main sources of environmental exposure to nitrogen dioxide. Also tobacco smoking partly contributes to the man-induced environmental release of nitrogen dioxide.

3.2.1 Production

Of the countries committed to the European Union only France produces nitrogen dioxide for commercial use. The production focuses mainly on liquid or solid N_2O_4, a dimer of nitrogen dioxide. The total production of the dimer is estimated to be several hundred tonnes a year.

The production of nitrogen dioxide as the final product is very limited. In laboratories, nitrogen dioxide can be produced by several methods, such as heating lead nitrate (SCO93).

3.2.2 Use

Nitrogen dioxide is primarily used as a chemical intermediate, such as in the production of nitric acid (SCO93). Additional applications are:

- for the nitration of organic compounds and explosives;
- for the manufacture of oxidized cellulose compounds;
- as a polymerisation inhibitor of acrylates;
- as a catalyst for sulphuric acid; and,
- as an oxidising agent for rocket fuels.

Chapter 4

Exposure

4.1 General population

Annual natural outdoor concentrations of nitrogen dioxide ranges between less than 0.001 mg/m^3 to more than 0.009 mg/m^3. In cities, the annual mean concentrations range from 0.02 to 0.09 mg/m^3, with one-hour peak values from 0.075 to 1.0 mg/m^3 (WHO00). In the Netherlands mean annual concentrations of nitrogen dioxide of 0.021, 0.038 and 0.042 mg/m^3 have been measured in the rural areas, cities and streets, respectively (RIV01).

Indoor levels of nitrogen dioxide range between 0.02 and 0.04 mg/m^3 in living rooms and up to 0.07 mg/m^3 in kitchens (measured in five European countries over 2 to 7 days). In addition, in the Netherlands, one-minute peak concentrations have been measured up to 3.8 mg/m^3 in kitchens with unvented gas cooking ranges (WHO00).

4.2 Working population

Occupational exposure to nitrogen dioxide is possible from exhausts of combustion engines. The main exposure sites are in the chemical industry (gas welding), the agriculture sector (silos), and, the mining industry (use of dynamite).

Data on the exposure levels of nitrogen dioxide in the chemical industry and other workplaces are limited. Furthermore, most of these data have been derived from samples taken from diesel exhausts. One such an example is a study with railroad workers (Wos89), in which work-related exposure levels varied between 0.03 and 0.24

mg/m^3. In another study, the highest individual shift exposure levels in personnel, working in a British coalmine, averaged 4.39 mg/m^3 (Rob84).

Investigators performed a study on the exposure of Parisian taxi drivers to automobile air pollutants, such as nitrogen dioxide, during their professional activity (Zag00). Inside the vehicles of 29 randomly selected non-smoking drivers, nitrogen oxides have been monitored by OGAWA passive samplers. These samplers were placed on the front of the passenger's seat, during the whole workday of 8 hours for a total of three days. After exposure, the samples were analysed so that exposure levels could be determined. The exposure levels were then compared to those recorded at the Paris air monitoring network fixed stations. Values of nitrogen dioxide that have been obtained inside the taxis averaged 0.14±0.04 mg/m^3, which was significantly higher than values found for: urban background (0.07±0.02 mg/m^3); near to the car values (0.08±0.02 mg/m^3); and, at places with high automobile traffic volume (0.12±0.02 mg/m^3).

In Norway, personal exposure measurements of nitrogen dioxide have been performed among 189 underground construction workers. Nitrogen dioxide concentrations ranged between 0.76 and 1.72 mg/m^3 (0.4 and 0.9 ppm). However, when workers passed through a blasting cloud, peak values of more than 19.1 mg/m^3 (10 ppm) were measured (Bak01).

Chapter 5

Kinetics

5.1 Absorption

Inhaled nitrogen dioxide reaches easily the lower respiratory tract (bronchioli and alveoli of the lungs). In addition, the substance is not only absorbed in the upper respiratory tract, but also throughout the lungs (WHO97). For instance, Wagner *et al*. (Wag70) showed that, depending on physical activity, healthy human volunteers absorbed approximately 75 to 90% of the inhaled nitrogen dioxide in the lungs.

Nitrogen dioxide dissolves easily in lung fluids and hydrates then slowly to nitrous (HNO_2) or nitric acid (HNO_3). These acids may dissociate into nitrite (NO_2^-) or nitrate (NO_3^-) ions, respectively. It are these subsequent acid and ion-products, which are rapidly taken up in the lung epithelium (Gol77, Pos89).

No data have been found concerning absorption of nitrogen dioxide through the skin.

5.2 Distribution

After passing the lungs, nitrogen dioxide products are distributed to all parts of the body via the bloodstream. Goldstein *et al.* (Gol77) exposed two female rhesus monkeys to radiolabelled nitrogen dioxide ($^{13}NO_2$) for 6 to 9 minutes through a facemask. The investigators found the radioactive tracer back in the circulation. They also have reported that the time-concentration relationship of ^{13}N in arterial blood correlated well with the time-concentration relationship in the lungs. In addition, the investigators

showed that the gas was distributed throughout the lungs. Also, they demonstrated that 50 to 60 % of the inspired nitrogen dioxide was retained during quiet respiration (inspired concentration of $^{13}NO_2$: 0.54 mg/m^3 in primate one, 0.30 mg/m^3 in primate two). The radioactive tracer remained in the lungs for approximately 20 minutes after stopping the exposure.

5.3 Biotransformation

Nitrite salts of nitrogen dioxide are oxidised by oxy-haemoglobulin to nitrate (Kos79).

Nitrogen dioxide acts as a strong oxidant; it oxidises unsaturated lipids and functional proteins, such as those present in cell membranes. This may result in loss of cell permeability and control. Furthermore, the substance activates peroxide detoxification pathways (WHO97).

5.4 Elimination

No human data have been found.

Saul and Archer (Sau83) observed that nitrogen dioxide or its products (nitrite, nitrate) were excreted as nitrate in the urine of Sprague-Dawley rats. Also, they showed that the amount of urinary nitrate correlated linearly to nitrogen dioxide exposure levels. The urinary values returned to normal after four days.

5.5 Possibilities for biological monitoring

Urinary nitrate excretion may correlate well with respiratory exposure to nitrogen dioxide. However, the use of nitrate as a biological exposure parameter is not useful, because considerable amounts of nitrate may originate from food.

5.6 Summary and evaluation

Information on the kinetics of nitrogen dioxide is limited. Nitrogen dioxide reaches easily the lower respiratory tract. In the lungs, it dissolves in lung fluids, forming several products, such as nitrous and nitric acid and their respective nitrite and nitrate salts. These products enter the bloodstream, and are then distributed throughout the body. In the body, nitrite is oxidised into nitrate, which is then rapidly excreted as urinary nitrate.

Nitrogen dioxide is a strong oxidant. As such, it may damage cellular membrane lipids and proteins.

Chapter 6

Effects

Numerous studies have been published on the adverse effects of nitrogen dioxide inhalation. In these studies, a broad range of exposure levels, exposure periods and parameters have been used. Since the purpose of this evaluation is to set an HBR-OEL, the committee restricted its evaluation to low exposure concentration data with known biological consequences. In addition, this chapter describes the relevant studies evaluated by the SCOEL (SCO93, SCO97) and the WHO (WHO97), supplemented with recent publications.

6.1 Observations in humans

6.1.1 Irritation and sensitisation

Nitrogen dioxide is a very irritating gas. The irritant effect of nitrogen dioxide is thought to be the result of the formation of nitrous and nitric acid on contact with water. These acids are known corrosive and irritant substances. Investigators described one case, in which exposure to nitrogen dioxide caused severe burns of the skin and eyes after even momentary contact (Boy63).

No data have been found on sensitisation of nitrogen dioxide in humans.

6.1.2 *Toxicity due to acute and short-term exposure*

Numerous controlled clinical studies have examined lung function, airway responsiveness to bronchoconstrictors and the defence against viral and bacterial airway infections in human subjects exposed to nitrogen dioxide.

Healthy people

A brief summary of the data evaluated by SCOEL and WHO is given in Table E.1 (single exposure) and Table E.2 (repeated exposure) in annex E.

Hazucha *et al*. (Haz83) exposed fifteen healthy, non-smoking volunteers to 0.19 mg/m^3 (0.1 ppm) nitrogen dioxide for one hour during rest. However, effects on measured lung function parameters were not observed.

Bylin *et al*. (Byl85) exposed eight healthy, non-smoking and eight asthmatic subjects (including three smokers) to clean air, 0.23, 0.46 and 0.91 mg/m^3 nitrogen dioxide for 20 minutes on four separate days. Before, during and after exposure, airway resistance (R_{aw}), thoracic gas volume (TGV) and specific airway resistance (SR_{aw}) were measured. Further, bronchial reactivity to inhaled histamine was measured after exposure to 0.91 mg/m^3 nitrogen dioxide and clean air. The results concerning asthmatic subjects are presented in the next section (*asthmatic people*). The healthy subjects experienced no or very little discomfort during exposure. Taken all the whole set of data from the different subjects into account, none of the tested lung parameters changed significantly during or after the exposure to nitrogen dioxide. However, statistical analysis of the separate data sets for each exposure, using the subjects as their own control, revealed statistically significant differences in some respects: the mean SR_{aw} obtained immediately following exposure to 0.46 mg/m^3 was significantly increased compared to the value obtained at 10 minutes of exposure ($p<0.03$); however, the SR_{aw} was significantly decreased during and after exposure to 0.91 mg/m^3 ($p=0.01$); on the other hand, at 0.91 mg/m^3, bronchial reactivity did not change significantly compared to clean air exposures.

Chaney *et al.* (Cha81) exposed nineteen healthy male volunteers to 0.38 mg/m^3 nitrogen dioxide for 2 hours during rest. Of the seven biochemical blood parameters measured all remained within their normal physiological ranges.

Rasmussen *et al.* (Ras90) exposed twenty healthy volunteers to clean air or to 0.19, 0.38, or 1.53 mg/m^3 nitrogen dioxide for two hours while exercising, for one day a week for a total of four consecutive weeks. No significant changes were observed on lung function, airway reactivity, alveolar permeability or nasal mucociliary clearance.

Five healthy, non-smoking volunteers were exposed to 1.15 mg/m^3 (0.6 ppm) nitrogen dioxide, for 2 hours on four separate days within a 6-day period. During exposure they exercised on a stationary cycle ergometer for 15 minutes, each half an hour of exposure. At least two weeks before the start of the exposure and after the end of the final exposure, individual blood and bronchoalveolar lavage fluid (BALF) were obtained. No significant changes in airway symptoms were observed after exposure to nitrogen dioxide. Furthermore, no differences in lymphocyte subtypes (BALF) were observed, except a slightly, but significantly, increased number of natural killer cells. Natural killer cells are thought to modulate killing of virus-infected cells. Changes in lymphocyte function were not studied. The authors noted the small number of participants and concluded that repeated exposure of healthy subjects to nitrogen dioxide at a concentration of 1.15 mg/m^3 was not associated with changes in lymphocyte subtypes in the blood and bronchoalveolar lavage fluid (Rub91).

The group of Frampton *et al.* conducted one study to assess effects of nitrogen dioxide exposure on pulmonary function, airway reactivity and respiratory defense against viruses in healthy volunteers. The results of this study were published in three separate papers (Fra89a, Fra89b and Fra91).

Healthy, non-smoking men and women were continuously exposed to nitrogen dioxide for three hours to a concentration of

- 1.1 mg/m^3 (0.6 ppm) (n=9);
- 0.1 mg/m^3 (0.05 ppm) with three 3.8 mg/m^3 (2 ppm) intermittent peak exposure periods of 15 minutes each (n=15);
- or 2.9 mg/m^3 (1.5 ppm) (n=15).

During exposure, all subjects exercised for 10 min of every 30 min on a bicycle ergometer. Each subject served as his or her own control.

Pulmonary function measured before, during and after exposure, was not altered in any of the exposure protocols. However, airway reactivity, measured by carbachol challenge, was increased in subjects continuously exposed to 2.9 mg/m^3, but not in the other two groups (Fra91).

Concerning respiratory defense against viruses, in four of nine subjects exposed to a concentration of 1.1 mg/m^3, the alveolar macrophages tended (not significant for the whole group, $p<0.07$) to inactivate influenza virus *in vitro* less effectively than cells collected after air-exposure. These alveolar macrophages were obtained by bronchoalveolar lavage, three and a half hours after exposure. In addition, in these four subjects also interleukine-1 production was increased, whereas this was not observed in the other five subjects. However, intermittent peak exposure did not alter the rate of viral inactivation or interleukine-1 production, nor did anyone in this group show any signs of susceptibility (Fra89a). The authors felt that although the observed changes in

virus inactivation did not reach significance, there was at least a strong indication of a response and further investigations are warranted.

Furthermore, in bronchoalveolar lavage fluids taken from all subjects 3.5 hours or 18 hours after the exposure (1.1 mg/m^3 exposed group only), no significant differences in percentages of macrophages, lymphocytes or neutrophils were found compared to the same subjects exposed to air (Fra89b). In addition, no changes in total protein or albumin were observed. However, continuous exposure to 1.1 mg/m^3 nitrogen dioxide was associated with significantly increased levels of antiprotease alpha-2-macroglobulin (α2M) when assessed 3.5 hours after exposure, but not 18 hours after exposure nor in the other exposure groups (Fra89b). The antiprotease is a secretary product of monocytes, fibroblasts and possibly alveolar macrophages. The enzyme may play a role in local control of lung protease activity.

In 2002, Frampton and his colleagues examined again the effects of nitrogen dioxide exposure on airway inflammation, blood cells and antiviral respiratory defence (Fra02). Healthy, non-smoking men (n=12) and women (n=9) were each exposed on separate occasions to clean air or to 1.1 or 2.9 mg/m^3 (0.6 and 1.5 ppm) nitrogen dioxide for three hours with intermittent moderate exercise. No remarkable effects on lung function, symptoms and on infection with either influenza or RSV virus were found following the exposure. Furthermore, the authors found no evidence for susceptible or nonsusceptible groups of subjects, which is in contrast to their previous study (Fra89a). Nitrogen dioxide exposure appeared to have a small effect on lymphocyte recovery and on lymphocyte subsets. Also the LDH release by airway epithelial cells was increased, although the viability of the epithelial cells was not changed. Overall, Frampton *et al*. concluded that all nitrogen dioxide effects observed in this study were small and unlikely to be of clinical significance for healthy subjects.

Beil and Ulmer (Bei76) examined the changes in airway resistance in sixteen male volunteers, who were exposed to clean air or to 1.8, 4.5, 9.0 and 13.5 mg/m^3 (1, 2.5, 5 and 7.5 ppm) nitrogen dioxide for 2 hours. Also, eight men were exposed to 9.0 mg/m^3 (5 ppm) for 14 hours. As a result of the exposures, airway resistance increased significantly when the volunteers were exposed to 4.5 mg/m^3 or higher. However, no dose-response relationship could be established. Also, no changes were found at any concentration in relation to the only other respiratory parameter measured, namely end-expiratory thoracic gas volume. The authors also examined the bronchial susceptibility to the bronchoconstricting agent acetylcholine, by measuring airway resistance. The agent was inhaled immediately after the exposure to nitrogen dioxide. Within two hours, airway resistance increased significantly when exposed to 13.5 mg/m^3, or within 14 hours when exposed to 9.0 mg/m^3, but no changes in airway resistance were observed at the other exposure levels.

The committee noted that most of the subjects were smokers. Smoking may be a source of bias. Additionally, the committee noted that there was no control 14-hour exposure to clean air.

Hackney *et al.* (Hac78) exposed fifteen healthy volunteers to 1.91 mg/m^3 (1 ppm) nitrogen dioxide for 2 consecutive days for 2 hours a day, while exercising. The authors observed no meaningful changes in conventional measures of lung function, including airway resistance. Some subjects reported minor increases in respiratory symptoms during exposure, but overall changes in symptoms were not statistically significant.

Goings *et al*. (Goi89) conducted a placebo-controlled, randomised, double-blinded study over three separate years to determine the effect of exposure to nitrogen dioxide on the susceptibility to virus infection. Healthy, non-smoking volunteers (n=21-23/ group, total 152) were exposed to clean air or to 1.9 mg/m^3 (1 ppm, year three), 3.6 mg/m^3 (2 ppm, year one and three) or 5.7 mg/m^3 (3 ppm, year two) nitrogen dioxide for three consecutive days for two hours a day. On the second day of exposure, each volunteer was intranassally inoculated with a non-transmissible cold-adapted influenza A/Korea/1/82 (H3N2) reassortant virus. Pulmonary function measurements (FEV_1, FVC) and non-specific airway reactivity to methacholine were unchanged after nitrogen dioxide exposure, virus infection, or both. The percentage virus-infected subjects was 57% (3.6 mg/m^3) versus 65% (air) in year one, 77% (5.7 mg/m^3) versus 71% (air) in year two, and 91% (both 1.9 and 3.6 mg/m^3) versus 71% (air) in year three. These differences were not statistically significant. Between the two groups exposed to 3.6 mg/m^3 (year one and three) significantly different responses were found for: total antibody; serum HAI antibody to A/Korea and to A/Philippines; serum ELISA IgG; and, nasal wash ELISA IgA. According to the authors, the reason for these different responses are not clear, but may be caused by the small number of participants in study or by the immunologic status of the volunteers. Overall, the authors suggested that exposure to nitrogen dioxide at a concentration of 1.9 and 3.6 mg/m^3 may be associated with increased susceptibility to influenza virus infection.

