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of Health Risks from Chemicals

142. Occupational exposure to chemicals and hearing impairment

Ann-Christin Johnson and Thais C Morata

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Preface

The main task of the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) is to produce criteria documents to be used by the regulatory authorities as the scientific basis for setting occupational exposure limits for chemical substances. For each document, NEG appoints one or several authors. An evaluation is made of all relevant published, peer-reviewed original literature found. The document aims at establishing dose-response/dose-effect relationships and defining a critical effect. No numerical values for occupational exposure limits are proposed. Whereas NEG adopts the document by consensus procedures, thereby granting the quality and conclusions, the authors are responsible for the factual content of the document.

The present document on *Occupational exposure to chemicals and hearing impairment* was developed within an agreement between the United States, National Institute for Occupational Safety and Health (NIOSH)[1] and NEG. The evaluation of the literature and the drafting of the document were done by Dr. Ann-Christin Johnson, Karolinska Institutet, Sweden and Dr. Thais C. Morata, NIOSH. The draft versions were discussed within NEG and the final version was accepted by the present NEG experts on December 15, 2009. Editorial work and technical editing were performed by the NEG secretariat. The following present and former experts participated in the elaboration of the document:

Present NEG experts	
Gunnar Johanson	Institute of Environmental Medicine, Karolinska Institutet, Sweden
Kristina Kjærheim	Cancer Registry of Norway
Anne Thoustrup Saber	National Research Centre for the Working Environment, Denmark
Tiina Santonen	Finnish Institute of Occupational Health, Finland
Vidar Skaug	National Institute of Occupational Health, Norway
Mattias Öberg	Institute of Environmental Medicine, Karolinska Institutet, Sweden
Former NEG experts	
Maria Albin	Department of Occupational and Environmental Medicine, Lund University Hospital, Sweden
Vidir Kristjansson	Administration of Occupational Safety and Health, Iceland
Kai Savolainen	Finnish Institute of Occupational Health, Finland
Karin Sørig Hougaard	National Research Centre for the Working Environment, Denmark
NEG secretariat	
Jill Järnberg and Anna-Karin Alexandrie	Swedish Work Environment Authority, Sweden

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All criteria documents produced by the Nordic Expert Group may be downloaded from *www.nordicexpertgroup.org*.

Gunnar Johanson, Chairman of NEG John Howard, M.D. Director, NIOSH

[1] Disclaimer: The findings and conclusions in this document are those of the authors and NEG. They do not necessarily represent the views of US NIOSH.

Contents

Abbreviations and acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
CI	confidence interval
CRA	cortical response audiometry
CYP	cytochrome P450
dB [1]	decibel
dBHL	decibel hearing level
EPA	Environmental Protection Agency
EU	European Union
HI [1]	hazard index (also called hygienic effect or additive effect)
Hz [1]	Hertz
Leq [1]	equivalent sound pressure level
LOAEL	lowest observed adverse effect level
MA	mandelic acid
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PCB	polychlorinated biphenyl
PGA	phenylglyoxylic acid
REL	recommended exposure level
ROS	reactive oxygen species
SCOEL	Scientific Committee on Occupational Exposure Limits
SPL [1]	sound pressure level
SD	standard deviation
TEOAE	transient evoked otoacoustic emissions
TWA [1]	time-weighted average
US	United States
WEI	work-life exposure index
WHO	World Health Organization

[1] See also “Terms as used in this document” (next page).

Terms as used in this document

Action level
A guideline used by many international occupational health bodies to express the level of a harmful or toxic substance/activity which requires medical surveillance, increased industrial hygiene monitoring or biological monitoring. For chemicals, it is usually 50 % of the occupational exposure limit. For noise, it indicates the sound level which, when reached or exceeded, necessitates implementation of activities to reduce the risk of noise-induced hearing loss. The new European noise directive has two exposure action levels (See Section 2.3).

Continuous noise
Noise of a constant level as measured over at least one second using the "slow" setting on a sound level meter. Note that a noise which is intermittent, e.g. on for over a second and then off for a period, would be both variable and continuous.

Decibel (dB)
A dimensionless unit expressing the relative loudness (intensity) of sound on a logarithmic scale. The decibel was named after Alexander Graham Bell.