Mohsenin (Moh88) exposed healthy, non-smoking volunteers (n=18) to 3.8 mg/m^3 nitrogen dioxide for one hour. Significantly increased airway reactivity to methacholine was observed. However, lung function was not changed.

Twelve healthy, non-smoking volunteers (eight men and four women) were exposed to clean air (once) or to 3.8 mg/m^3 (2 ppm) nitrogen dioxide for 4 hours on four consecutive days. During the exposures, light exercise on a bicycle ergometer was alternated with rest in 15-min intervals. Lung functions measurements (FEV_1 and FVC) were made before and immediately after the end of each exposure. In addition, bronchoscopy with endobronchial biopsies, bronchial wash (BW) and bronchoalveolar lavage (BAL) were carried out 1.5 hours after the air exposure and after the last exposure with nitrogen dioxide. The subjects, showed significant decrements in FEV_1

and FVC after the first exposure with nitrogen dioxide, but this attenuated with repeated exposures. Furthermore, repeated exposure resulted in: a significantly decreased number of neutrophils in the bronchial epithelium; a twofold increase of neutrophils in bronchial wash; and, a 1.5-fold increase in myeloperoxidase. However, the antioxidant status of the airway fluid was unchanged. These findings suggested that migration and activation of neutrophils in the airways occurred, representing inflammation, whereas changes in pulmonary function and antioxidants are resolved. Based on these results, the authors concluded that nitrogen dioxide is a proinflammatory air pollutant under conditions of repeated exposure (Blo99).

In a counter-balanced, single-blind, repeated-measures designed study, eleven men and four women, all healthy and non-smoking, were exposed to (filtered) air or to 3.8 mg/m^3 (2ppm) nitrogen dioxide for four hours a day for three consecutive days. During exposure they exercised four times for 30 min, followed by a 30 min rest period. Bronchoscopical analysis, performed each day 18 hours after exposure to nitrogen dioxide, revealed significantly increased percentages of neutrophils (bronchial fraction) and significantly decreased percentages of CD4+ T-cells (bronchoalveolar lavage) as compared to filtered air exposure. The authors concluded that exposure to nitrogen dioxide resulted in bronchial inflammation and minimal changes in bronchoalveolar lavage T-helper cells (Sol00).

Rasmussen *et al.* (Ras92), exposed fourteen healthy volunteers to clean air or to 4.4 mg/m^3 nitrogen dioxide for five hours. The subjects served as their own control. No effects on mucus membrane irritation or lung function during or after exposure were observed. However, 11 hours after the exposure started, alveolar permeability decreased statistically significant. Apart from that, serum glutathione peroxidase activity decreased with 14%, as measured 24 hours after the exposure.

Sandström *et al.* (San91) exposed eighteen healthy, non-smoking volunteers to 7.52 mg/m^3 nitrogen dioxide for 20 minutes daily for 12 days. The exposure included a 15-min exercise. From bronchoalveolar lavage analysis, the authors concluded that exposure to nitrogen dioxide induced inflammation in the lungs.

Asthmatic people

Bylin *et al.* (Byl85) exposed eight healthy, non-smoking and eight asthmatic subjects (including three smokers) to clean air or nitrogen dioxide (0.23, 0.46 and 0.91 mg/m^3). For study details see foregoing section. The results concerning asthmatic subjects are presented here. The asthmatics experienced no or very little discomfort during the nitrogen dioxide exposure. However, the investigators observed the following: consistently higher baseline values for the lung function variables (R_{aw}, TGV, and SR_{aw}) in asthmatics than in normal subjects; significantly increased bronchial reactivity at 20

minutes of exposure to 0.91 mg/m^3 (p=0.04); a tendency to increased airway resistance at low exposures, but decreased airway resistance at 0.91 mg/m^3; and, a significantly decreased TGV during exposure to 0.91 mg/m^3 (p=0.02).

Svartengren *et al.* (Sva00) exposed persons with mild allergic asthma (n=20), inside a car, for 30 minutes in a Stockholm city road tunnel. The median nitrogen dioxide level during exposure was 0.31 mg/m^3 (range 0.21-0.46 mg/m^3). Also median levels of particles were measured. As a control, the volunteers were also exposed to much lower pollution levels in a suburban area. Four hours after exposure all subjects inhaled a low dose of allergen. Subjective symptoms during the tunnel exposure were not pronounced. Subjects exposed to more than 0.3 mg/m^3 nitrogen dioxide or 0.1 mg/m^3 $PM_{2.5}$ (aerodynamic diameter of particle 2.5 micron) had a more pronounced early reaction to the allergen than in the control situation. Furthermore, subjects exposed to nitrogen dioxide showed late reactions, such as lowered lung function and more asthma symptoms, compared to control exposure. The authors concluded that air pollution in road tunnels may significantly enhance asthmatic reactions to subsequently inhaled allergens.

Jenkins *et al.* (Jen99) exposed non-smoking, mildly atopic, asthmatic volunteers (n=11/group) to : clean air; 0.38 mg/m^3 (200 ppb) nitrogen dioxide; ≈0.20 mg/m^3 (100 ppb) O_3; or, 0.38 mg/m^3 nitrogen dioxide combined with ≈0.20 mg/m^3 O_3, for 6 hours, followed immediately by bronchial allergen challenge. Subsequently, ten volunteers from each group were exposed to: 0.76 mg/m^3 (400 ppb) nitrogen dioxide; ≈0.40 mg/m^3 O_3; or, to 0.76 mg/m^3 nitrogen dioxide combined with ≈0.40 mg/m^3 O_3, for 3 hours. Also these exposures were immediately followed by bronchial allergen challenge. The challenge consisted of inhalation of increasing concentrations of *D. pteronyssinus* until a fall in FEV_1 of larger than 20% of the baseline value was observed (dose expressed as allergen $PD_{20}FEV_1$). In none of the groups exposed for 6 hours were the airway responses changed compared to air controls. In contrast, in all of the groups exposed for three hours, the sensitivity to inhaled allergen was significantly increased compared to air controls. The authors concluded that nitrogen dioxide-induced airway response to allergen in mild atopic asthmatics may be dependent on a threshold concentration rather than the total amount of NO_2 inhaled over a period of time.

Strand *et al.* (Str98) exposed non-smoking subjects (n=16) with mild asthma and allergy to birch or grass pollen, to clean air or to 0.5 mg/m^3 nitrogen dioxide for 30 minutes at rest on 4 subsequent days. Four hours after exposure, an individually determined nonsymptomatic dose of birch and timothy allergen extracts was inhaled. The subjects served as their own controls. Before, during and at several time points after exposure and allergen inhalation, lung function tests were performed and questionnaires filled in for subjective symptom recording. No nitrogen dioxide associated symptoms were reported. Also, nitrogen dioxide did not affect lung function (SR_{aw} and FEV_1)

before allergen inhalation. However, after allergen inhalation, FEV_1 decreased in both nitrogen dioxide and air exposed subjects, although the decrease was more pronounced in the nitrogen dioxide exposed group. The authors concluded that even though the effects were small, repeated short-term exposure to low nitrogen dioxide concentrations enhanced the airway response to a nonsymptomatic allergen dose.

Barck *et al.* (Bar02) performed a comparable study, with the main differences being: the number of subjects (n=13); the study duration (single exposure, not repeated); and, the inclusion of bronchoscopy with bronchial wash (BW) and bronchoalveolar lavage (BAL) performed 19 hours after allergen inhalation. Concerning lung function, FEV_1 statistically decreased after allergen inhalation in both control and nitrogen dioxide exposure groups; however, between the groups it did not differ. Furthermore, nitrogen dioxide plus allergen inhalation enhanced the percentage neutrophils in both BW and BAL fluids compared to air plus allergen inhalation. Also, nitrogen dioxide exposure plus allergen inhalation increased eosinophil cationic protein in BW, whereas this was not found after air exposure. Based on these results, the authors suggested that low concentrations of nitrogen dioxide can enhance allergic inflammatory reactions in the airways without causing symptoms or pulmonary dysfunction.

In a double-blinded, randomised study, nine non-smoking subjects with clinically stable asthma were exposed to filtered air or 0.57 mg/m^3 (0.3 ppm) nitrogen dioxide for 30 minutes on two separate days at least 1 week apart. Each subject exercised during the first 20 minutes of each exposure. Specific airway resistance and FEV_1/FVC were measured before, 5 minutes after and 1 hour after completions of the air or nitrogen dioxide exposures. The authors did not find any association between exposure to nitrogen dioxide and changes in the pulmonary tests (Rub90).

6.1.3 *Case reports*

Several case reports have been published on nitrogen dioxide exposures around accidents with either nitric acid, fire in nitrogen containing organic matter or silo accidents. Accidental exposure led to pulmonary diseases such as lung oedema, bronchiolitis obliterans or pneumonia. In addition, the number of fatalities was high. Exposure levels are usually not known or given.

6.1.4 *Epidemiological studies*

Many epidemiological studies on the health effects of nitrogen dioxide have been conducted. These include indoor and outdoor studies of the general population, with special interest to children. Also associations between the use of gas for cooking or heating and the frequency of respiratory illness have been investigated. There is some

evidence that nitrogen dioxide exposure may induce respiratory illnesses. However, a major difficulty in the analysis of these population-based studies is the presence of many uncertainties, due to: measurement error; misclassification on the health outcome; missing covariates (smoking, gender, socioeconomic status); selection bias; and, internal inconsistency (WHO97).

Furthermore, epidemiological studies on nitrogen dioxide health effects in the workplace are limited, because of the presence of several sources of bias, such as: the absence of a job history; the presence of susceptible people; missing data concerning smoking habits; and, co-occurring pollutants. Concerning the latter, in practice, occupational exposure to nitrogen dioxide exclusively is very rare (Lun86). It is almost always accompanied by exposure to additional combustion products or air pollutants, such as sulphur dioxide, particles and mineral dust. In addition, one of these combustion products, nitric oxide (NO), may attribute to the health effects of nitrogen dioxide, because it oxidises easily in air (oxygen) into nitrogen dioxide (Mat80).

Overall, the committee considers the epidemiological data of nitrogen dioxide insufficient for quantitative risk assessment in an occupational environment.

6.1.5 Other relevant studies

Other relevant data were not found.

6.2 Animal experiments

A wide range of animal species, experimental designs and analyses methods has been used to study the adverse effects of inhaled nitrogen dioxide. These studies have shown a variety of responses in the respiratory tract, such as in the host defence mechanisms (mucociliary clearance, alveolar macrophages, cell mediated immunity, interaction with infectious agents), lung biochemistry (lipid peroxidation, lung proteins and enzymes, antioxidant defence systems) and morphology.

6.2.1 Irritation and sensitisation

Nitrogen dioxide is irritating to the eyes and nose (SCO93). Presumably, the irritation is caused by nitric or nitrous acid, formed by contact with water on the epithelial surface.

Ohashi *et al*. (Oha98) reported on the modifying effects of nitrogen dioxide on allergic inflammation in the tracheal mucosa. Female Hartley guinea pigs (n=10/group) were exposed to 17.2 mg/m^3 (9 ppm) nitrogen dioxide for 6 hours a day for two weeks. The animals were sensitised with anti-benzyl penicilloil bovine gamma globulin antigens. In the non-sensitised groups, ciliary activity did not differ between the NO_2-

exposed and the non-exposed group. However, in the sensitised groups, ciliary activity was significantly reduced in the NO_2-exposed group compared to the non-exposed group. The authors concluded that exposure to nitrogen dioxide does not cause prominent epithelial injury by itself, but could promote epithelial injury and hyperresponsiveness in the allergic airways by airborne allergens or irritants.

6.2.2 Toxicity due to acute exposure

Lethal concentrations

Several animal studies have been conducted to assess lethal concentrations of nitrogen dioxide. These studies have shown that mice and rats died, as a result of pulmonary oedema, within 2 to 5 minutes after exposure when exposed to 1,910 - 3,820 mg/m^3 (1,000-2,000 ppm) and around 955 mg/m^3 (500 ppm), respectively (Gra54; Car62; Hil77).

Non-lethal concentrations

A summary of single-exposure studies in animals, including the evaluations by SCOEL (SCO93) and WHO (WHO97), is given in annex F, Table F3. In short, animals were exposed to concentrations of nitrogen dioxide of as low as 1.0 mg/m^3 to more than 20.0 mg/m^3 for minutes to hours. The effect parameters measured concerned lung morphology; lung inflammation parameters; interaction with infectious agents; alveolar macrophage activity; lipid peroxidation; and, lung proteins and enzymes.

Illing *et al.* (Ill80) exposed female, CD-1 Swiss albino mice (n=16/group) to clean air or 1.9 and 5.7 mg/m^3 (1 or 3 ppm) nitrogen dioxide for 3 hours. Half of the animals exercised during exposure. Immediately after the exposure, the animals were challenged with an aerosol of viable *Streptococcus pyogenes* and then observed for incidence of mortality in a period of 15 days. In mice exposed to 5.7 mg/m^3, exercise significantly enhanced mortality compared to control animals (p<0.05). The survival time of mice exposed to 1.9 mg/m^3, with or without exercise, did not differ from control exposure.

Kakinoki *et al*. (Kak98) studied the effects of nitrogen dioxide on the defence functions of the airway epithelium. New Zealand white rabbits (n=26/group) were exposed to clean air or to 5.7 mg/m^3 (3 ppm) nitrogen dioxide for 24 hours. Nitrogen dioxide exposure significantly lowered ciliary activity and mucociliary transport compared to control exposure. Additionally, epithelial permeability was significantly increased. The authors suggested that nitrogen dioxide might be involved to some extent in the clinical manifestation of airway allergic disorders through epithelial dysfunction,

because dysfunction of both the junctional barrier and the mucociliary system allow easier entry of allergen molecules to the airway parenchyma,

Gilmour *et al*. (Gil96) exposed female, Brown Norway rats (n=10/group) to 9.6 mg/m^3 (5 ppm) nitrogen dioxide for 3 hours. As a result, the immune (antigen specific IgE, local IgA, and IgG) and inflammatory (inflammatory cells in lungs) response to dust mite antigen increased significantly.

Six male, Sprague-Dawley rats were exposed to 34.8 mg/m^3 (18 ppm) nitrogen dioxide for 12 hours. The exposure elevated the total amount of rTI_{40} in the bronchoalveolar fluid compared to non-exposed animals (McE97). rTl_{40} is a recently detected protein, which is localised exclusively in the rat alveolar epithelial type I cells of the lungs. The protein is used as a biochemical marker for mild injury and alteration of lung barrier function.

Papi *et al.* (Pap99) exposed male, Hartley guinea pigs (n=6-7/group) to air or to 34.8 mg/m^3 (18 ppm) nitrogen dioxide for 4 hours through a tracheal cannula. Nitrogen dioxide exposure caused intraluminal airway inflammation. This was characterised by: an influx of both eosinophils and neurtrophils; airway microvascular leakage; and, airway smooth muscle hyperresponsiveness to electrical stimulation, acetylcholine A and neurokinin A. The authors concluded that nitrogen dioxide exposure induced important inflammatory responses that mimic acute bronchitis.

6.2.3 *Toxicity due to short-term exposure*

A summary of short-term studies in animals, including the evaluations by SCOEL (SCO93) and WHO (WHO97), is given in annex F, Table F2.

Kobayashi and Miura reported on concentration and time-dependent increases in specific airway resistance and hyperresponsiveness by subchronic nitrogen dioxide exposure (Kob95). Harley guinea-pigs were continuously exposed to filtered air; 0.11; 0.96; 1.91; 3.82; or, 7.64 mg/m^3 (0.06; 0.5; 1.0; 2.0; or, 4.0 ppm) nitrogen dioxide for 6 and 12 weeks. In addition, before and after exposure, airway responsiveness to inhaled histamine and specific airway resistance was measured. As a result of the exposure airway hyperresponiveness and airway resistance increased concentration and time-dependently. These observations were significantly different from air exposure animals from 1.91 mg/m^3 onwards.

Ehrlich *et al.* (Ehr79) studied the effects of repeated exposure to peak concentrations of nitrogen dioxide on the pulmonary resistance to bacterial infection. Female CD_2F_1 mice were continuously exposed to filtered air or 0.19 mg/m^3 nitrogen dioxide for 1, 2, 3, and 6 months. During this exposure period, daily 3-hour exposures (5 days a week) to 0.94 mg/m^3 were performed. However, no control-group was included that had been

continuously exposed to 0.19 mg/m^3 without peak-exposure. Immediately after ending the final exposure, animals were challenged with *Streptococcus* aerosol by inhalation. Mice exposed to filtered air and peaks of 0.94 mg/m^3 had lowered number of alveolar macrophages in the first two months of exposure, and increased mortality and decreased survival time after 3 months of exposure. In addition, mice exposed to 0.19 mg/m^3 and peaks of 0.94 mg/m^3 showed only increased mortality and decreased survival time at 6 months of exposure. The authors concluded that intermittent exposure to nitrogen dioxide may alter the host's resistance to respiratory infection.

Overall, concerning peak-exposure studies, a brief summary of data on repeated peak exposure that is evaluated by WHO, is given in Table F4 in annex F.

The group of Richters studied the immunological effects of nitrogen dioxide, of which the results have been published in three different papers. In the first paper, male C57BL/6J mice (n=170 total) were exposed to 0.5-0.7 mg/m^3 nitrogen dioxide for 6 to 16 weeks (8 h/day, 5 days/week). This resulted in decreased subpopulations of several T-lymphocytes (spleen, blood) and natural killer cells (Kur89). In the second study, female, AKR/*cum* mice (n=36) and male, C57BL/6J mice (n=20) were exposed to nitrogen dioxide at a concentration of 0.5 mg/m^3 (0.25 ppm) for 7 weeks or to 0.7 mg/m^3 (0.35 ppm) for 12 weeks (7 h/day, 5 days/week). Again, T-lymphocyte cell populations and the number of natural killer cells decreased (Ric88). Comparable effects were observed in female C57BL/6J *cum* mice (n=9; n=10 control) exposed to 7.6 mg/m^3 (4 ppm) for 8 hours only (Dam89). According to the authors, changes in cell populations, such as observed in their studies, could affect directly the immune response against viral infections.

A series of experiments have been published in various Japanese journals, performed by the same group of investigators. In two reviews, written in English, Nakajima *et al.* reported on the study design and results of these experiments (Nak79, Nak80). In three experiments, female mice (JCL:ICR) were continuously exposed to: 0.57-0.96 mg/m^3 (0.3-0.5 ppm) nitrogen dioxide for 6 months; 0.96-1.53 mg/m^3 (0.5-0.8 ppm) for 1 month; and, to 1.34-1.53 mg/m^3 (0.7-0.8 ppm) for 1 month (n=12/group), respectively. In another experiment, mice were allowed to recover for 3 months after being continuously exposed to 1.91-2.87 mg/m^3 (1.0-1.5 ppm). All experiments included control groups, which were exposed to purified air. Immediately after ending the exposure, all animals were subjected to histological examinations. Mice exposed to nitrogen dioxide showed pulmonary lesions, such as proliferation of bronchiolar and alveolar epithelium, and loss of cilia. These lesions disappeared in mice, which were allowed to recover in clean air for 3 months. In another experiment, twelve mice infected with influenza virus after exposure to 0.96-1.91 mg/m^3 (0.5-1.0 ppm) for 39 days, showed adenomatous proliferation of bronchial and bronchiolar epithelium. No

details were given on the control group and on the number of animals in most of the experiments.

Sherwin and Richters (She82) exposed male Swiss Webster mice (n=60/group) to clean air or to 0.65 mg/m^3 (0.34 ppm) nitrogen dioxide for 6 hours per day, 5 days per week, for 6 weeks. Following exposure, lung tissue was isolated and prepared for quantitative image analysis. The investigators observed the following abnormalities: increased number of type II alveolar cells; small increased size of type II cells; and possibly slight atelectasis and/or oedema of the alveolar walls. Also, the authors reported on the possibility of an susceptible subpopulation of animals.

Chang and her colleagues performed two studies. In the first study, adult rats (n=8) were exposed to 0.96 or 3.8 mg/m^3 (0.5 or 2.0 ppm) nitrogen dioxide for 6 weeks (23 h/day, 7days/week). The exposure included two daily hour spikes to three times the background levels (0.96 to 2.9 mg/m^3 (1.5 ppm) and 3.8 to 11.5 mg/m^3 (6.0 ppm)), which were applied Monday through Friday. Morphometric analysis of the proximal alveolar regions of the lungs revealed changes in: the alveolar macrophages; the alveolar interstitium; and, in the epithelium (spreading and hypertrophy of type II epithelial cells) in all exposed animals (p<0.05). In addition, in the high-dose group only, the number of type I epithelial cells were increased (Cha86).

In the second study with the same exposure design, structural changes in the terminal bronchioles of the lungs were described. In the high-dose group, exposure to nitrogen dioxide caused injury to the cilia, the ciliated cells and possibly affected Clara cell differentiation. However, in the low-dose group, no morphologically measurable injuries in the terminal brionchioles were found. The authors compared the injuries occurring in the terminal bronchioles with the injuries occurring in the proximal alveolar regions, and concluded that cells in the proximal alveolar regions were more susceptible to epithelial injury caused by nitrogen dioxide than cells in the terminal bronchioles (Cha88).

Rombout *et al*. (Rom86) continuously exposed male Wistar rats (n=3/group) to 0.96, 2.5, 5.0 or 20.0 mg/m^3 nitrogen dioxide for up to 28 days. No morphological changes in the lungs were observed in the two lowest-dose groups. In addition, animals exposed to 5 mg/m^3 showed only minimal morphological alterations, including hypertrophy of the epithelial cells and loss of cilia. However, these kinds of injuries were prominent in the highest-dose group. All animals recovered almost completely from the injuries within eight days.

Bermúdez *et al.* (Ber99) continuously exposed male Sprague-Dawley rats (n=4/ group) to filtered air or to 2.3 mg/m^3 (1.2 ppm) nitrogen dioxide for three days. Analyses of the bronchoalveolar lavage fluid revealed no significant changes in the number of lung cells and the activity of lactate dehydrogenase.

Ohashi *et al*. (Oha94) investigated the effects of nitrogen dioxide on the epithelium of the nasal mucosa in female Hartley guinea pigs. The animals (n=10/group) were exposed to filtered clean air or to 6.6 or 16.9 mg/m^3 (3 or 9 ppm) nitrogen dioxide for 6 hours a day and 6 days per week for a total of two weeks. A day after the last exposure, all animals were sacrificed to obtain nose epithelium samples. Half of these samples were used to examine ciliary activity, for electron microscopic examination and to count the numbers of eosinophils. Exposure to nitrogen dioxide affected ciliary activity in a dose-dependent matter: low dose, 681±44 beat/min; high dose, 552±45 beats/min; and, control, 775±38 beats/min (p<0.01). Additionally, the number of eosinophils increased significantly from 0.1 per 100 µm epithelial length (control) to 2.1±1.1 (low dose) and 5.7±1.8 per 100 µm (high dose). Also the morphology worsened by increasing nitrogen dioxide exposure. Based on these results, the investigators suggested that nitrogen dioxide may contribute to hyperresponsiveness in the nose and may be involved in the manifestation of airway allergic disorders.

Hooftman *et al*. (Hoo88) examined the histopathology of the respiratory tract, including nasal cavity, larynx, trachea and lungs, in male SPF-reared albino rats after exposure to nitrogen dioxide. The animals (n=10/group) were exposed to clean air or to 7.6, 19.1 or 47.8 mg/m^3 (4, 10 or 25 ppm) nitrogen dioxide for 6 hours a day, 5 days a week, for 7, 14, or 21 days. Treatment-related histopathological changes were found in the lungs only. These changes included: hypertrophia and/or hyperplasia of small bronchi and bronchioli; increased cellularity of walls of respiratory bronchioli, alveolar ducts and/or adjacent alveoli; and, – after 7 days of exposure only – inflammatory cell infiltrates. All these changes were statistically significant different at an exposure concentration of 19.1 and 47.8 mg/m^3, whereas no differences were found between the lowest-dose group and control animals. In addition, the authors examined alveolar macrophage function and several biochemical parameters in lung lavage fluids. The morphology of the alveolar macrophages changed at all exposure levels. In addition, the number of macrophages increased at the two highest dose levels, at all measurement days. However, the phagocytic capacity decreased at the highest exposure level only at 14 and 21 days. Of the biochemical parameters tested, a statistically significant increase was only found in the gamma-glutamyl transferase (GGT) level in the two highest dose groups at 14 and 21 days.

Barth *et al*. (Bar99) exposed male Sprague-Dawley rats (n=5/group, n=10 controls) to 9.6, 19.1 or 38.2 mg/m^3 (5, 10 or 20 ppm, respectively) nitrogen dioxide for 3 or 25 days (hours/day not given). Following the three-day exposure period, injuries in the epithelium of the bronchioli were found in all exposed animals. These injuries increased from mild to severe with increasing exposure concentrations, except for morphological alterations found in Clara cells, which were dose independent. Following the 25-day exposure period, morphological changes were only observed in the two highest-dose

groups. This time, no morphological alterations in Clara cells were found in any of the exposed groups. According to the authors, the different outcomes between the three- and 25-day exposure periods may be explained by adaptation of the airway epithelium to oxidative stress.

Mauderly *et al.* (Mau87) examined age-related differences in the physiological responses of rats to inhaled automotive emissions. Only the results of nitrogen dioxide in adult rats are presented here. Male Fischer F344 rats (n=48/group, at least 10 animals/ group were used for each effect parameter measured) were exposed to filtered air or to 18.2 mg/m^3 (9.5 ppm) nitrogen dioxide for 7 hours a day, 5 days a week, over 6 months. Immediately after ending the exposure, health effects were measured, which included: pulmonary function; pulmonary immune responses; respiratory tract clearance; airway fluid enzymes and protein analysis; cytology; and, lung morphometry and histopathology. No significant exposure-related mortality in any groups was observed. Also respiratory function and body weight gain were comparable between the exposed and the control group. In addition, in exposed animals no changes were observed in: histopathology; mean internal surface area of the lung; and, in the rate of particle clearance from the lungs. However, in exposed animals, significant increases in lung weight/body weight ratios and significant reductions in lung volume/lung weight ratios were observed. Furthermore, in the lavage fluids of nitrogen dioxide-exposed rats, increased levels of acid phosphatase, ALP, LDH and GSH-P were measured. In addition, the percentage of neutrophils in the lavage fluids increased almost six-fold in exposed animals compared to controls. Based on these results, the authors suggested that pulmonary oedema had developed in nitrogen dioxide-exposed animals, although respiratory function appeared normal.

Van Bree *et al.* (Bre00) continuously exposed male Wistar rats (n=6/group) to clean air or to 20 mg/m^3 (10.6 ppm) nitrogen dioxide for four days. Immediately after exposure, the animals were sacrificed, and lung tissue prepared for biochemical and morphometrical analyses. Nitrogen dioxide exposure resulted in elevated levels of glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione peroxidase, and in a higher number of type II epithelial cells and alveolar macrophages.

Farman *et al*. (Far99) exposed male Sprague-Dawley rats (n=4/group) to filtered air or to 27.5 mg/m^3 (14.4 ppm) nitrogen dioxide for 6 hours a day and seven days a week for up to 90 days. Histopathological findings within the central acinus of the lungs in nitrogen dioxide-exposed animals included mild epithelial hypertrophy and very slight interstitial thickening. Concerning the bronchealveolar duct junction, a slight thickening of the interstitium was measured. Overall, the lesions in animals exposed for 90 days were milder than at 7 days of exposure.

The committee noted that in some of the foregoing animal studies adaptation did occur and that to a certain exposure level the observed effects were reversible.

6.2.4 *Toxicity due to long-term exposure and carcinogenicity*

Chronic toxicity

A summary of long-term non-carcinogenic studies in animals, including the evaluations by SCOEL (SCO93) and WHO (WHO97), is given in annex F, Table F1.

A comprehensive Japanese study on the effects of low levels of nitrogen dioxide exposure on rats has been described in three separate papers. The study included biochemical and morphological observations. Male Wistar rats (n=24/group/exposure period) were continuously exposed to filtered air or to nitrogen dioxide at concentrations of 0.08, 0.76 or 7.6 mg/m^3 (0.04, 0.4 and 4.0 ppm) for 9, 18 or 27 months. Three to four rats per group were randomly selected for morphological examinations. The remaining rats were used for studying effects on various biochemical parameters, such as lipid peroxidation and glutathione peroxidase.

Kubota *et al.* (Kub87) reported on their morphological observations. At 7.6 mg/m^3 morphological changes in the lungs of rats were observed after 9 months of exposure. Examinations with the light microscope revealed: hypertrophy and hyperplasia of bronchial mucosa; and, thickening of walls in the area through the bronchopulmonary junction of the alveolar duct, with cell infiltration and increase of Clara cells. The lesions progressed according to the prolongation of exposure. After 27 months of exposure, lesions in the area from bronchopulmonary junction to the proximal alveoli progressed more severely, and the interstitial fibrosis and hyperplasia of epithelium seemed to progress steadily. The alveolar structure was maintained and no emphysema developed. Appearance of macrophages was normal during the exposure period. At 0.76 mg/m^3, the lesions were milder than at 7.6 mg/m^3 and they occurred later. The morphological changes were detectable at 18 months by electron microscope, but no definite alterations were evident by light microscope until month 27. No remarkable morphological changes throughout the entire exposure period were observed in animals exposed to 0.08 mg/m^3.

The committee considers the morphological changes observed at 0.76 mg/m^3 as a pre-phase of fibrosis.

No significant biochemical changes were observed in groups exposed to 0.08 mg/m^3. However, at 0.76 and 7.6 mg/m^3 lipid peroxidation increased significantly. Also, significant changes were found on several enzyme activities, such as: glutathione peroxidase; glucose-6-phosphate dehydrogenase; and, glutathione-S-transferases. Overall, these changes were more prominent at 7.6 mg/m^3 than at 0.76 mg/m^3.

Furthermore, all these changes were observed at 18 months of exposure. Results after 27 months of exposure are not included because of very few survivors (Sag84, Sag87).

Concerning lung morphology, the same group of researchers performed a co-carcinogenesis study with the same experimental design as of the previous one (Ich91). In more detail, Wistar rats (effective number of animals per group = 10) were continuously exposed to clean air, 0.08, 0.76 and 7.6 mg/m^3 (0.04, 0.4 and 4.0 ppm) nitrogen dioxide for 18 months. Only the results of the animals exposed to nitrogen dioxide alone are presented here. Morphological analysis with the light microscope revealed: slight hyperplasia of alveolar cells; slight hypertrophy, proliferation and slight hyperplasia of bronchiolar epithelial cells; and, thickening of alveolar duct walls in animals exposed to 7.6 mg/m^3. No lung pathology was observed in rats exposed to the other exposure levels. In addition, no lung tumours were present.

Hyde *et al.* (Hyd78) used beagle dogs to study pulmonary effects of nitrogen dioxide after long–term exposure. Beagle dogs (n=10/group) were daily exposed to: 0.26 mg/m^3 nitrogen dioxide plus 2.05 mg/m^3 nitric oxide (NO); 1.2 mg/m^3 nitrogen dioxide plus 0.31 mg/m^3 nitric oxide; or, to filtered air for 86 months (16 hours/day). The dogs were allowed to breath clean air for a further 32 to 36 months before they were sacrificed. Morphologic examination of the lungs revealed that dogs exposed to 1.2 mg/m^3 nitrogen dioxide plus a low concentration of nitric oxide had significantly larger lungs with enlarged air spaces and evidence of destruction of alveolar walls. These effects were not observed in dogs from the other two exposure groups. The types of lesions observed in dogs are indicative of emphysema of the type seen in human lungs (see also review by Gillespie *et al*. (Gil80)).

The committee noted that no morphological measurements were carried out during or immediately after stopping the exposure with NO_2/NO. Hence, the committee cannot conclude whether or not some recovery of tissue damage has occurred. Furthermore, the investigators limited exposure to co-exposure only. However, at high NO-exposure no effects were observed, indicating that nitrogen dioxide was responsible for the observed effects.

Miller *et al*. (Mil87) exposed female CD-1 mice (n=21-28/group) to clean, filtered air or to 0.38 mg/m^3 (0.2 ppm) nitrogen dioxide for 16, 32 or 52 weeks (23h/d, 7d/w). Following these exposure periods, animals were challenged with *Streptococcus zooepidemicus* to examine antibacterial host defence. Also, pulmonary function and lung morphology were checked. The authors reported that exposure to nitrogen dioxide did not alter substantially any of the parameters measured.

Hayashi et al. (Hay87) studied the morphological effects of nitrogen dioxide on the rat lung. Male Wistar rats (n total =86; probably 4-8 animals/group) were continuously exposed to clean air or to 0.96 mg/m^3 (0.5 ppm) nitrogen dioxide for up to 19 months. Morphological changes were examined both by light and electron microscopy. As a

result of the nitrogen dioxide exposure, in groups exposed more than 4 months showed swelling of type II alveolar cells. In the 6-month group, the width of the alveolar wall increased. In addition, in the 19-month group, slight fibrous thickening of the pleura, although no fibrosis was observed in the lung parenchyma. Also, interstitial edema was noticed in the alveolar walls after 4 months of exposure, which progressed with prolonged exposure time.

Ehrlich and Henry (Ehr68) and Blair *et al.* (Bla69) performed antibacterial host defences and histopathologic examinations, respectively, on lung tissues obtained from mice, which were exposed to nitrogen dioxide over different time periods. Female, Swiss albino mice were exposed to clean air or to 0.96 mg/m^3 (0.5 ppm) nitrogen dioxide for 6, 16, and 24 hours daily, for 1, 3, 6, 9, and 12 months. Ehrlich and Henry observed increased susceptibility to bacteria, as demonstrated by enhanced mortality, of mice exposed for three months or longer. In addition, Blair *et al*. observed early bronchial inflammation with reduction of distal airway size and a concomitant expansion of alveoli, which progressed with prolonged exposure. Examination of heart, kidney, and spleen did not reveal any pathology.

Mauderly *et al.* (Mau89) exposed male Fischer F344 rats (n=46/group) to 18.2 mg/m^3 (9.5 ppm) nitrogen dioxide or clean air for 7 hours a day, 5 days a week for 24 months. Health effects were evaluated after 12, 18, and 24 months of exposure. The measurements included: respiratory function (n=16/time point); lung clearance; pulmonary immune responses (n=8 after 24 months of exposure); biochemistry and cytology of airway lavage fluid; and, lung morphometry and histopathology (n=8/time point). Nitrogen dioxide exposure caused: mild epithelial hyperplasia and a thickening of the walls of terminal bronchioles; an extension of bronchiolar epithelium into proximal alveoli; and, inflammation in proximal alveoli. On the other hand, lung volume and weight and the lung collagen content were increased. Also, lavage fluid indicators of cell damage and oxidant protective mechanisms were increased.