A-weighted decibels, dBA or dB(A). A-weighting is the most commonly used of a family of curves defined in various standards relating to the measurement of perceived loudness, as opposed to actual sound intensity. The others are B, C and D-weighting (for dBB, dBC and dBD). The A-weighting is the most used in noise measurements since its corrections are aimed to replicate the sensitivity of the average human ear to sound at different frequencies.

Equivalent sound pressure level (Leq)
The steady sound level that, over a specified period of time, would produce the same energy equivalence as the fluctuating sound level actually occurring. Occupational exposure limits for a hazard expressed as an 8-hour time-weighted average value includes the total exposure during a shift exposure. For noise, a single number gives the value in decibels that represents the equivalent average level of the actual changing noise levels. When the exchange rate (see below) of 3 dB is used in this calculation, the average noise level is called the *Leq*.

Exchange rate
The amount of decrease (or increase) in noise level which would allow doubling (or require halving) of the exposure time in order to have the same risk. The 3-dB exchange rate is also known as the "equal-energy" exchange rate because the equivalent acoustic energy is preserved when the sound level changes by 3 dB and the exposure duration changes by a corresponding factor of 2. Most countries use a 3-dB exchange rate, thus, if the intensity of an exposure increases by 3 dB, the dose doubles or the allowable time is halved.

Hazardous noise
Any sound for which any combination of frequency, intensity or duration is capable of causing permanent hearing loss in a specified population.

Hazard Index (HI)
A single chemical hazard index (also called hygienic or additive effect) is the ratio of a hazardous air pollutant concentration divided by its reference concentration, or safe exposure level. If this "hazard index" exceeds one, people are exposed to levels of that substance that may pose health risks. A cumulative hazard index or total hazard index is the result of the summation of the hazard quotients for all chemicals to which an individual is exposed. It is calculated according to the formula HI = C1/T1 + C2/T2 + C3/T3 … where C1, C2, C3, etc. are the measured exposure levels of the different agents, and T1, T2, T3, etc. are the individual occupational exposure limits of the corresponding agent. If the hazard index exceeds 1, the total exposure load is considered excessive.

Hearing loss
Hearing loss is often characterised by the area of the auditory system responsible for the loss. For example, when injury or a medical condition affects the outer or middle ear (i.e. from the pinna, ear canal and ear drum to the cavity behind the ear drum - which includes the ossicles) the resulting hearing loss is referred to as a *conductive hearing loss*. When an injury or medical condition affects the inner ear or the auditory nerve that connects the inner ear to the brain (i.e. the cochlea and the vestibulo-cochlear nerve) the resulting hearing loss is referred to as a *sensorineural loss.* Because noise can damage the hair cells located in the cochlea, it causes a sensorineural hearing loss (see also Section 3.1). Hearing loss that results from damage or impairment to the central nervous system, especially the brain itself, is called *central hearing loss*. Unless stated otherwise, hearing loss means sensorineural hearing loss in this document.

Mid- and high-frequency hearing loss. Hearing loss can be defined by audiometric frequency bands, but these definitions are species specific. In humans, the terms mid- and high-frequency hearing loss, refer to hearing losses affecting frequencies at 1-3 kHz and above 3 kHz, respectively. In rats, high-frequency hearing loss is usually defined as affecting frequencies above 16 kHz, whereas a hearing loss at 4-12 kHz is considered as a mid-frequency hearing loss. Other animal models may have other definitions depending on the hearing frequency range of that particular species.

Hearing threshold level
The hearing level, above a reference value, at which a specified sound or tone is heard by an ear in a specified fraction of the trials. It corresponds to the minimum sound level of a pure tone that an ear can hear. The International Organization for Standardization (ISO) specifies in ISO 389 a standard reference zero dB for the scale of hearing threshold level applicable to air conduction audiometers, which

corresponds to the threshold of hearing in the mid-frequencies for young adults. Audiometric zero was determined by the average hearing of young adults who have never been exposed to loud noise or suffered ear disease or injury. However, in the clinic, because people differ considerably in their hearing, hearing thresholds up to 25 dB are considered to be in the normal range.