Carcinogenicity

Several investigators have evaluated the carcinogenic effects of nitrogen dioxide. However, none of these studies meet the current OECD standards in setting up carcinogenicity studies. This not only concerns the number of animals used, the use of susceptible animal strains and the exposure duration, but also poor reporting on the histopathology of tumours, poor reporting on additional signs of toxicity, and the absence of statistical evaluation.

Kubota *et al.* (Kub87) did not report on any sign of tumour development in the lungs or in other parts of the body of rats, which were continuously exposed to 0.08, 0.76 or 7.6 mg/m^3 nitrogen dioxide for up to 27 months. Also Mauderley *et al.* (Mau89), who

exposed rats to 18.2 mg/m^3 nitrogen dioxide for 24 months, did not report on signs of tumour development. However, both studies were not set up as carcinogenicity studies.

In addition, Wagner *et al*. (Wag65) used various animal species (rats, mice, hamsters, guinea pigs, rabbits and mongrel-dogs) to study the long-term effects of nitrogen dioxide at low exposure levels. The animals were exposed to 1.9, 9.4 or 47.8 mg/m^3 (1, 5 or 25 ppm) nitrogen dioxide, 6 hours per day and 5 days per week for 10 to 18 months. The investigators suggested that nitrogen dioxide accelerated the development of tumours in CAF1/Jax mice (mice strain that has spontaneously high pulmonary tumour rates) after exposure to 9.4 mg/m^3 for 12 months (7/10 had developed tumours compared to 4/10 in control animals). However, no differences in tumour development were observed after 14 and 16 months of exposure. Additionally, in none of the other animal species any sign of tumour development was observed.

In another study, Adkins *et al.* (Adk86) reported a small but statistically significant increase in frequency and incidence of spontaneously occurring pulmonary adenomas in female A/J mice (n=30/group; strain with spontaneously high pulmonary tumour rates) after exposure to 18.9 mg/m^3 (10 ppm) nitrogen dioxide for 6 hours per day and 5 days per week for six months. The increase was only observed when compared to pooled control groups. Exposure to 1.9 and 9.4 mg/m^3 (1 and 5 ppm) nitrogen dioxide had no effect.

More recently, Ichinose *et al*. (Ich91) published the histopathological results of a tumour promotion study. Male Wistar rats were continuously exposed to clean air or to 0.08, 0.76 and 7.6 mg/m^3 nitrogen dioxide for 17 months. Before exposure to nitrogen dioxide, the animals received a single intraperitoneal injection of N-bis(2-hydroxypropyl) nitrosoamine (BHPN) (n=40/group), a known inducer of tumours in the respiratory tract in rats, or an injection of saline (n=10/group for lung histopathology, n=20/group for nasal cavity histopathology). Immediately after exposure, all rats were killed for histopathologic examinations on several organs.

The mortality did not differ between the nitrogen dioxide-exposed groups with or without BHPN treatment (2.5% in all groups without BHPN treatment; 22.5-32.5% in all groups with BHPN treatment), nor did it differ with control animals (2.5% without nitrogen dioxide and BHPN; 30% with BHPN only). There were no lung tumours or tumours in the nasal cavity in any group exposed to nitrogen dioxide alone (n=10/group). Only one animal exposed to 7.6 mg/m^3 nitrogen dioxide showed slight to moderate alveolar cell hyperplasia. With BHPN treatment, a slight non-significant increase in lung adenomas (4/40) was observed in the group exposed to 7.6 mg/m^3 compared to the control group (1/40). In the same group one animal had an adenocarcinoma in the lung, which was not found in any of the other exposure or control groups. The incidence of tumours in the nasal cavity in the groups treated with BHPN was almost 100%. This finding was independent of the exposure level of nitrogen

dioxide given, and included the group exposed to clean air. Almost all these tumours were identified as malignant adenocarcinomas. Tumours, including lymphomas, were found in several other organs, but the authors could not correlate these findings with nitrogen dioxide or with BHPN exposure. Based on these findings, the authors suggested that nitrogen dioxide can promote the development of lung tumours initiated by BHPN at a concentration of 7.6 mg/m^3 or higher.

The WHO document (WHO97) reported on two tumour-promotion studies, which are written in Russian and Japanese. In the Russian study, rats were continuously exposed for lifetime to the carcinogen dimethylamine in combination with 2 or 3 mg/m^3 nitrogen dioxide. Despite the co-exposure, no tumours were observed (Benemansky *et al*., 1981, see WHO97). In the Japanese study, nitrogen dioxide (9.4-18.8 mg/m^3; 2 h/day, 5 days/week for 50 weeks) did not enhance tumour development in conventional mice receiving five weekly injections of 4-nitroquinoline-1-oxide, a lung tumour specific carcinogen (Ide and Otsu, 1973, see WHO97). Both studies show many shortcomings, such as inappropriate statistical analyses and the absence of control groups.

6.2.5 *Genotoxicity*

In vitro studies in bacteria

Nitrogen dioxide induced mutations in *Salmonella typhimurium* (Big87, Vic88) and *Escherichia coli* (Kos86) with (TA100) or without the presence of a metabolic activation system (TA100, TA1535). Victorin and Ståhlberg (Vic88) reported that nitrogen dioxide was not only mutagenic in TA100 at a concentration of 9.6, 19.1 and 28.7 mg/m^3 (5, 10 and 15 ppm; dynamic flow-through exposure system), but also bacteriotoxic at concentrations from around 19.1 mg/m^3.

In vitro studies in mammalian cells

In a well-reported study by Shiraishi and Bandow (Shi85), statistically significant, but not dose-related, increases in SCEs were observed in Chinese hamster V79 cells after exposure to 1.9, 3.8, 7.6, 11.5 and 15.3 mg/m^3 (1, 2, 4, 6 and 8 ppm) gaseous nitrogen dioxide for 2 hours. No SCEs were observed at a dose of 0 and 0.96 mg/m^3 (0 and 0.5 ppm).

Tsuda *et al.* (Tsu81) investigated the ability of nitrogen dioxide to induce chromosomal aberrations and sister chromatid exchanges (SCEs) in Chinese hamster V79 cells. Cells were exposed to 0.0, 9.6, 19.1, 38.2, 96 and 192 mg/m^3 (0, 5, 10, 20, 50 and 100 ppm) gaseous nitrogen dioxide (v/v in N_2) for 10 minutes. A significant dose-

related increase of chromosomal aberrations was observed at a concentration of 9.6 mg/m^3 and higher compared to control. Also, a relatively small increase in the frequency of SCEs was observed (increase factors compared to control were 1.3, 1.7, 1.6, 2.2 and 2.5 at 9.6, 19.1, 38.2, 96 and 192 mg/m^3, respectively). No details were provided on cytotoxicity.

Görsdorf *et al.* (Gör90) exposed Chinese hamster V79 cells to gaseous nitrogen dioxide at concentrations of 0-955 mg/m^3 (0-500 ppm) (v/v in N_2) for 5 to 30 minutes. Cells were harvested immediately after exposure. The threshold for cytotoxicity was determined to be around 382 mg/m^3 (200 ppm) after 20 minutes of exposure. Statistically significant increases in single strand breaks were observed at 19.1 mg/m^3 (10 ppm) onwards for 20 minutes of exposure. This increase was dose- and time related.

Walles *et al*. (Wal95) isolated alveolar macrophages from the lungs of NMRI mice, and exposed these cells in culture dishes without lid to 38.2 mg/m^3 (20 ppm) nitrogen dioxide for 2 hours. The level of DNA single strand breaks, as measured by the alkaline unwinding technique, increased significantly compared to control ($p<0.01$).

In vivo studies in *Drosophila melanogaster*

Victorin *et al.* (Vic90) investigated the genotoxic activity of nitrogen dioxide *in vivo* in the somatic mutation and recombination test in *Drosophila melanogaster* (the wing spot test). Larvae of the *Drosophila* flies were exposed to 95.5 mg/m^3 (50 ppm) nitrogen dioxide for up to 19 hours. Seven days later, the wings (n=20-40/group) were analysed under a microscope and scored for the number of spots. Nitrogen dioxide was not genotoxic. The authors noted that the present *in vivo* data do not confirm the results obtained earlier with bacteria (Vic88).

Inoue *et al*. (Ino81) used the standard sex-linked recessive lethal test to study whether high concentrations of nitrogen dioxide (285-535 or 134-1,070 mg/m^3 (150-280 or 70-560 ppm)) induces lethal mutations in male germ cells of *Drosophila melanogaster* in the presence or absence of methylurea or ethylurea. Nitrogen dioxide per se did not enhance the mutation frequency significantly. However, nitrogen dioxide enhanced significantly the mutation frequency in flies pre-treated with methylurea compared to controls ($p<0.01$). The authors suggested that nitrogen dioxide may participate in the formation of carcinogenic nitroso-compounds, but that nitrogen dioxide per se is at most a weak mutagen in *Drosophila.*

In vivo studies in mammals

Gooch *et al*. (Goo77) exposed male C3H mice (number of animals not given) to 0.2, 1.9, 9.5 and 19.1 mg/m^3 (0.1, 1, 5 and 10 ppm) nitrogen dioxide for 6 hours. Arterial blood

from several animals was sampled and pooled immediately, 1 week, and 2 weeks following exposure. Furthermore, spermatocyte preparations were made 8 weeks after exposure. No increase was observed in either chromatid- or chromosome-type aberrations in leucocytes or spermatocytes for any exposure. No concurrent controls were presented; instead the authors used control data from a previous study.

Bermúdez *et al.* (Ber99) continuously exposed male Sprague-Dawley rats (n=4/group) to filtered air or to 2.26 mg/m^3 (1.2 ppm) for 3 days, to study the occurrence of DNA single strand breaks in alveolar macrophages. Alveolar macrophages were obtained by bronchoalveolar lavage; the percentage of macrophages of the total cell number thus recovered was 98% and 99% of exposed and control animals, respectively. Cell viability did not differ statistically between both groups (control, 92%). Exposure of rats to 2.26 mg/m^3 nitrogen dioxide did not cause a significant increase in DNA single-strand breaks as measured by the alkaline elution technique.

Isomura *et al.* (Iso84) exposed male Sprague-Dawley rats (n=5/group; n=40 controls) to clean air or to nitrogen dioxide at concentrations of 15.3, 28.7, 40.1 and 53.5 mg/m^3 (8, 15, 21 or 27 ppm) for three hours. Eighteen hours later, the animals were killed and primary lung cells were isolated and prepared for further culture over a 5-day period. Cell survival in cells from exposed animals did not differ from control (plating efficiency: 10 - 15%). The mutation frequency increased dose-dependently and this was statistically significant from 28.7 mg/m^3 (15 ppm) upwards. Also the number of chromosomal aberrations was increased at 15.3 and 53.5 mg/m^3 compared to control.

Victorin *et al.* (Vic90) investigated the genotoxic activity of nitrogen dioxide *in vivo* in the mouse bone marrow micronucleus assay. Male NMRI mice (n=3/group) were exposed to clean air or to 38.2 mg/m^3 (20 ppm) nitrogen dioxide for 23 hours. Seven hours later, the animals were killed and bone marrow smears were prepared. Polychromatic erythrocytes were examined for the presence of micronuclei. Nitrogen dioxide was not genotoxic. The authors noted that the data of this study did not confirm the results obtained earlier with bacteria (Vic88). The authors also suggested that the present negative results probably reflect the fact that reactive nitrogen dioxide products may be inactivated *in vivo* before they reach target cells in the bone marrow.

For this reason, the same group of investigators decided to study genotoxic effects in lung tissue – the primary target of nitrogen dioxide - of mice after *in vivo* exposure (Wal95). This time, male NMRI mice (n=3-5/group) were exposed to 57.3 and 95.5 mg/m^3 (30 and 50 ppm) nitrogen dioxide for 16 hours and 5 hours, respectively. Following exposure, animals were killed and lung tissue obtained. Isolated nuclei obtained from these lung tissues were tested for the presence of DNA single strand breaks, using two techniques: the alkaline unwinding technique, and the alkaline elution technique. Using the unwinding technique, there was a statistically significant increase in single strand breaks at both exposure levels (p<0.01). However, no increase of single

strand breaks was found using the alkaline elution technique. The authors speculated that formation of DNA-DNA cross-links in the exposed samples could have resulted in the differing sensitivities of the two methods used to assay single strand breaks. DNA inter-strand cross-links can obscure the single strand breaks in the alkaline unwinding technique.

Summary and evaluation of the genotoxic data

Mutagenicity and genotoxicity data obtained from *in vitro* studies suggest that nitrogen dioxide is genotoxic under certain conditions.

In the literature, some suggestions have been made concerning the possible genotoxic mechanism of nitrogen dioxide. Nitrogen dioxide forms nitrous and nitric acids in aqueous solutions, which are converted into the more stable nitrite (NO_2^-) and nitrate (NO_3^-) ions.

One mechanism may be that nitrous and nitric acid locally reduce the pH. According to some investigators, lowered pH may induce genotoxicity. However, Tsuda *et al*. (Tsu81) found no changes in pH at the surface of plastic dishes, even after exposure to 191 mg/m^3 (100 ppm) nitrogen dioxide.

In addition, Tsuda *et al*. (Tsu81) and Görsdorf *et al*. (Gör90) measured the ability of $NaNO_2$ and $NaNO_3$ to produce chromosomal aberrations, SCEs and single strand breaks in their *in vitro* cell systems. Tsuda *et al*. observed increases of chromosomal aberrations and SCEs at concentrations of 50 mM $NaNO_2$ or higher. For comparison and using the same experimental conditions, the authors found that 191 mg/m^3 (100 ppm) nitrogen dioxide led to a dissolved concentration of 0.66 mM $NaNO_2$. Görsdorf *et al*. did not find single strand breaks in V79 cells after exposure to 1 mM $NaNO_2$ or $NaNO_3$.

Also an indirect acting mechanism is proposed. Nitrite is known to react with amines to produce nitrosamines (under acidic conditions), which are known carcinogens. The formation of nitrosamines after exposure to nitrogen dioxide *in vivo* in the presence of added amines was demonstrated by several investigators (Iqb84, WHO97). However, the results of these studies were not considered reliable as for how much nitrosamines are formed without adding 'artificial' amines and whether or not these low amounts detected can induce genotoxicity.

Overall, the committee is of the opinion that the *in vitro* data on the genotoxicity of nitrogen dioxide give rise to concern. However, the mechanism by which nitrogen dioxide might act as a genotoxic compound is unknown. It is possible that antioxidant defense systems, which are present in and at the epithelial surface of the respiratory tract, may protect against nitrogen dioxide-induced DNA damage and therefore prevent the formation of tumours. This hypothesis may explain why *in vivo* data did not show convincing evidence for a genotoxic potential.

6.2.6 *Reproduction toxicity*

A few investigators have examined the effects of inhaled nitrogen dioxide on the reproduction and development in rats.

Two studies were published in Russian, but thoroughly reviewed in English by Barlow and Sullivan (Bar82). One of these studies was published by Gofmekler *et al.* (Gof77). Female rats (strain and number of dams was not given) were continuously exposed to clean air or to 0.03, 0.08 or 0.82 mg/m^3 (0.018, 0.045 and 0.43 ppm) nitrogen dioxide between gestations days 1 and 21. Foetal body and liver weights decreased in a dose-related manner. However, intra-uterine mortality increased ($p<0.05$). No further details were given.

In the other Russian study, Shalamberidze and Tsereteli (Sha71) exposed female rats (n=7-10/group) to 0, 0.13 or 2.4 mg/m^3 (0, 0.7 or 1.25 ppm) nitrogen dioxide (12 h/day, daily) for 3 months, to study effects on the oestrus cycle and the reproductive capacity. Exposure to 2.4 mg/m^3 increased the oestrous cycle length (was reversible), reduced the mean litter size and decreased the body weights of the litters. No further details were given.

The group of Tabacova *et al.* (Tab85) conducted a study on postnatal development. Pregnant Wistar rats (n=20/group) were exposed to filtered air, 0.05, 0.1, 1.0 or 10 mg/m^3 (approximates 0.03 – 5.2 ppm) nitrogen dioxide for 6 hours a day, 7 days a week throughout gestation. Offspring were studied for 2 months post-exposure. In the two highest-dose groups, a significant lack of normal neuromotor development was observed and open field activity was reduced. No significant postnatal developmental effects were observed in the two lowest-dose groups.

The committee noted the inadequate reporting of the preceding studies. For this reason, no firm conclusion can be made on the reproduction toxicity potential of nitrogen dioxide.

6.3 Other studies

Other relevant data were not found.

6.4 Summary

Numerous studies have been published on the adverse effects of nitrogen dioxide inhalation. In these studies, a broad range of exposure levels, exposure periods and parameters have been used. Since the purpose of this evaluation is to set an HBR-OEL,

the committee restricted its evaluation to low exposure concentration data with known biological consequences.

Human data

Nitrogen dioxide is an irritating gas that affects the respiratory tract, in particular the epithelial surface of the deeper parts of the lungs. Therefore, many human studies, in particular controlled laboratory studies, have been directed to pulmonary effects. A summary by type of study is given below.

A range of controlled laboratory studies in healthy humans has been performed on the adverse pulmonary effects of nitrogen dioxide after single exposure. The duration of the exposure varied from 15 minutes to 6 hours. In addition, in some studies, volunteers exercised on a bicycle ergometer during exposure. The main effects resulting from exposure consisted of increased airway resistance and changes in respiratory host defense. These adverse effects were clearly present at exposure levels from 3.8 mg/m^3 upwards. However, below 3.8 mg/m^3, data became inconsistent. The lowest significant effect, namely increased airway reactivity against carbachol, was found at 2.9 mg/m^3. Between 1.1 and 2.9 mg/m^3 only a few human laboratory studies have been performed. However, all these showed some limitations, such as a low number of volunteers, inclusion of smokers and the absence of a detailed report. On the other hand, at around 1.0 mg/m^3 not only many more studies were performed, but also more volunteers participated in those studies. At 1.0 mg/m^3, no significant respiratory effects were found.

One controlled laboratory study has also included peak exposure. In this study, a group of healthy volunteers was exposed to a ‘background’ nitrogen dioxide concentration of 0.1 mg/m^3 for three hours. During these 3 hours, they were also exposed three times to 3.8 mg/m^3 for 15 minutes (peak exposure). However, peak exposure did not affect lung function or pulmonary host defense mechanisms.

Some of these controlled studies concerned repeated exposure for several consecutive days. However, the number of these types of studies was limited.

Except laboratory studies, the committee has found many epidemiological studies. Most of these have been conducted in the general population. Overall, these studies give some evidence that the substance may induce respiratory illness. However, in most of these studies confounders were present. These comprised: the inclusion of vulnerable people; absence of job history; missing data concerning smoking habits; and, co-occurrence of other combustion products and pollutants.

Lastly, there have been a few case reports of human health effects following acute or accidental exposure to high concentrations of nitrogen dioxide. Accidental exposure resulted in lung oedema, bronchiolitis obliterans and pneumonia. However, none of these symptoms could be related to a certain exposure level.

Animal data

Numerous animal data are available on the adverse effects of nitrogen dioxide to concentrations of as low as 0.11 to 3.8 mg/m^3 after single or repeated short-term exposure. In most of these studies, animals have been continuously exposed to nitrogen dioxide. The exposure resulted in a variety of responses in the respiratory tract, including: lowered host defense mechanisms; changes in lung biochemistry; and, changes in morphology. These respiratory effects were consistently found from 1.0 mg/m^3 upwards. Below this concentration, the data were less consistent; with the lowest reliable effect being observed at 0.65 mg/m^3. At this level morphological changes in the lung of mice were observed by quantitative image analysis.

Concerning the severity of the observed effects, some investigations have shown that at least part of these short-term responses were reversible. For instance, morphological changes observed in rats exposed to 5.3 mg/m^3 nitrogen dioxide for 28 days, disappeared after the exposure stopped. Additional data suggested that adaptation did occur.

Regarding chronic or long-term exposure, a lot of animal studies have been performed. In animals that have been exposed for at least 12 months, effects such as: lowered survival rates; lowered host defense activity in the lungs; and, lung tissue pathology were found. These effects have been consistently reported at 0.96 mg/m^3 upwards. In addition, in one comprehensive long-term study, rats were continuously exposed to nitrogen dioxide at a concentration of 0.08, 0.76 or 7.6 mg/m^3 or clean air for up to 27 months. In the lungs of these exposed animals, slight (at 0.76 mg/m^3) to severe (at 7.6 mg/m^3) signs of hyperplasia and fibrosis, and changes in various antioxidant enzyme activities were observed. However, no remarkable morphological or biochemical effects were observed in animals exposed to 0.08 mg/m^3. In another study, beagle dogs developed signs of emphysema after being exposed to 1.2 mg/m^3 nitrogen dioxide (plus a low concentration of nitrogen monoxide) for 86 months with a recovery period of 32 to 36 months. No such signs were observed in beagle dogs that have been exposed to 0.26 mg/m^3 nitrogen dioxide (plus a high concentration of nitric oxide). Furthermore, Miller *et al*. (Mil87) found no treatment-related changes in lung morphology, lung function and pulmonary host defense in animals, which have been exposed to 0.38 mg/m^3 for up to 12 months.

As for peak exposure, several short-term and long-term animals studies have been carried out in mice and rats. Most of these studies have been focussed on the pulmonary host defense to infectious agents. Following peak exposure for several days to several months, nitrogen dioxide clearly lowered the pulmonary host defence to infectious agents.

Except for pulmonary effects, also extrapulmonary effects have been described. For instance, T-lymphocyte populations in spleen and liver and the number of natural killer cells were decreased in animals that have been exposed to 0.48 mg/m^3 nitrogen dioxide for a few weeks. In theory, these changes could affect the immune systems ability to fight against viral infections. However, it cannot be excluded that these immunologic effects were normal adaptive physiological reactions.

Carcinogenicity and genotoxicity

Studies on the carcinogenic or tumour promoting potential of nitrogen dioxide are limited and give no reliable information. On the other hand, additional *in vivo* and *in vitro* data have provided some evidence of genotoxicity. However, the committee noted the insufficient reporting of particularly the *in vivo* data.

Reproduction toxicity

Only limited investigations have been carried out on the reproduction toxicity of nitrogen dioxide.

Chapter 7

Existing guidelines, standards and evaluations

7.1 General population

The WHO has set health-based guidance values for nitrogen dioxide. The WHO wrote:

On the basis of human controlled exposure studies, the recommended short-term guidance value is for a one-hour average NO_2 daily maximum concentration of 200 $\mu g/m^3$ (0.11 ppm). The recommended long-term guidance value, based on epidemiological studies of increased risk of respiratory illness in children, is 40 $\mu g/m^3$ (0.023 ppm) annual average.

These values are defined as the concentration of nitrogen dioxide, below which no adverse effects to human health are expected and are based on human clinical data (WHO97, WHO00).

7.2 Working population

The existing occupational exposure limits of nitrogen dioxide are summarised in Table 7.1 (see next page).

The following evaluations were given:

The Netherlands

In 1985, the 'Werkgroep van Deskundigen' (WGD) published a criteria document concerning nitrogen dioxide and recommended a health-based occupational exposure limit of 0.5 mg/m^3 (8 h TWA) and a short-term exposure limit of 1 mg/m^3 (15 min TWA) (DEC85). However, due to socio-economic constraints, a legally binding OEL has been set at 4 mg NO_2/m^3 (8 h TWA) (SZW02).

In 1985 the WGD concluded in its criteria document that:

The critical organ is the respiratory tract, and in practice inhalation is also the sole route of exposure.

The following effects were shown in human subjects after short-term exposure: aberrations of the lung functions, the no observed adverse effect level (NOAEL) on young healthy adults was estimated to be 1 to 2 mg/m^3 NO_2, after 60 to 120 min of exposure, at rest or with physical exercise. For adults with increased susceptibility, the NOAEL was estimated to be about 0.2 to 0.9 mg/m^3 NO_2, after 60 to 120 min of exposure, and with physical exercise. For this group of subjects the lowest observed adverse effect level (LOAEL) was estimated to be 1 to 3 mg/m^3 NO_2. Variations between dose-effect relationships have been observed; the no observed adverse effect level for older-aged subjects with chronic non-specific lung disease is lower than that for healthy young adults.

Experimental animal data showed species specificity, guinea pigs being the most sensitive experimental animals.

Experimental animal data showed that exposure to relatively high levels of NO_2 caused adverse effects on the liver, immune system and reproduction. There were no indications that NO_2 induced mutagenicity or carcinogenicity. On the other hand, there was evidence that exposure to NO_2 might promote the production of nitrosoamines, and also increase the risk of metastasis in the respiratory tract.

European Commission

Recently, the SCOEL recommended a limit for nitrogen dioxide of 0.4 mg/m^3 (0.2 ppm; 8 h TWA), and a STEL of 1.0 mg/m^3 (0.5 ppm; 15 min TWA). The committee disclosed that it can be discussed whether or not an 8-hour TWA can be derived and whether such a limit would make sense, as the primary danger of nitrogen dioxide is the acute effect on the airway system, which was found after short-term exposure to sufficiently high nitrogen dioxide concentrations. However, one should not forget the biochemical and morphological changes, which were observed in rats after continuous exposure to 0.75 mg NO_2/m^3 (0.4 ppm) for 18 to 27 months.

In addition, the SCOEL established a LOAEL of 1.1 mg NO_2/m^3 after single exposure for three hours in healthy subjects (with respect to the efficiency of macrophages to inactivate influenza virus). Several studies pointed to a LOAEL of 0.6 mg/m^3 for

obstructive effects on the lung function of people suffering from bronchial asthma or chronic bronchitis. A meta-analysis indicated that some asthmatics might have increased bronchial reactivity at concentrations below 0.4 mg/m^3. Taking into account all these data the committee recommended the current limit of nitrogen dioxide (SCO93; SCO97). See also annex D for the evaluation of the SCOEL.

Table 7.1 Foreign Occupational Exposure Limits (OELs).

country -organisation	OEL mg/m^3	ppm	TWA	type of OEL	note	year of adoption	reference
The Netherlands							
- Ministery	4	2	8-h	legal MAC	-	-	SZW02
- DECOS	0.5	0.25	8-h	HBR-OEL	-	1985	DEC85
	1.0	0.5	15-min	(STEL)	-	1985	DEC85
Germany							
- DFG	9.5	5	8-h	MAK	-	-	DFG01
	9.5	5	15-min	Peak Limitation Category	max. 4 shifts; 1-h interval		
Sweden	4	2	8-h	OEL	-	1990	SNB00
	10	5	-	Ceiling			
Denmark	5.6	3	8-h	OEL	-	-	Arb96
	9.4	5		Ceiling			
The United King-dom							
- HSE	5.7	3	8-h	OES	-	-	HSE02
	9.6	5	15-min	STEL			
European Union							
- SCOEL	0.4	0.2	8-h	OEL	-	1997	SCO97
	1.0	0.5	15-min	STEL			
The USA							
- ACGIH	5.6	3	8-h	TLV	A4	1981	ACG02
	9.4	5	15-min	(STEL)			
- OSHA	9.0	5	-	PEL (Ceiling)	-	-	ACG02
- NIOSH	1.8	1	15-min	REL (STEL)	-	-	ACG02

Sweden

In their consensus report, the Swedish National Board of Occupational Safety and Health considered that the effect on the respiratory system due to short-term exposure to nitrogen dioxide, in the form of increased pulmonary resistance, was the critical effect

(Lun86). Asthmatics and individuals with respiratory diseases were particularly susceptible.

The USA: ACGIH

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a TLV for nitrogen dioxide of 5.6 mg/m^3 (3 ppm; 8 h TWA), and a STEL of 9.4 mg/m^3 (5 ppm) (ACG02). In addition, on the basis of information from animal studies, a ceiling of 9.4 mg/m^3 was recommended. This level was considered sufficiently low to protect against immediate injury or adverse physiological effects from prolonged daily exposures.

Chapter 8

Hazard assessment

8.1 Hazard identification

Epidemiological studies and numerous laboratory studies on humans and animals have been performed to identify the adverse health effects of inhaled nitrogen dioxide. These include single and repeated exposure studies, as well as peak exposure studies. The following paragraphs contain short evaluations on the relevant toxic effects of nitrogen dioxide after single and repeated exposure.

Health effects after single exposure

Concerning humans, various controlled clinical studies in healthy volunteers have shown that nitrogen dioxide is harmful to the upper and lower respiratory tract (bronchioli and alveoli). These effects include: irritation to the nose and throat; increased airway resistance and airway reactivity; and, lowered pulmonary host defense, during or shortly after the exposure was stopped (see Table E1 in annex E). Also eye irritation has been described. All these effects were consistently observed when volunteers were exposed to 3.8 mg/m^3 or higher. Based on an extensive database, the no-effect threshold can be set at 1.0 mg/m^3.

The acute adverse health effects found in humans after a single exposure have also been described in animals (see Table F3 in annex F). In addition, in animals, a variety of additional effects have been observed, such as: changes in lipid peroxidation; changes in

pulmonary antioxidant enzyme activities; and, changes in lung morphology. These effects were first observed in the exposure range of 1 to 20 mg/m^3.

In several human and animal studies, the effects of peak exposure have been investigated. However, although this may reflect the occupational situation, the committee considers these studies inadequate for derivation of an HBR-OEL, since it cannot separate effects of peak exposure from those of the 'background' exposure. This makes it impossible to assess their respective contribution to the complete set of health effects.

Health effects after repeated exposure

A few controlled laboratory studies in human volunteers have been performed with repeated nitrogen dioxide exposure for 2 to 12 days (see Table E2 in annex E). These studies showed increased pulmonary inflammation at around 3.8 mg/m^3 or higher. However, the number of repeated-exposure studies is limited, such that no clear dose-response relationships can be established.

In addition, the epidemiological studies described in this document are not useful for quantitative risk assessment of chronic nitrogen dioxide exposure. This is due to the many confounding factors present in these studies, such as the absence of job history, the co-occurrence of other toxic substances, and the presence of vulnerable people, among them children. Despite these confounders, some association between nitrogen dioxide exposure and respiratory illness have been reported.

In animals, a lot of short-term and long-term repeated-dose studies have been performed (see Table F1 and F2 in annex F). All these studies support the findings found in humans and animals after single exposure. These included: irritated eyes, nose and throat; decreased lung function; and, changes in the antioxidant defense system. In addition, some of the effects worsened with extended exposure. For instance, repeated exposure led to increased mortality and decreased survival time after challenge with an infectious agent. Also, morphological changes of the lungs were more severe and resulted in hyperplasia, fibrosis or emphysema, of which the last two are known to be (partly) non-reversible. All these effects occurred consistently when the animals were exposed to 0.96 mg/m^3 nitrogen dioxide or higher. However, also below this concentration respiratory effects have been reported. These included morphological changes in the lungs at 0.65 mg/m^3 (subchronic exposure (She82)) and at 0.76 mg/m^3 (chronic exposure (Kub87)). However, part of the low-exposure data is less reliable for several reasons, including: insufficient reporting; limitations in study design; and, the question whether the observed effects were just normal adaptive physiological reactions or pathological ones. Finally, several investigators have reported findings reflecting adaptation or (complete) recovery of the induced effects after repeated exposure.

Carcinogenicity

Studies on the carcinogenic or tumour promoting potential of nitrogen dioxide are limited and give no conclusive information. On the other hand, additional *in vivo* and *in vitro* data have provided some evidence of genotoxicity. However, the committee noted the insufficient reporting of particularly the *in vivo* data. Overall, the committee concludes that the carcinogenic and genotoxic properties of nitrogen dioxide were insufficiently investigated. Therefore, according to the EU guidelines, the committee recommends not classifying nitrogen dioxide.

Reproduction toxicity

Animal studies on the fertility and developmental toxicity of inhaled nitrogen dioxide are of insufficient quality and, therefore, no firm conclusions can be drawn.

Conclusion

Taking the whole set of available data into account, the committee considers the nitrogen dioxide effects on the deeper parts of the respiratory tract as the most sensitive. These include increased airway resistance, enhanced susceptibility to bacterial or viral airway infections, and, on long-term, irreversible damage of the lung tissue. It is evident that the respiratory effects are primarily local effects, and that part of these effects are not only observed after repeated exposure, but also immediately or during a brief exposure. To prevent workers from these harmful acute and long-term effects, a 15-minute as well as an 8-hour HBR-OEL is warranted.

8.2 Quantitative hazard assessment

Recommendation of an HBR-OEL, 15-min TWA (STEL)

In deriving a STEL, the committee has access to a large set of human data that focussed on short-term single-exposure studies. In addition, most of these studies have been performed within a narrow range of nitrogen dioxide concentrations. Although within this narrow range results have been sometimes contradictory, overall the results are consistent. For this reason, the committee proposes a STEL based on the complete set of human single-exposure studies instead of a single study.

Concerning the derivation of the STEL, in the clinical studies on healthy volunteers toxicological significant effects on lung function or respiratory resistance were observed from 3.8 mg/m^3 upwards. The lowest significant effect, namely increased airway

reactivity against carbachol, was found at 2.9 mg/m^3 (Fra91). Between 1.1 and 2.9 mg/m^3 only a few human laboratory studies have been performed. However, all these showed some limitations. These include a low number of volunteers, inclusion of smokers and the absence of a detailed report. On the other hand, at around 1.0 mg/m^3 not only many more studies were performed, but also more volunteers participated in those studies; in none of these studies, significant respiratory effects were found. Taking into account all these facts, the committee considers 1.0 mg/m^3 as the NOAEL. Compensation for differences between individuals is not necessary, because of the very large and consistent set of data (see Table E1 in annex E). Taking these considerations into account, the committee recommends an HBR-OEL for nitrogen dioxide of 1.0 mg/m^3, as a 15-minutes time weighted average concentration (STEL).

Concerning previous recommendations, this STEL value is similar to the one recommended by DECOS in 1985 and by the SCOEL in 1997.

Recommendation of an HBR-OEL, 8-hour TWA

Unfortunately, epidemiological data are limited and insufficient to be useful in deriving an HBR-OEL, because epidemiological studies were almost always accompanied by co-exposure with other (toxic) substances. In addition, clinical human data on repeated exposure showed limitations in study design. For instance, the exposure periods were short and the types of effects studied were limited to (semi)acute effects. On the other hand, a large number of animal studies with subchronic and chronic effects are available. Therefore, the committee decided to derive an HBR-OEL from animal data.

Table 8.1 and 8.2 summarize the available information on chronic low exposure to nitrogen dioxide and its effects on lung morphology, lung function and pulmonary host defense.

Table 8.1 Nitrogen dioxide effects on lung morphology in animals after (sub)chronic exposure.