Hertz (Hz)
The Hertz is a unit of frequency. One Hertz simply means one cycle per second (typically what is being counted is a complete cycle). Hertz can be prefixed and commonly used multiples are kHz (kilohertz), MHz (megahertz), etc. The frequency range for human hearing lies between approximately 20 and 20 000 Hz. The sensitivity of the human ear drops off sharply below about 500 Hz and above 4 000 Hz. Different animal species have different hearing frequency ranges. Guinea pigs have the same frequency range as humans (20 Hz-20 kHz), whereas rats hear between 500 Hz and 40 kHz. Bats can hear above 100 kHz.

Noise
Any unwanted sound.

Noise dose
The noise exposure expressed as a percentage of the allowable daily exposure. If 85 dBA is the maximum permissible level, an 8-hour exposure to a continuous 85-dBA noise would equal a 100 % dose. If a 3-dB exchange rate is used in conjunction with an 85-dBA maximum permissible level, a 50 % dose would equal a 2-hour exposure to 88 dBA or an 8-hour exposure to 82 dBA.

Noise-induced hearing loss
A sensorineural hearing loss attributed to noise exposure, bilaterally symmetrical and often irreversible. In humans, it has its onset in the frequency range between 3 and 6 kHz and for which no other aetiology can be determined.

Ototoxic
A term typically associated with drugs or other substances that are toxic to auditory and/or vestibular systems, affecting the senses of hearing and/or balance.

Ototraumatic
A broader term than the term ototoxic. As used in hearing loss prevention, ototraumatic refers to the potential of an agent (e.g. noise, drugs or industrial chemicals) to cause permanent hearing loss subsequent to acute or prolonged exposure.

Sound pressure level (SPL)
A measure of the ratio of the pressure of a sound wave relative to a reference sound pressure. Sound pressure level in decibels is typically referenced to 20 mPa.

When used alone (e.g. 90 dB SPL), a given decibel level implies an unweighted sound pressure level.

Time-weighted average (TWA) concerning noise
A normalised 8-hour average sound level expressed in dBA which is computed so that the resulting average would be equivalent to an exposure resulting from a constant noise level over an 8-hour period.

Tinnitus
Tinnitus is a perception of sound that has no external source. It is normal for almost all people to perceive a transient noise in the ear either spontaneously or associated with temporary hearing loss after exposure to loud noise. These temporary auditory sensations are reversible and resolved after a few minutes. For a sound without an external source to be defined as tinnitus it has to last at least 5 minutes per day more than once a week. For most patients with tinnitus, the internal sound is constantly present. The prevalence of tinnitus is 10-15 % in adult populations.

Tinnitus is often associated with noise exposure and hearing loss and usually of neurophysiological origin. Tinnitus can also be generated by vascular, muscular or teeth disorders. Another underlying cause of tinnitus is depressive disorders. Whatever the cause of tinnitus is, signals are processed in the central auditory system and perceived as a sound.

1. Introduction and problem identification

Noise is often present in occupational settings where also chemical exposures occur. As a consequence, hearing disorders observed in several occupations are often attributed to noise exposure alone and not much consideration, if any, is given to the possibility of involvement of other agents. The term occupational or work-related hearing loss has been used as a synonym for noise-induced hearing loss, which may not be accurate. Current standard hearing conservation practices do not take into account the potential risk to hearing posed by chemical exposures.

Before the 1980s, no research programme had systematically focused on chemical-induced hearing loss and only isolated studies reported such effects. This scenario started changing following reports from groups dedicated to investigations of the neurotoxic properties of chemicals (309). Since then, progress has been considerable towards understanding the effects of certain environmental and occupational chemicals on the auditory system and their interactions with noise (62, 121, 208, 225, 229, 251, 257, 351).

Chemicals such as organic solvents, metals and asphyxiants are known for their neurotoxic effects on both the central and peripheral nervous systems. Researchers therefore hypothesised that these agents could injure the sensory cells and peripheral nerve endings of the cochlea (23). A more central effect on the auditory system could also be expected due to the general neurotoxicity of these classes of chemicals.