Level (mg/m^3)	Animal species	Exposure duration	Lung morphology	Ref.
0.08 0.76 7.6	Rat n=2-3/ group	Continuous for 9, 18 and 12 months	*After 9 mo*: Hypertrophia and hyperplasia of bronchial mucosa, and thickening of walls through the bronchopulmonary junction of alveolar duct with cell infiltration and increase in Clara cells at 7.6 mg/m^3. Observations were made by light microscope. No morphological changes were observed at 0.08 and 0.76 mg/m^3. *After 18 mo*: Progression of the lesions observed after 9 months of exposure at 7.6 mg/m^3. No morphological changes were observed with light microscope at 0.76 mg/m^3, but a tendency towards epithelial changes and interstitial edema of alveolar wall were detected with electron microscope. No remarkable changes in lung morphology were found at 0.08 mg/m^3. *After 27 mo*: Interstitial fibrosis and hyperplasia of epithelium progressed steadily at 7.6 mg/m^3, but alveolar structure was maintained and no emphysema developed. Slight but definite alteration of the epithelium became evident at 0.76 mg/m^3 with light and electron microscope. Lung morphology at 0.08 mg/m^3 did not show remarkable changes.	Kub87
0.26 (+ 2.05 mg/m^3 NO) 1.2 (+ 0.31 mg/m^3 NO)	Beagle dog (n=6/ group)	16 h/d, daily for 86 mo, followed by a recovery period of 32 to 36 months	*Filtered air*: no remarkable morphological changes. *0.26 mg/m³ (+ 2.05 mg/m³ NO)*: no remarkable morphological changes. *1.2 mg/m³ (+ 0.31 mg/m³ NO)*: emphysema (larger lungs with enlarged spaces and evidence of destruction of alveolar walls.	Hyd78
0.38	Mouse n=12	Continuous for 4, 6 and 12 months	No treatment related pathological lesions found.	Mil87
0.65	Mouse n=60	6 h/d, 5 d/w for 6 weeks	Type 2 cell hypertrophy and hyperplasia; mean linear intercept and amount of alveolar wall area were increased (quantitative image analysis).	She82
0.96	Rat n=86 total	Continuous for up to 19 months	*After 4 mo*: Various histological changes observed: swelling of type-II alveolar cells and interstitial edema. *After 6 mo*: Increased thickness of alveolar septa. *After 19 mo*: Fibrous pleural thickening.	Hay87
0.96	Mouse n= up to 5/ group	Continuous, 18 h/d or 6 h/d for 12 months	*After 3 mo*: Pneumonitis and increased alveolar size; loss of cilia with 24h/d exposure; increased susceptibility to airborne *Klebsiella* and decreased clearance. *After 6 mo*: Pneumonitis, cilia loss, increased alveolar size, bronchial / bronchiolar inflammation. *After 12 mo*: Reduced capacity to clear viable bacteria.	Ehr68, Bla69
0.96 1.88 7.52	Rat n=2/sex/ group	Continuous for 7 months	*0.96 mg/m³*: Swelling of cilia in terminal bronchial epithelia; and, hyperplasia of type II cells in alveolar septa. Authors suggest that early injury may be repaired. *1.88 mg/m³*: Cilia loss in terminal bronchioles; hyperplasia of type II cells; and, interstitial edema. *7.52 mg/m³*: Cilia loss in terminal bronchioles; hyperplasia of type II cells, interstitial edema; no. of lamellar bodies in type II cells decreased.	WHO97 (Yamamoto and Takahashi, 1984)

Table 8.2 Nitrogen dioxide effects on lung function in animals after (sub)chronic exposure.

Level (mg/m^3)	Animal species	Exposure duration	Lung function and interaction with infectious agents	Ref.
0.11 0.96 1.91 3.82 7.64	Guinea-pig n=12-15/ group	Continuous for 6 and 12 weeks	Specific airway resistance was significantly increased at 3.82 and 7.64 mg/m^3. Furthermore, airway responsiveness to inhaled histamine was significantly increased at 1.91 (12 weeks), 3.82 (6 and 12 weeks) and 7.64 mg/m^3. No effects on lung function were observed at 0.11 and 0.96 mg/m^3.	Kob95
0.38	Mouse n=12	Continuous for 4, 6 and 12 months	Lung function did not alter substantially, although multiple-breath nitrogen wash-out was significantly decreased. Mortality and relative mean survival time not affected after infectious challenge.	Mil87

The descriptions of the pathological effects made by several investigators are of concern to the committee, because these suggest that lung fibrosis may develop. Lung fibrosis is a slowly progressive disease that can end in life-threatening pulmonary and cardiac diseases. Therefore, the committee is of the opinion that lung fibrosis should be prevented. Unfortunately, all of the most relevant studies have some shortcomings, as will be discussed below in more detail for some of them.

In deriving an HBR-OEL, the long-term study of Kubota *et al.* (Kub87) is of primary interest, because they used a range of different exposure concentrations, including a very low one. Furthermore, the reported lesions (hyperplasia, fibrosis) were more pronounced when increasing the exposure level. The committee is concerned about these observations, despite some limitations in this study, such as the low number of animals and the absence of a matched-control group at 27 months. Nevertheless, from the Kubota-study a No-Observed-Adverse-Effect-Level (NOAEL) of 0.08 mg/m^3 and a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 0.76 mg/m^3 can be derived. Regarding this LOAEL, also the (short-term) study by Sherwin (She82) supports the finding that at around this level (0.65 mg/m^3) respiratory tract effects with possible long-term consequences do occur. Regarding the above-mentioned NOAEL, the committee is aware that the outdoor concentration in certain urban areas is close to this value.

In deriving an HBR-OEL, the committee should take a NOAEL as a starting point, unless the NOAEL is not known. Concerning the NOAEL of 0.08 mg/m^3 of the Kubota-study, there is a large gap (factor 10) between this NOAEL and the LOAEL of the same study. Due to this large gap, the "real" NOAEL may be higher. This is supported by data reported by other investigators. For instance, Miller *et al.* (Mil87) found no treatment-related changes in lung morphology, lung function and pulmonary host defense in animals, which have been exposed to 0.38 mg/m^3 for up to 12 months. In addition, Hyde *et al*. (Hyd78) found no remarkable changes in lung morphology in beagle dogs, which

have been exposed to 0.26 mg/m^3 (with co-exposure to 2.05 mg/m^3 nitrogen monoxide) for 86 months. The committee subsequently surveyed the available long-term animal studies (see Tables 8.1 and 8.2) and concluded that at or below 0.38 mg/m^3 no relevant toxicity occurred (acknowledging the shortcomings of each study). These data clearly show that the NOAEL of 0.08 mg/m^3 is indeed too low, and confirm that the ten-fold gap in the concentration range of the Kubota-study was too wide. Hence, taking all the animal data into accounting, the committee sets the NOAEL at 0.38 mg/m^3.

For the establishment of the HBR-OEL, several aspects have to be considered, because the toxicity data should be extrapolated to workers. One of these aspects is the difference between animals and humans. The committee noted that in assessing a NOAEL three different animal species were taken into consideration. These species responded with pulmonary effects during and after nitrogen dioxide exposure within a narrow concentration range. For this reason, the committee decided not to add an additional extrapolation for interspecies differences.

Also differences among people should be taken into account, requiring an additional uncertainty factor of three for inter-individual differences.

Another aspect is the experimental condition. The NOAEL of 0.38 mg/m^3 is established from animal data concerning mainly exposure for 24 hours a day and 7 days a week, which is much more than the exposure pattern of the worker (8 hours a day, 5 days a week). The observed effect fibrosis is an irreversible endpoint of an inflammation process that over time fails to be repaired, leading to tissue damage (*e.g.* cytotoxicity). In general, early tissue damage can be repaired, unless the damage is too severe or the time needed for complete repair is absent or too short. Consequently, when early tissue damage is repaired, fibrosis will not develop. Concerning nitrogen dioxide and the animal studies, because the animals were mainly continuously exposed, they did not have time to recover from their lung injuries. As a result the injuries progressed over time leading to lung fibrosis. However, workers are exposed for shorter periods during their working week and thus may have time to recover. Therefore, the committee takes the view that workers are less at risk than the animals used in those laboratory studies. This aspect should be included in deriving an HBR-OEL. However, it is difficult to assess the exact height of the uncertainty factor as to the effect of exposure time on the human NOAEL. Nevertheless, taking the law of Haber into account, the committee is of the opinion that this uncertainty counterbalances the uncertainty of intraspecies differences.

Considering all these aspects, the committee recommends an HBR-OEL for nitrogen dioxide of 0.4 mg/m^3 (rounded up), as an 8-hour TWA.

Concerning previous recommendations, this HBR-OEL is the same as the one recommended by the SCOEL in 1997, but slightly lower than the one recommended by DECOS in 1985 (0.5 mg/m^3). However, in 1985, fewer data were available.

8.3 Groups at extra risk

Based on controlled clinical studies, the committee is of the opinion that workers with chronic obstructive airway diseases, such as chronic bronchitis, may be more susceptible to nitrogen dioxide exposure than healthy workers. Also, workers with bronchial asthma, when exposed to common allergens at the same time, may be more susceptible. At present, it is unknown whether people with abnormalities in their immune system should also be classified as a group at extra risk.

8.4 Health-based recommended Occupational Exposure Limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for nitrogen dioxide of 0.4 mg/m^3, as an eight-hour time weighted averaged concentration (8-h TWA), and of 1.0 mg/m^3 as a 15-minutes time weighted average concentration (15-min TWA, STEL).

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A	Request for advice
B	The Committee
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E	Human data retrieved by DECOS, including data evaluated by the SCOEL (SCO93) and WHO (WHO97)
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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of

genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- LJNGM Bloemen
  epidemiologist; Dow Benelux BV, Terneuzen
- PJ Boogaard
  toxicologist; Shell International B.V., The Hague
- PJ Borm
  professor of inhalation toxicology; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; IRAS, Utrecht University, Utrecht
- G Mulder, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Dutch Centre for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen.

- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by AAE Wibowo, PhD, of the Coronel Institute, Academic Medical Center of Amsterdam, the Netherlands, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: F Smith and R Aksel-Gauri.
Lay-out: M Javanmardi.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2002 for public review. The following organisations and persons have commented on the draft document:

- E Dybing and P Schwarze, Norwegian Institute of Public Health, Norway
- M Costigan, Health & Safety Executive, United Kingdom
- RD Zumwalde, National Institute for Occupational Safety and Health, USA
- JA Hazelzet, Erasmus Universiteit, The Netherlands
- JJH Koning, VNO-NCW, The Netherlands

Annex **D**

Recommendations from Scientific Committee on Occupational Exposure Limits for nitrogen dioxide

CAS No. 10102-44-0 EUR 18216 SCOEL/SUM/53D June 1997

8 hour TWA	:	0.2 p pmm (0.4 mg/m^3)
STEL (15 mins)	:	0.5 p pmm (1.0 mg/m^3)
Additional classification	:	-

Substance

Nitrogen dioxide	:	NO_2
Synonyms	:	Nitrogen peroxide,
EINECS No	:	233-272-6
EEC No	:	07-002-00-0
Classification	:	T+; R26 Xi; R37
CAS No	:	10102-44-0
MWt	:	46.01
Conversion factor (20 ºC, 101 kPa)	:	1.91 mg/m^3= 1 ppm

Occurrence/use

Depending upon the temperature, nitrogen dioxide is a colourless solid, yellow liquid or reddish-brown gas with an irritating odour. It has a MPt of -9.3 ºC, a BPt of 21.2 ºC and a vapour pressure of 52 kPa at 80 ºC. 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).

Nitrogen dioxide 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 with exposure to exhaust from combustion engines in confined areas. It is also produced by various industrial emissions and in tobacco smoke. Combined exposure to nitrogen dioxide and nitric oxide is ubiquitous. Oxidation of nitric oxide to nitrogen dioxide may easily occur in some circumstances.

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.

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 (Frampton *et al.*, 1989). This value is considered to be a LOAEL. An increase in blood glutathione content has been 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.

Controlled clinical studies in patients suffering from bronchial asthma have demonstrated a slight but statistically significant constrictive effect on lung function at a concentration of 0.3 ppm (0.6 mg/m^3) (Bauer *et al*., 1986; Koenig *et a*l., 1988; Avol *et al*., 1989; Roger *et a*l., 1990). A decrease in lung function at 0.3 ppm (0.6 mg/m^3) has likewise been shown in patients suffering from chronic bronchitis (Morrow *et al*., 1992). Other studies performed at this level of exposure have failed to demonstrate a similar effect (Avol *et al*., 1988; Rasmussen *et al*., 1990; Roger *et al*., 1990). An increase in non-specific airway responsiveness in asthmatic subjects has been reported at NO_2 levels as low as 0.1 ppm (0.2 mg/m^3) (Orehek *et al*., 1976). Three other studies have not been able to show any effect at this level (Bylin, 1993). Some trials have found an increase in airway responsiveness at concentrations of about 0.3 ppm (0.6 mg/m^3) (Bauer et al., 1986; Bylin *et a*l., 1988). A meta-analysis by Folinsbee (1992) of available data on subjects experimentally exposed to levels below 0.2 ppm (0.4 mg/m^3) concluded that there is a statistically significant effect on bronchial responsiveness in asthmatics above 0.1 ppm (0.2 mg/m^3). Studies have not been performed on subjects with severe asthma, who presumably are most sensitive to NO_2.

Reports on the effects of long term occupational exposure relate to mixed exposures and are therefore not considered appropriate for establishing occupational exposure limits.

Exposure of rats to 0.4 ppm (0.8 mg/m^3) NO_2 continuously for 27 months resulted in biochemical and morphological changes in the lung (Sagai *et al*., 1984; Kubota *et al*., 1987). This study is not considered to be a suitable basis for proposing occupational exposure limits because the exposure was continuous.

NO_2 showed no evidence of carcinogenicity in NMRI mice at a dose level of 40 ppm (76 mg/m^3) (Henschier and Ross, 1966). Adkins *et a*l. (1986) observed a small increase in lung adenomas in A/J mice exposed to 10 ppm (19 mg/m^3) NO_2 for 6 months. Because the A/J mouse is susceptible to lung adenomas, and the effect was not dose related, this result is not considered to be biologically significant.

NO_2 is mutagenic in bacteria (Biggart and Rinehart, 1987) and clastogenic in mammalian cells *in vitro* (Gorsdorf *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 nitrogen dioxide 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 are required to evaluate a possible local genotoxicity on epithelia of the airways.

Effects of NO_2 on the immune system have been also observed. Short-term exposure of mice to 0.25 ppm (0.5 mg/m^3) resulted in a significant decrease in peripheral blood Iymphocytes (Kuraitis and Richters, 1989). Short-term exposure of healthy volunteers to 0.6 ppm (1.1 mg/m^3) NO_2 resulted in a small rise in the proportion of natural killer cells in the bronchoalveolar lavage fluid (Rubinstein *et al*., 1990).

Reproductive toxicology of NO_2 has not been adequately investigated.

Recommendation

Effects on lung function in healthy people are associated with exposures in the region of 1 ppm or more. However, individuals with compromised respiratory function (asthmatics or persons with chronic bronchitis) represent a large and increasing proportion of the working population, which has to be considered in setting an occupational exposure limit for the general workforce. In specific instances, such as in the mining industry where all workers are subject to strict medical surveillance programmes, consideration of this vulnerable subpopulation may not be required, but an OEL needs to consider the general working population.

In this context, the study of Frampton *et al*. (1989), indicates a LOAEL of 0.6 ppm (1.1 mg/m^3) in healthy volunteers exposed for 3 hours, with respect to the efficiency of macrophages to inactivate influenza virus. Several studies point to a LOAEL of 0.3 ppm (0.6 mg/m^3) for obstructive effects on the lung function of persons suffering from bronchial asthma or chronic bronchitis (Bauer *et al*., 1986; Koenig *et al*., 1988; Avol *et al*., 1989; Roger *et al*., 1990; Morrow *et al*., 1992). The meta-analysis by Folinsbee (1992) indicates that

some asthmatics may have an increased bronchial reactivity at concentrations below 0.2 ppm (0.4 mg/m^3). Taking into account all the available data, the recommended 8-hour TWA for nitrogen dioxide is 0.2 ppm (0.4 mg/m^3). A STEL (15 mins) of 0.5 ppm (1.0 mg/m^3) was proposed to limit peaks in exposure, which could result in irritation.

It should be noted that the proposed values should afford protection to most, but not all, individuals suffering from bronchial asthma or chronic bronchitis.

No 'skin' notation was considered to be necessary.

No difficulties are foreseen with measurements relating to the 8 hour TWA, but further validation may be required for measurement of the STEL (15 mins).

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Annex **E**

Human data retrieved by DECOS, including data evaluated by SCOEL (SCO93) and WHO (WHO97)

Table E1. Respiratory tract effects of nitrogen dioxide exposure in healthy volunteers after single exposure.

No. of subjects	Concentration (mg/m^3)	Experimental design	Observed effects	Ref.
n=15	0.10 plus three 3.8 mg/m^3 peak exposures	3 hours with exercise	No changes in pulmonary function and airway reactivity. No differences in percentage of macrophages, lymphocytes and neutrophils in BALF compared to control.	Fra91 Fra89a Fra89b
n=6	0.19	4 hours	No changes in lung function and airway responsiveness.	WHO97 (Sackner *et al*. 1980)
n=15	0.19	1 hour	No changes in pulmonary function.	Haz83
n=10	0.23	1 hour	No effects on lung function.	WHO97 (Koenig *et al*. 1985)
n=10	0.23	40 minutes with exercise	No effects on respiratory resistance.	WHO97 (Koenig *et al*. 1987a,b)
n=6	0.28	2 hours with exercise	No changes in pulmonary function. No symptoms reported.	WHO97 (Kagawa and Tsuru, 1979, Johnson *et al*. 1990)

n=10	0.34	40 minutes	No effects on respiratory resistance.	WHO97 (Koenig *et al*. 1987a,b)
n=9	0.34	30 minutes with exercise	No changes in lung function.	WHO97 (Kim *et al*. 1991)
n=6	0.56	4 hours	No changes in lung function and airway responsiveness.	WHO97 (Sackner *et al*. 1980)
n=6	0.56	2 hours with exercise	No changes in specific airway resistance.	WHO97 (Kagawa 1986)
n=20	0.56	3 hours and 45 minutes with exercise	No effects on lung function and airway reactivity. No symptoms reported.	WHO97 (Morrow and Utell 1989)
n=9	0.56	30 minutes with exercise	No changes in lung function.	WHO97 (Kim *et al*. 1991)
n=10	0.94	2 hours with exercise	No changes in airway resistance and spirometry. Decreased quasistatic lung compliance, which may be due to awareness of the exposure.	WHO97 (Kerr *et al*. 1979)
n=6	0.94	4 hours	No changes in lung function and airway responsiveness.	WHO97 (Sackner *et al*. 1980)
n=10	0.94	4 hours with exercise	No changes in pulmonary function and airway resistance.	WHO97 (Stacy *et al*. 1983)
n=16	1.1	2 hours with exercise	No statistically significant changes in lung function.	WHO97 (Drechsler-Parks *et al*. 1987)
n=40	1.1	1 hour with exercise	No changes in spirometry and airway resistance.	WHO97 (Adams *et al* 1987)
n=9	1.1	3 hours with exercise	No changes in pulmonary function and airway reactivity. No differences in percentage of macrophages, lymphocytes and neutrophils in BALF compared to control. In four of nine subjects alveolar macrophage tended to be less active against virus, suggesting the presence of a susceptible subgroup.	Fra91 Fra89a Fra89b
n=21	1.1	3 hours with exercise	No changes in pulmonary function and infection rate with virus. No evidence for a susceptible subgroup was found.	Fra02

n=10	1.2	2 hours with exercise	No changes in pulmonary function.	WHO97 (Folinsbee *et al*. 1978)
n=5	1.3	1 hour	No effects on airway conductance.	WHO97 (Toyama *et al*. 1981)
n=10	1.3 to 3.8	10 minutes	Increased airway resistance. No further details presented. Study in Japanese only.	WHO97 (Suzuki and Ishikawa 1965)
n=16, including smokers	1.8	2 hours	No changes in pulmonary function and airway resistance.	Bei76
n=6	1.9	4 hours	No changes in lung function and airway responsiveness.	WHO97 (Sackner *et al*. 1980)
n=8	1.9	3 hours with exercise	No effects on pulmonary host defences.	WHO97 (Jorres *et al*. 1989)
n=15	2.9	3 hours with exercise	No changes in pulmonary function. Airway reactivity was increased compared to control. No differences in percentage of macrophages, lymphocytes and neutrophils in BALF compared to control.	Fra91 Fra89a Fra89b
n=21	2.9	3 hours with exercise	No changes in pulmonary function and infection rate with virus. No evidence for a susceptible subgroup was found.	Fra02
n=18	3.8	1 hour	No changes in pulmonary function. Airway reactivity was significantly increased.	Moh88
n=12	3.8	6 hours with exercise	Immediate and 18-h post-BAL increase in PMN.	WHO97 (Frampton *et al*. 1992)
n=10	3.8	4 hours with exercise	Increased bronchial PMN's and decreased macrophage phagocytosis.	WHO97 (Devlin *et al*. 1992; Becker *et al*. 1993)
n=8	4.2	20 minutes with exercise	Increased levels of mast cells in BALF.	WHO97 (Sandström *et al* 1989)
n=14	4.3	5 hours	No changes in lung function. Alveolar permeability and serum glutathione peroxidase were decreased significantly.	Ras92
n=16, including smokers	4.5	2 hours	No changes in pulmonary function. Small but statistically significant increase in airway resistance.	Bei76

n=25	7.5	75 minutes with exercise	No change in specific airway resistance. Small but significant decrease in blood pressure.	WHO97 (Linn and Hackney 1983; Linn *et al*. 1985)
n=8	7.5	20 minutes with exercise	Increased levels of mast cells and increased numbers of lymphocytes in BALF.	WHO97 (Sandström *et al.* 1989)
n=16, including smokers	9.0	2 hours	No changes in pulmonary function. Small but statistically significant increase in airway resistance.	Bei76
n=16	9.4	15 minutes	Decreased diffusing capacity for carbon monoxide.	WHO97 (Von Nieding *et al.* 1973)
n=11	9.4	2 hours with exercise	Increased resistance (60%), which remained elevated for 60 minutes.	WHO97 (Von Nieding *et al.* 1977)
n=11	9.4	2 houres with exercise	Increased resistance (60%), which remained elevated for 60 minutes.	WHO97 (Von Nieding *et al.* 1979)
n=8	10.3	20 minutes with exercise	Increased levels of mast cells and increased numbers of lymphocytes in BALF.	WHO97 (Sandström *et al.* 1989)
n=16, including smokers	13.5	2 hours	No changes in pulmonary function. Small but statistically significant increase in airway resistance.	Bei76

Table E2. Respiratory tract effects of nitrogen dioxide in healthy volunteers after repeated exposure.

Number of subjects	concentration (mg/m^3)	experimental design	Observed effects	Ref.
n=20	0.19	2 hours on four consecutive days, with exercise	No changes in pulmonary function, airway reactivity, alveolar permeability and mucociliary clearance.	Ras90
n=8	0.23	20 min on three consecutive days.	No changes in pulmonary function and no effects on bronchial reactivity.	Byl85
n=20	0.38	2 hours on four consecutive days, with exercise	No changes in pulmonary function, airway reactivity, alveolar permeability and mucociliary clearance.	Ras90
n=8	0.46	20 min on four consecutive days	No changes in pulmonary function.	Byl85
n=8	0.91	20 min on four consecutive days.	No changes in pulmonary function and no effects on bronchial reactivity.	Byl85
n=5	1.2	2 hours on four consecutive days, with exercise.	No airway symptoms observed. Further, no differences were observed in lymphocyte subtypes in BALF.	Rub91
n=20	1.5	2 hours on four consecutive days, with exercise	No changes in pulmonary function, airway reactivity, alveolar permeability and mucociliary clearance.	Ras90
n=15	1.9	2 hours on two consecutive days, with exercise	No changes in pulmonary function and airway resistance.	Hac78
n=21-23	1.9	2 hours on three consecutive days	No changes in pulmonary function and non-specific airway reactivity.	Goi89
n=21-23	3.6	2 hours on three consecutive days	No changes in pulmonary function and non-specific airway reactivity.	Goi89
n=12	3.8	4 hours on four consecutive days, with exercise	After first day, significant decrements in FEV1 and FVC were observed; this attenuated with repeated exposure. Findings suggest development of inflammation, whereas pulmonary function and antioxidant status are resolved.	Blo99
n=15	3.8	4 hours on three consecutive days, with exercise	Signs of bronchial inflammation were observed as well as minimal changes in BALF T-helper cells.	Sol00
n=21-23	5.7	2 hours on three consecutive days	No changes in pulmonary function and non-specific airway reactivity.	Goi89
n=18	7.5	20 minutes, daily for 12 days, with exercise	Signs of inflammation in the lungs were observed.	San91

Annex **F**

Animal data retrieved by DECOS, including data evaluated by SCOEL (SCO93) and WHO (WHO97)

Table F1. Respiratory tract effects of *long-term* nitrogen dioxide exposure in animals.

animal species	dosis (mg/m^3)	dosis (ppm)*	duration	parameter	type of effect	Ref.
Rat	0.076 0.76 7.64	0.04 0.4 4.0	continuous, 9, 18 en 27 mo	Lung morphology, biochemical parameters	0.04: No effect. 0.4: pre-phase fibrosis, increase of lipid peroxidation. 4.0: fibrosis, increase of lipid peroxidation.	Kub87, Sag84, Sag87
Mouse	0.38	0.2	continuous, 4, 6 and 12 months	Lung morphology, lung function and interaction with infectious agents	No treatment related pathological lesions found. Lung function did not alter substantially, although multiple-breath nitrogen wash-out was significantly decreased. Mortality and relative mean survival time not affected after infectious challenge.	Mil87
Mouse	0.96	0.5	intermittent (6 or 18 h/day) or continuous up to 12 mo	Lung morphology and interaction with infectious agents	After 3 mo: pneumonitis and increased alveolar size; loss of cilia with 24h/d exposure; increased susceptibility to airborne *Klebsiella* and decreased clearance. After 6 mo: pneumonitis, cilia loss, increase alveolar size, bronchial/ bronchiolar inflammation. After 12 mo: reduced capacity to clear viable bacteria.	Ehr68 Bla69

Rat	0.96	0.5	continuous, 7 or 15 days; 1, 2, 4, 6, 12 of 19 mo	Lung morphology	After 4 mo: various histological changes observed: swelling of type-II alveolar cells and interstitial edema. After 6 mo: increased thickness of alveolar septa. After 19 mo: fibrous pleural thickening.	Hay87
Mouse	0.96	0.5	continuous; up to 12 mo	Immunological parameters	Linear decrease in PHA-induced mitogenesis with duration.	WHO97 (Maigetter *et al.*, 1978)
Rat	1.53	0.8	continuous, lifetime (up to 33 mo)	Lung morphology and lung function.	Minimal changes (slight enlargement of alveoli and alveolar ducts; some rounding of bronchial and bronchiolar epithelial cells). No emphysema or other morphologic changes were observed. Respiratory rates were uniformly elevated throughout the lives. Three exposed animals developed subcutaneous spindle cell tumours containing foci of calcium. One animal developed a ganglioneuroma of the adrenal.	WHO97 (Freeman *et al*, 1966; Haydon *et al.*, 1965)
Guinea-pig	1.91	1.0	6 h/day, 5 days/ week, 18 mo	Lung morphology	Mild thickness of alveolar septa due to inflammation; some alveolar dilatation.	Wag65
Rat	3.82	2.0	continuous, 2 year	Lung morphology	Changes in cilia of terminal bronchioli epithelium.	WHO97 (Stephens *et al.*, 1971, 1972)
Rat	3.82	2.0	continuous, up to 360 days	Lung morphology	No change in turnover of terminal bronchiolar epithelial cells.	WHO97 (Evans *et al.*, 1972)
Rat	3.82	2.0	continuous, 14 mo	Lung morphology	Minimal effect; some terminal bronchiolar epithelial hypertrophy.	WHO97 (Furiosi *et al.*, 1973)
Rat	3.82	2.0	continuous, life-time (up to 763 days); 1.0 ppm for 1st 69 days, then 2.0 ppm	Lung morphology	Alveolar distension, especially near alveolar duct level; increased variability in alveolar size; loss of cilia and hypertrophy in terminal bronchiolar cells; no inflammation.	WHO97 (Freeman *et al.*, 1968)
Rat	3.82	2.0	continuous, 112-763 days	Emphysema	No effect.	WHO97 (Freeman *et al.*, 1968)

Rat	5.54	2.9	continuous (5 days/week) for 9 mo	Lung lipid metabolism	Decrease in total lipid, saturated fatty acid content (in BALF), increase surface tension of BALF, decrease in lung compliance.	WHO97 (Arner *et al.*, 1973)
Rat	7.64	4.0	continuous for 16 mo	Lung morphology	Bronchial epithelial hyperplasia.	WHO97 (Haydon, 1965)

mo, month(s); BALF, bronchoalveolar lavage fluid; FEF_{25}, forced expiratory flow at 25% of forced vital capacity.

* 1ppm ≈ 1.91 mg/$m^{3.}$

Table F2. Respiratory tract effects of *short-term* nitrogen dioxide exposure in animals.

animal species	dose (mg/m^3)	dose (ppm)*	duration	parameter	type of effect	Reference
guinea-pig	0.11 - 0.96 – 1.91 – 3.82 – 7.64	0.06 - 0.5 – 1.0 – 2.0 – 4.0	continuous for 6 and 12 weeks	Lung function and hyperresponsiveness to inhaled histamine.	No effect on airway responsiveness at 0.11 and 0.96 mg/m^3. Airway responsiveness was significantly increased at 1.91 (12 weeks), 3.82 (6 and 12 weeks) and 7.64 mg/m^3 (6 and 12 weeks). Effects were time and concentration dependent. Specific airway resistance was significantly increased as 3.82 and 7.64 mg/m^3 (12 weeks).	Kob95
Rat (different ages)	0.19 – 0.96 – 5.35 – 16.81	0.11 – 0.46 – 2.8 – 8.8	continuous, for 1 month	Lung morphology	From 0.19 mg/m^3 upwards: body and lung weight normal; slight changes in morphology (light microscope); several morphological responses (electron microscope). Results are difficult to explain consistently.	WHO97 (Kyono 1982)
Mouse	0.48 0.67	0.25 0.35	7-h/day, 5 days/ week for 7 weeks (0.25 ppm) or 12 weeks (0.35 ppm)	Effects on immune system	Reduced percentage of total T-cell population, certain T-cell subpopulations and natural killer cells.	Ric88 Kur89
Mouse	Between 0.57 and 0.96	Between 0.3 and 0.5	continuous, for 3 months	Interaction with infectious agents	3 mo: High incidence of adenomatous proliferation of peropheral and bronchial epithelial cells. 6 mo: no enhancement of effect of exposure and virus. NO_2 alone and virus alone caused less severe alterations.	WHO97 (Motomiya *et al*., 1973, in Japanese)
Rabbit	0.57 – 1.91	0.3 – 1.0	2-h/day, 14 days	Effects on alveolar macrophages	Increase in alveolar clearance; decreased AM phagocytic capacity at 0.3 ppm, increase at 1.0 ppm after 2 days of exposure. No effect on cell number or viability. No effects from 6 days of exposure on.	WHO97 (Schlesinger *et al*., 1987)

Mouse	0.65	0.34	6-h/day, 5days/ week for 6 weeks; 2-h/day for 1 to 14 days	Lung morphology	Data suggest type 2 cell hypertrophy and hyperplasia; increase in mean linear intercept and amount of alveolar wall area (quantitative image analysis).	She82
Rat	0.76 – 2.29 – 7.64	0.4 – 1.2 – 4.0	continuous for 4 months	Effects on lung lipid metabolism and on lung proteins and enzymes	Duration-dependent increase in ethane exhalation, TBA-reactive substances and activities of antioxidant enzymes; peak increase in early weeks of exposure, return towards control in mid-exposure and increase late in exposure. Concentration dependent effects.	WHO97 (Ichinose and Sagai, 1982)
Rat	0.76 – 2.3 – 7.64	0.4 – 1.2 – 4.0	continuous for 1 to 12 weeks	Lung proteins and enzymes	Complex concentration and duration dependence of effects on cytochrome P-450 and succinate-cytochrome C reductase levels.	WHO97 (Takahashi *et al*., 1986)
Mouse	0.76 – 3.06	0.4 – 1.6	continuous for 4 weeks	Effects on immune system	Decrease in primary PFC response at 0.4 ppm; increase in secondary PFC response at 1.6 ppm.	WHO97 (Fujimaki *et al.*, 1982)
Mouse	0.86	0.45	continuous, 7 h/ day for 4 weeks	Effects on lung proteins and enzymes	No changes in lung serotonin levels.	WHO97 (Sherwin *et al.*, 1986)
Rat	0.96 – 1.88 – 7.52	0.5 – 1.0 – 4.0	continuous for 7 months	Lung morphology (electron microscope)	At 0.96 mg/m^3: swelling of cilia in terminal bronchial epithelia; and, hyperplasia of type II cells in alveolar septa. Authors suggest that early injury may be repaired. At 1.88 mg/m^3: cilia loss in terminal bronchioles; hyperplasia of type II cells; and, interstitial oedema. At 7.52 mg/m^3: cilia loss in terminal bronchioles; hyperplasia of type II cells, interstitial oedema; decrease in number of lamellar bodies in type II cells.	WHO97 (Yamamoto and Taka-hashi, 1984)

Mouse	0.96	0.5	3-h/day for 3 months	Interaction with infectious agents	Increase in mortality with reduction in mean survival time.	WHO97 (Ehrlich *et al.*, 1979)
Mouse	0.96 – 1.91	0.5 – 1.0	continuous for 39 days	Interaction with infectious agents	Increased susceptibility to infection.	WHO97 (Ito *et al*., 19 71)
Mouse	0.96 – 1.91 – 2.87	0.5 – 1.0 – 1.5	continuous for 3 months	Interaction with infectious agents	No effects, but control mortality was high.	WHO97 (McGrath et al., 1985)
Guinea-pig, dog, rabbit, rat, monkey	1.01	0.53	continuous for 90 days	Lung morphology	No lung pathology. Hematological findings and gross pathologic findings did not differ from control animals. Mortality was within the range of control animals.	WHO97 (Steadman *et al*., 1961, 1966)
Rat	1.01 – 2.54 – 2.08	0.53 – 1.33 – 2.66	continuous, 28 days	Lung morphology	0.53 and 1.33 ppm: No pathology. 2.66 ppm: Focal thickening of centriacinar septa by 2 days; progressive loss of cilia and abnormal cilia in trachea and main bronchi at > 4 days; hypertrophy of bronchiolar epithelium at > 8 days. At days 16 and 28, all epithelial cells hypertrophied.	WHO97 (Rombout *et al.*, 1986)
Mouse	1.34/1.53 – 1.91/2.87	0.7/0.8 – 1.0/1.5	continuous, 1 mo	Lung morphology	0.7/0.8 ppm: Mucous hypersecretion, terminal bronchiolar epithelial hyperplasia, shortening of cilia. 1.0/1.5 ppm: Terminal bronchial epithelial hyperplasia; some alveolar enlargement.	Nak79 Nak80
Rabbit	1.92 – 19.2	1.0 – 10.0	2 h/d, 14 days	Effects on alveolar macrophages	Acceleration in alveolar clearance at both concentrations; response at 1.0 ppm was higher than at 10.0 ppm.	WHO97 (Vollmuth *et al.*, 1986)
Monkey	1.91	1.0	493 days	Effects on immune system	Challenged five times with virus. Exposure caused an earlier and greater increase in serum neutralization antibody titres to the virus then control animals.	WHO97 (Fenters *et al.*, 1973)