A 20-year longitudinal study of hearing sensitivity in 319 employees revealed that a large proportion of the workers in the chemical division showed a hearing loss severe enough to be regarded and compensated as a work-related hearing loss (23 %) as compared to groups working in non-chemical environments (5-8 %). This effect was found despite the lower noise levels in the chemical division (80-90 dBA) when compared to the other divisions (95-100 dBA). Thus, the exposure to industrial solvents was suggested as an additional causative factor for the observed hearing losses (30).

Since the early 1980s, a few research groups began investigating the ototoxic properties of chemical agents systematically, and ototoxic properties have been identified among metals, solvents, asphyxiants, organotins, nitriles, polychlorinated biphenyls (PCBs) and pesticides. It has also been shown that if these chemicals occur in sufficiently high concentrations, hearing may be affected despite the lack of exposure to noise. Increased prevalence of hearing loss has been reported following occupational as well as environmental exposures, including ingestion of contaminated fish and water and environmental exposures to lead or mercury. Reports on the auditory effects of exposures to chemicals in the (outdoor) environment as well as reports on intentional or accidental inhalation are not included in this document.

The objective of the present document is to describe the currently available evidence regarding exposure to chemicals found in the workplace and their auditory effects. No observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) in this document relate to effects

on hearing if not stated otherwise and may thus be lower for other end-points. When chemical exposure at a certain level does not potentiate noise-induced hearing loss, that level is regarded as the NOAEL for the combined exposure to the chemical and noise.

2. Occurrence of occupational hearing loss

2.1 Estimates of noise-exposed working population

It is difficult to estimate the number of workers exposed to potentially hazardous noise in the Nordic countries, in Europe and in the world. Most scientific studies focus on noise levels from different workplaces or economic sectors. To get an overall picture from different countries or regions, self-report questionnaire surveys are often used. In the year 2000, such surveys were collected in the 15 European Union (EU) member states (EU-15) and in 2001 in the 9 member states (EU-9) that joined the EU in 2004. The results from the questions on noise exposure at work from these surveys were published by the European Agency for Safety and Health at Work (104). Self-estimated noise levels were based on assumptions like "if it is necessary to shout to converse with someone 2 metres away in the workplace, noise levels are potentially hazardous". The surveys showed that in the year 2000, about one third of the working population in Europe (29 % in EU-15 and 35 % in EU-9) was exposed to hearing damaging noise at least 25 % of their work time. The figures for all day exposures were 11 % for EU-15 and 15 % for EU-9. The figures from EU-15 were similar for Denmark and Finland, the two Nordic countries whose data appear in the report. The Statistics Norway estimated the percentage of the working population exposed to damaging noise for most of their working hours to be 7 % (354). The latest Swedish work environment survey showed that 30 and 15 % of the men and women, respectively, were exposed to noise that made conversation impossible more than 25 % of their work shift (self-estimated figures) (360).

In a recent publication, the proportion of the global population exposed to occupational noise was estimated (274). The calculations were performed using data from the United States (US) National Institute for Occupational Safety and Health (NIOSH) (282). Between 1981 and 1989, NIOSH conducted nationwide surveys in which inspectors visited and conducted measurements on various workplaces in the US. These surveys provided the basis for an estimation of the proportion of workers exposed to noise above 85 dBA. The data suggest that 12 % of service workers, 20 % of fishermen, agriculture and forestry workers, 18-22 % of construction and manufacturing workers and 85 % of workers in the mining industry were commonly exposed to noise levels above 85 dBA during working hours. Nelson *et al* (274) combined these data with several scientific studies of occupational noise exposure from third world countries and adjusted the values by the distribution of work force in different occupational settings and regions of the world according to a method established by the World Health Organization

(WHO) (275). Since the noise levels in third world countries according to the cited studies were higher than those in the US data, the proportions of exposed workers were estimated to be higher in regions outside the developed countries even when the criterion of noise-induced hearing loss was set to > 41 dB (274).

2.2 Estimates of noise-induced hearing loss

In Europe, 4 068 cases of noise-induced hearing loss were recognised as an occupational disease in 2001 in ten of the member states. Extrapolation of these data to EU-15 makes 6 700 cases per year (188). Thus, noise-induced hearing loss is the 4th most common recognised occupational injury in Europe. The total prevalence is approximately 4.7 in 100 000 workers. This is not an exact figure of the prevalence since the European countries have different criteria for recognising and reporting occupational diseases. In Sweden, the number of recognised occupational noise-induced hearing loss cases has been around 1 200 each year during the late 1990s. This is about 7 % of the total number of occupational diseases and makes noise-induced hearing loss the 4th most common occupational condition in Sweden (361, 363, 364). Approximately the same figures appear in the other Nordic countries. In Denmark, around 400 cases are recognised annually (367) and in Finland, 800 cases (188).

Nelson *et al* estimated that the prevalence of noise-induced hearing loss (> 41 dB) attributable to occupational exposure in the world was 16 % of the work force (22 % in males and 11 % in females, all ages and regions) ranging from 9 % in Europe and the US to 18-19 % in Africa and South East Asia. The highest prevalences were found in the age groups between 15 to 30 years, in the eastern European countries, countries from the former Soviet Union, China and South East Asia (274).

2.3 Regulations for noise exposure in Europe

In 2003, the EU passed a new noise directive concerning noise exposure at workplaces (108). In summary, two exposure action levels and one exposure limit level were given. The lower exposure action level is 80 dBA Leq8h (time-weighted average (TWA) of the noise exposure levels for a nominal 8-hour working day). At this level, workers are entitled to a hearing test and to information about hearing conservation and the risk of hearing loss. Hearing protection should be provided on demand. The upper exposure action level is 85 dBA Leq8h at which technical measures to reduce noise exposure and hearing conservation programmes including obligatory use of hearing protection should be implemented. The exposure limit value is 87 dBA Leq8h measured *inside* the hearing protectors. The directive indicates that this value must not be exceeded under any circumstances.

According to the new EU directive, the employer is obligated to give particular attention to any effects on workers' health and safety due to interactions between noise and work-related ototoxic substances, and between noise and vibrations (108).

The EU directive has been implemented in Finland (289). The new directive was taken also in Denmark (83), Sweden (362) and Norway (285), but in these countries the old threshold limit value of 85 dBA Leq8h was kept.

3. Definitions

In this session, general descriptions are given on hearing loss, the effects of noise and ototoxic substances.

Abbreviations used and the definitions of terms used in the document are found at the beginning of the document. Descriptions of methods used to assess auditory effects are presented in Chapter 4.

3.1 Hearing loss

The sense of hearing is essential for communication between people and hearing loss is a common handicap that can severely affect the well-being of the individual. The physiology of hearing is rather complex and it is not within the scope of this review to explain that mechanism. However, for clarity, it is necessary to mention some aspects of hearing physiology. The inner ear houses the cochlea (Figure 1), the structure responsible for the conversion of the physical sound waves of different frequencies and different amplitudes into electrical nerve signals. The cochlea is tonotopically organised, which means that sounds of different frequencies stimulate different regions of the cochlea. These regions are also connected to different parts of the cochlear nerve. This tonotopic organisation is present also in the different nuclei in the brainstem, as well as in the auditory cortex in the central nervous system where the nerve signals are perceived.

Hearing loss can be divided in conductive hearing loss and sensorineural hearing loss (92). Conductive hearing loss is the impairment of the sound conduction on the way to the inner ear. Sensorineural hearing loss is defined as hearing loss caused by changes in the cochlea, the auditory nerve or the auditory nervous system. Hearing loss that results from damage or impairment to the central nervous system, especially the brain itself, are sometimes also referred to as central hearing loss. The sensorineural hearing loss may affect only certain frequency regions of our hearing due to the tonotopical organisation of the hearing system. Unless stated otherwise, hearing loss means sensorineural hearing loss in this document. If the impairment affects *only* the auditory nerves or the brain itself, we refer to it as a central hearing loss. The most common form of sensorineural hearing loss involves structural effects in the cochlea. Cochlear hearing loss is mostly caused by an injury to the outer hair cells of the cochlea. This damage usually develops gradually and starts at the high frequencies of hearing from where it progresses towards the lower frequencies.