Guinea-pig	1.91	1.0	6 months	Effects on immune system	Decreased activity of complements after virus challenge.	WHO97 (Kosmider *et al*., 1973)
Rat	1.91 – 9.55	1.0 – 5.0	15 weeks	Effects on lung proteins and enzymes	Changes in BALF and lung tissue levels of enzymes early in exposure; resolved by 15 weeks.	WHO97 (Gregory *et al.*, 1983)
Mouse	2.87	1.5	continuous or intermittent (7-h/ day), 7 days/week, 2 weeks	Interaction with infectious agents	After 1 week, mortality with continuous exposure was greater than that for intermittent exposure; after 2 weeks, no significant differences between continuous and intermittent exposure.	WHO97 (Gardner *et al.*., 1979)
Mouse	2.87	1.5	7-h/day, 4, 5 and 7 days	Interaction with infectious agents	Elevated temperature (32 °C) increased mortality after 7 days.	WHO97 (Gardner *et al.*, 1982)
Mouse	2.87 – 9.55	1.5 – 5.0	continuous, 6, 14 or 21 days	Effects on immune system	Reduction in number of splenic PFCs. No effect on cell-mediated immune system.	WHO97 (Lefkowitz *et al*., 1986)
Mouse	2.87 up to 53.48	1.5 up to 28.0	intermittent and continuous up to 4 mo	Effects in infectious agents	Decreased resistance to, especially after intermittent exposure.	WHO97 (Coffin *et al.*, 1977; Gardener *et al.*, 1977)
Hamster	3.82	2.0	8-h/day, 5 days/ week, 8 weeks	Effects on pulmonary function; lung morphology; emphysema	No changes in vital capacity or lung compliance following exposure in normal animals. Moderate alveolar enlargement, primarily at bronchioloar-alveolar duct junction; decrease internal surface area lung; no lesions in bronchial, bronchiolar, alveolar duct or alveolar epithelium; no change in macrophage number. No emphysema.	WHO97 (Lafuma *et al.*, 1987)

mo, month(s); BALF, bronchoalveolar lavage fluid; AM, alveolar macrophage, TBA, thiobarbituric acid; PFC, plaque-forming cell.

* 1ppm ≈ 1.91 mg/m^3.

Table F3. Respiratory tract effects of nitrogen dioxide exposure in animals after *single* exposure.

animal species	dose (mg/m^3)	dose (ppm)	duration	parameter	type of effect	Reference (from WHO97)
Rat	0.94	0.5	4 hours	Lung morphology	Loss of cytoplastic granules and rupture of mast cells.	Thomas *et al.*, 1967
Rat	0.94	0.5	10, 30 and 45 min; 1, 4, 6, 10 and 24 hours	Lung inflammation parameters	Statistically significant increase in histamine content after 45 minutes. At the same time there was an increase in the number of mast cells directly related to the length of the exposure period.	Hayashi *et al.*, 1987
Rat	1.88	1.0	1 hours	Lung morphology	Degranulation and decreased number of mast cells.	Thomas *et al.*, 1967
Rabbit	1.88	1.0	2 hours	Lipid peroxidation	Elevated thromboxane B_2.	Schlesinger *et al.*, 1990
Mouse	1.88	1.0	3 hours	Interaction with infectious agents	No change in mortality.	Ill80
Mouse	1.88	1.0	17 hours	- Alveolar macrophage activity - Interaction with infectious agents	- Bactericidal activity not affected. - No difference in number of bacteria deposited.	Goldstein *et al.*, 1974
Mouse	2.82	1.5	2 hours	Interaction with infectious agents	No change in mortality.	Purvis & Ehrlich, 1966; Ehrlich, 1979
Mouse	3.57	1.9	4 hours	Interaction with infectious agents	Physical removal of bacteria unchanged.	Goldstein *et al.*, 1973
Mouse	3.76	2.0	3 hours	Interaction with infectious agents	Increased mortality.	Ehrlich *et al.*, 1977; Ehrlich, 1980
Mouse	4.32	2.3	17 hours	- Alveolar macrophage activity - Interaction with infectious agents	- Bactericidal activity significantly decreased by 6%. - No difference in number of bacteria deposited.	Goldstein *et al.*, 1974
Mouse	4.7	2.5	2 hours	Interaction with infectious agents	No change in mortality.	Purvis & Ehrlich, 1966; Ehrlich, 1979
Mouse	4.7	2.5	4 hours	Interaction with infectious agents	No change in bactericidal activity.	Jakab, 1987
Rabbit	5.64	3.0	2 hours	Lipid peroxidation	Depressed thromboxane B_2.	Schlesinger *et al.*, 1990

Rabbit	5.64	3.0	3 hours	Alveolar macrophage activity	Increased swelling of alveolar macrophages.	Dowell *et al*., 1971
Mouse	5.64	3.0	3 hours	Interaction with infectious agents	Increased mortality	Ill80
Mouse	6.58	3.5	2 hours	Interaction with infectious agents	Increased mortality.	Purvis & Ehrlich, 1966; Ehrlich, 1979; Ehrlich, 1975
Rat	6.77	3.6	2 hours	Alveolar macrophage activity	Enhanced alveolar macrophage agglutination with concanavaline A.	Goldstein *et al.*, 1977a
Guinea-pig	8.46	4.5	16 hours	Lung proteins and enzymes	Increased BAL protein content in vitamin C-depleted animals.	Hatch *et al.*, 1986
Guinea-pig	9.02	4.8	3 hours	Lung proteins and enzymes	Increased BAL protein content in vitamin C-depleted animals.	Hatch *et al.*, 1986
Rabbit	9.4	5.0	3 hours	Alveolar macrophage activity	No change in alveolar macrophage resistance to pox virus.	Acton & Myrvik, 1972
Mouse	9.4	5.0	12 hours	Immune system	No effect on primary and secondary splenic 'plaque-forming cell' response.	Fujimaki & Shimizu, 1981
Mouse	9.4	5.0	2 hours	Interaction with infectious agents	Increased mortality. Also, when challenged 1 and 6 hours following exposure.	Purvis & Ehrlich, 1966; Ehrlich, 1979
Guinea-pig	9.4	5.0	3 hours	Lipid peroxidation; Lung proteins and enzymes	Increased lung lipid content and BAL protein in vitamin C-depleted animals, 18 hours and 15 hours following exposure, respectively.	Selgrade *et al.*, 1981
Mouse	9.4	5.0	4 hours	Interaction with infectious agents	Increase in incidence and severity of pneumonia lesions, and decrease in number of organisms needed to induce pneumonia.	Parker *et al*., 1989
Mouse	12.4	6.6	17 hours	- Alveolar macrophage activity - Interaction with infectious agents	- Bactericidal activity significantly decreased by 35%. - No difference in number of bacteria deposited.	Goldstein *et al.*, 1974
Mouse	13.16	7.0	4 hours	Interaction with infectious agents	7% lower bactericidal activity	Goldstein *et al.*, 1973
Mouse	17.3	9.2	4 hours	Interaction with infectious agents	14% lower bactericidal activity	Goldstein *et al.*, 1973
Mouse	18.8	10.0	2 hours	Interaction with infectious agents	Increased mortality. Also, when challenged 1 and 6 hours following nitrogen dioxide exposure.	Purvis & Ehrlich, 1966; Ehrlich, 1979

Rabbit	18.8	10.0	2 hours	Lipid peroxidation	Depressed thromboxane B_2 and 6-keto-prostaglandin F_{1alfa}	Schlesinger *et al.*, 1990
Mouse	19.0	10.0	4 hours	Interaction with infectious agents	Increase in incidence and severity of pneumonia lesions, and decrease in number of organisms needed to induce pneumonia. Also increased mortality in C57BL/6N strain.	Parker *et al*., 1989

Table F4. Respiratory tract effects of nitrogen dioxide exposure in animals after *peak* exposure.

animal species	dose (mg/m^3)	dose (ppm)	duration	parameter	type of effect	Reference
Mouse	0.00 base + 0.94 peak; 0.19 base + 0.94 peak	0.00 base + 0.5 peak; 0.10 base + 0.5 peak	continuous base + 3 h/d, 5 d/w peak for 1, 2, 3, and 6 months	Interaction with infectious agents	0.00 mg/m^3 + peak at 1 mo: decreased number of alveolar macrophages; not seen at 2 and 3 months. 0.00 mg/m^3 + peak at 2 mo: decreased percentage of phagocytosis. 0.00 mg/m^3 + peak at 3 and 6 mo: increased mortality and decreased survival time. 0.19 mg/m^3 + peak at 6 mo: increased mortality and decreased survival time.	Ehrlich and Findley, 1979
Mouse	0.096 base + 0.19 peak; 0.96 base + 1.91 peak	0.05 base + 0.1 peak; 0.5 base + 1.0 peak	continuous, with 1-h peak, twice/ day (5 days/week), 15 days	Interaction with infectious agents	No effect at 0.05 plus 0.1 peak. Increased mortality at 0.5 base plus 1.0 peak.	WHO97 (Gardner *et al*., 1980, 1982; Graham 1987)
Mouse	0.19 base; 0.48 – 0.96 – 1.91 peak	0.1 base; 0.25 – 0.5 – 1.0 peak	continuous; peak for 3 h/day, 5 days/week, up to 12 mo	Immunological parameters	Peak exposure: variable suppression of splenic T and B cell responsiveness to mitogens; suppression not related to concentration or duration.	WHO97 (Maigetter *et al*., 1978)
Mouse	0.19/0.96 base + 1.91 peak; 0.96/3.82 base + 3.82 peak	0.1/0.5 base + 1.0 peak; 0.5/2.0 base + 2.0 peak	base, continuous; peaks, 7 h/d, 5 d/w for 21 weeks	Effects on alveolar macrophages	No observable effects on alveolar macrophages.	WHO97 (Aranyi *et al*., 1976)
Mouse	0.19 base + 1.91 peaks	0.1 base + 1.0 peaks	daily, 6 mo (peaks: 2 h/d)	Lung morphology	Equivocal results (LM and SEM): dilated airspaces and alveolar wall destruction (emphysema).	WHO (Port *et al*., 1977)

Mouse	0.38 base + 1.53 peak	0.2 base + 0.8 peak	continuous base with 1-h peak twice/day (5 days/ week) for 1 year; 23 h/day base (7 days/week), 1-h peaks twice/day, 32 and 52 weeks	Interaction with infectious agents pulmonary function	Peak plus baseline caused greater mortality than baseline. decreased vital capacity following base +peak exposure and tendency toward decreased respiratory system compliance in base+spike, all compared to base and control.	WHO97 (Miller *et al.*, 1987)
Rat	0.96 base + 2.87 peak	0.5 base + 1.5 peak	22 h/day (7 days/ week), 2-h peak (5 d/wk); 1, 3, 12, 52 and 78 weeks	Pulmonary function	Decreased FEF_{25} and frequency of breathing following 78-week exposure. At other time points no effects.	WHO97 (Tepper *et al.*, 1993)
Mouse	0.96 base + 2.87 peak	0.5 base + 1.5 peak	22 h/day, 7 days/ week; 6-h ramped peak (5d/w) 1, 3, 13, 52 and 78 weeks	Immunological parameters	No effect on B- en T-cell responsiveness. No histological changes in lymphoid tissue.	WHO97 (Selgrade *et al.*, 1991)
Rat (7 weeks old)	0.96 base + 2.87 peak; 1.91 base + 5.73 peak; 3.82 base + 11.46 peak	0.5 base + 1.5 peak; 1.0 base + 3.0 peak; 2.0 base + 6.0 peak	23-h/day (7 days/ weak) base, 1-h peaks twice/day (5 days/week); 1, 3 and 6 weeks	Pulmonary function	Decreased body weight and lung compliance following 6-week exposure to 2.0 ppm + spike. Animals recovered 3 weeks after exposure.	WHO97 (Stevens *et al.*, 1988)
Rat (6 weeks old)	0.96 base + 2.87 peak; 3.82 base + 11.46 peak	0.5 base + 1.5 peak; 2.0 base + 6.0 peak	23-h/day (7 days/ weak) base plus 1-h peaks (twice/ day, 5 days/week) for 6 weeks	Lung morphology	In proximal alveolar region: type 2 cells spread over more surface area; type 2 cell hypertrophy and increase in number of alveolar macrophages. In terminal bronchiolar region: no effects on percentage distribution of ciliated cells and Clara cells.	WHO97 (Crapo 1984, Chang 1986, Chang 1988)
Mouse	0.96 base + 3.82 peak	0.5 base + 2.0 peak	continuous base with 1-h/day (5 days/week) peak, 3 mo	Effects on immune system	Decreased serum neutralizing antibody after virus vaccination, changes in serum Immunoglobolins.	WHO97 (Ehrlich *et al.*, 1975)
Rat	1.91 base + 9.55 peak	1.0 base + 5.0 peak	7-h/day, 5 days/ week base, with one 1.5-h peak/ day, 15 weeks	Effects on alveolar macrophages	Several animals showed areas of subpleural alveolar macrophage accumulation plus focal hyperinflation. Increased glutathione-peroxidase in lavage fluid.	WHO97 (Gregory *et al.*, 1983)

Annex **G**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at which inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{10}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{10}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion (v/v)10^{-9}

ppm	parts per million (v/v)10^{-6}
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit
STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice a day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	guinea pig maximisation test

GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration
MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	Relative Risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography

IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Additional abbreviations in the present report

FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
SR_{aw}	specific airway resistance

Annex **H**

DECOS documents

Aanpassing van grenswaarden bij flexibele werktijden	2001/06OSH
Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Azathioprine	1999/04OSH
Aziridine (ethyl imine)	2000/13OSH
Azobisisobutyronitril	2002/01OSH
1,2,3-Benzotriazole	2000/14OSH
Bisphenol A and its diglycidylether	1996/02WGD
Bromoethane	1998/10WGD
1,2-and t-Butanol	1994/10WGD
n-, iso-, sec-, tert-Butylacetaten	2001/03OSH
β-Butyrolactone	1999/05OSH
Cadmium and inorganic cadmium compounds	1995/04WGD
Calculating cancer risk	1995/06WGD
Carbadox	1999/06OSH
Carbon disulphide	1994/08
Chlorine dioxide	1995/07WGD
p-Chloroaniline	1998/09WGD
4-Chloro-o-toluidine	1998/08WGD
Chlorotrimethylilane	2001/05OSH
Chromium and its inorganic compounds	1998/01WGD
Chromium VI and its compounds	2001/01OSH

Cresols	1998/15WGD
Copper sulphate	1999/01OSH
1996-1997 WGD-rapporten/1996-1997 DECOS reports	1999/01WGD
1,2-Dibromoethane	1999/07OSH
1,2-Dichloroethane	1997/01WGD
Diethylsulphate	1999/08/OSH
Diglycidyl resorcinol ether	1999/09OSH
Diphenylamine	1997/05WGD
	1998/03WGD
Epichlorohydrin (1-Chloro-2,3-epoxypropane)	2000/10OSH
1,2-Epoxybutane	1998/11WGD
1,2-Ethanediamine	1996/03WGD
Ethyleneglycol ethers	1996/01WGD
Ethylene oxide	2001/11OSH
Ethylene thiourea	1999/03OSH
Formaldehyde	2003/02OSH
Formamide and dimethylformamide	1995/08WGD
Halothane	2002/14OSH
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Hydrogen cyanide, sodium cyanide, and potassium cyanide	2002/15OSH
Isopropyl acetate	1997/04WGD
Lactate esters	2001/04OSH
Lindane	2001/07OSH
Man made mineral fibers	1995/02WGD
Manganese and its compounds	2001/02OSH
2-Methylaziridine (propylene imine)	1999/10OSH
Methyl Methacrylate	1994/09
Methacrylates. Ethyl methacrylate, n-butyl methacrylate and isobutyl methacrylate	1994/11
Methyl-t-butylether	1994/23
Methyl chloride	1995/01WGD
4,4'-Methylene bis (2-Chloroaniline)	2000/09OSH
4,4'-Methylene dianiline	2000/11OSH
Metronidazole	1999/11OSH
2-Nitropropane	1999/13OSH
N-Nitrosodimethylamine (NDMA)	1999/12OSH
2-Nitrotoluene	1998/12WGD
Pentaerythritol	1997/06WGD
Phenol	1996/04WGD
o-Phenylenediamine	1998/06WGD
Piperidine	1997/08WGD
Procarbazine hydrochloride	1999/14OSH

1- and 2-Propanol	1994/24
Propylene oxide	1997/02WGD
Ronidazole	1998/05WGD
Styrene	1998/07WGD
Styrene	2001/08OSH
Tetrachloroethylene (PER)	2003/01OSH
Quartz	1998/02WGD
Toluene	2001/09OSH
1,1,1-Trichloroethane	1995/03WGD
1,2,3-Trichloropropane	1994/25
1,2,3-Trichloropropane	1998/14WGD
Urethane (ethyl carbamate)	2000/12OSH
Vinylbromide	1999/15OSH
Xylene	2001/10OSH
Wood dust	1998/13WGD