Age-related changes and exposure to noise are the most common causes of damage to cochlear hair cells. Sensorineural hearing loss may also be hereditary,

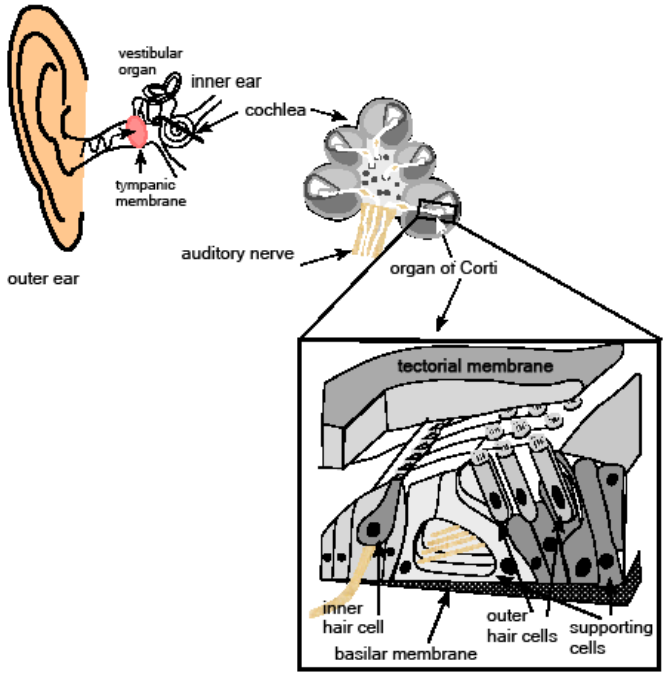

Figure 1. Overview and details of the anatomy in the inner ear.

either manifested as complete deafness or as a gradual deterioration during early life. Hearing loss may be caused by diseases such as Menière's disease or by viral infections that affect the auditory nerve. Damage to the auditory nervous system may also happen as a consequence of a benign or non-cancerous growth that arises from the vestibulo-cochlear nerve (called acoustic neuroma, neurinoma or vestibular schwannoma (270).

Even if ageing and noise exposure are the most common causes, several other factors such as exposure to ototoxic substances may also cause hearing loss. The hearing loss caused by noise can be potentiated or additive to the effects caused by exposure to e.g. chemical agents.

3.2 Noise

Sound is a prerequisite for oral communication between people and it provides us with many pleasant experiences that are essential to our well-being. However, sound can also disturb our work, sleep and communication, cause annoyance and even damage our physical health. Unwanted, unpleasant or loud sound is defined as noise (Cambridge advanced learner's dictionary). When sound is measured at workplaces, an assessment is made of its potential effects on humans. These effects include elevated blood pressure, annoyance, disturbed performance, stress,

speech interference and tinnitus but the far best documented health effect of loud sounds is irreversible hearing damage, i.e. noise-induced hearing loss (381).

The damaging properties of noise exposure to hearing depend partly on the characteristics of the sound reaching the sensory structures in the inner ear of the person exposed. However, a great variation in individual susceptibility exists. The characteristics of noise considered as critical are the intensity (Figure 2), usually measured as sound pressure level (SPL) in decibels (dB, a logarithmic measurement unit that describes a sound's relative loudness), sound spectrum (distribution of sound energy by frequency), duration and temporal distribution during a typical workday, and the expected cumulative exposure over a given duration of days, weeks or years (1).

The variability in susceptibility to noise-induced hearing loss may be due to both endogenous and exogenous factors. Among the endogenous factors that have been shown to influence the degree of hearing loss, genetic factors, health status, and physical characteristics of the ear should be mentioned. Exogenous factors, in addition to noise and ototoxic drugs and chemicals that are the main subject of this review, are e.g. vibrations and smoking (314, 372). Also physical exercise has been shown to increase the susceptibility to noise (87, 224).

In some environments, in particular at work, noise can reach damaging levels to the ear. With 10 or more years of noise exposure, 8 % of the workers exposed to 85 dBA, 22 % of the workers exposed to 90 dBA, 38 % of the workers exposed to 95 dBA and 44 % of those exposed to 100 dBA are estimated to develop hearing

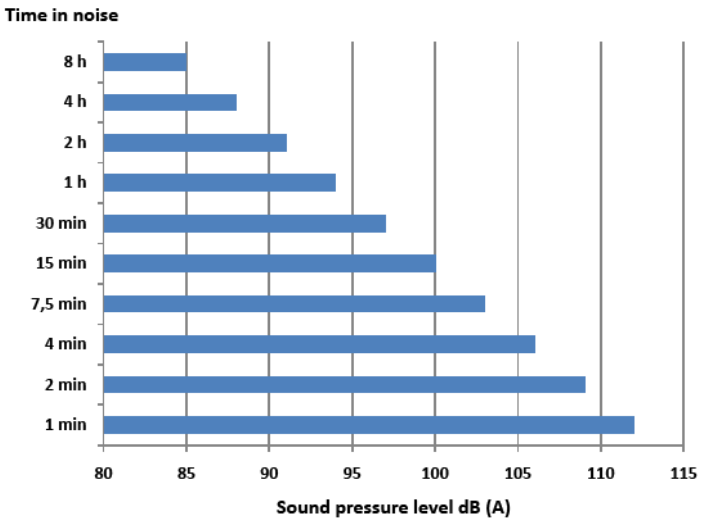

Figure 2. The principle of equal energy. If the permissible noise level of 85 dB is allowed for 8 hours, then the double noise level 85 + 3 dB = 88 dB can be allowed for 4 hours, etc. The principle is described in ISO 1999:1990 (175).

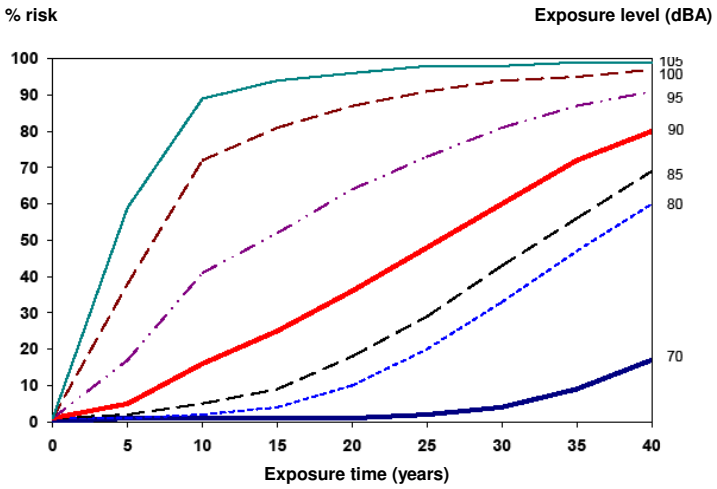

Figure 3. Calculated percentages of the population at risk for developing noise-induced hearing loss at different exposure levels and exposure time in years. Calculations made according to ISO 1999:1990 (175).

impairment (302). The population at risk can also be calculated according to the International Organization for Standardization (ISO) who describes a practical relation of occupational noise exposure (dBA) and an estimation of the percentage of personnel at risk for a noise-induced hearing loss (≥25 dB, averaged from 0.5, 1 and 2 kHz) based on duration of exposure within a normal 40-hour working week (Figure 3) (175).

Noise-induced hearing loss is a specific condition with established symptoms and objective findings (21, 337). It is an irreversible hearing loss, often bilateral and sensorineural with damage mainly to the cells in the peripheral auditory organ, which are responsible for transforming the sound waves into neural signals. Noise-induced hearing loss develops gradually after a long period (8-10 years) of exposure to intense levels of noise. This means exposure to continuous noise levels greater than 85 dBA for 8 hours/day or exposure to impact noise (a noise that arises as the result of the impact between two objects), even if for shorter periods, sufficient to cause the degree and pattern of hearing loss found in pure-tone audiometry. The results are displayed as an audiogram. An audiogram indicates the individual's hearing detection thresholds. The results are given in decibels, which indicate the intensity, or how loud a sound has to be for the listener to be able to detect it. Thresholds up to 20-25 decibel hearing level (dBHL) are considered as normal. Several frequencies are tested. Frequency determines the pitch of a sound. Noise-induced hearing loss is usually not a profound hearing loss but may reach up to 75 dBHL in the higher frequencies such as 4 and 6 kHz and up to 40 dBHL in the lower frequencies of 1 and 2 kHz. An example audiogram of noise-induced hearing loss is shown in Figure 4.

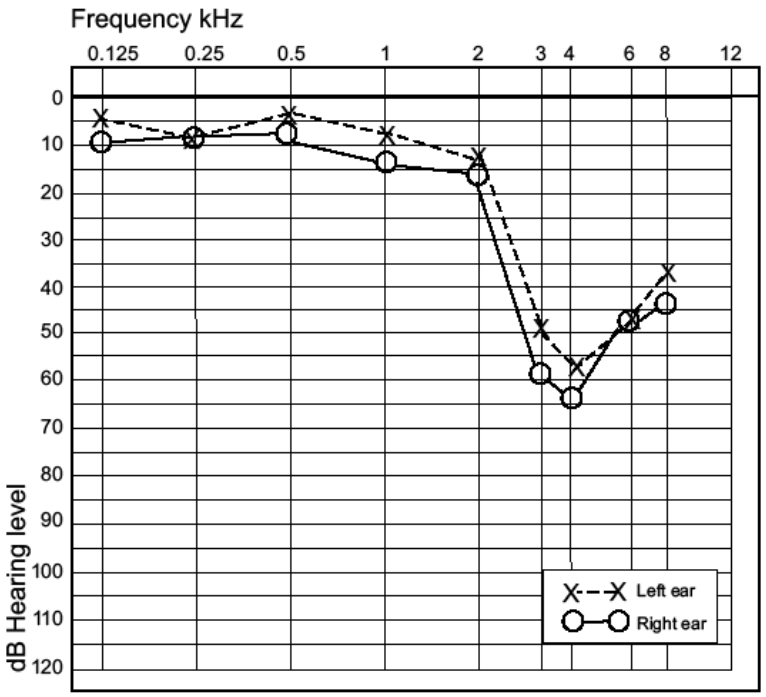

Figure 4. Typical audiogram showing a noise-induced hearing loss.

A noise-induced hearing loss usually develops most rapidly during the first 6-10 years of exposure, and the rate of loss decreases as hearing thresholds increase, in contrast to age-related loss. In a noise-exposed population, a marked individual variability is seen within groups exposed to the same noise levels regardless of age differences. A common scientific opinion is that hearing loss due to noise exposure should not continue to progress if the patient is removed from noise exposure. There is however limited knowledge about how noise-induced hearing impairment is influenced by, or interacts with, age-related hearing impairment. In a recent interesting animal study, Kujawa and Lieberman showed that mice exposed to noise at different ages (4-124 weeks) demonstrated differences in their sensitivity to noise exposure. The mice exposed when young acquired a larger hearing impairment in comparison to elderly mice. Another finding was that when the young mice aged, and their hearing was measured between 8 and up to 96 weeks after the noise exposure, they had a more divergent and more severe age-related hearing loss than the non-noise exposed mice of the same age. According to the authors, the results show that sub-lethal changes caused by the noise exposure made the mice more sensitive to age-related hearing changes (198).

The degree of hearing loss is usually defined by the average value of the audiometric measure dBHL for a range of frequencies (Table 1).

Table 1. The World Health Organization classification system for hearing impairment using the frequencies 0, 0.5, 1, 2 and 4 kHz and the audiometric values shown below (382).

Grade of impairment	Corresponding audiometric ISO value	Performance
Slight hearing impairment	26-40 dBHL	Able to hear and repeat words spoken in normal voice at 1 metre.
Moderate hearing impairment	41-60 dBHL	Able to hear and repeat words spoken in raised voice at 1 metre.
Severe hearing impairment	61-80 dBHL	Able to hear some words when shouted into better ear.
Profound hearing impairment	≥81 dBHL	Unable to hear and understand even shouting.

dBHL: decibel hearing level, ISO: the International Organization for Standardization.

Many studies use slight impairment (26-40 dBHL) as a definition for noise-induced hearing loss since early detection is essential to any preventive initiative. WHO uses moderate hearing impairment or worse (≥41 dBHL) as a definition for hearing loss since this is easier to detect in e.g. self-report studies (274).

3.3 Ototoxicity

Ototoxicity is a selective organ toxicity directed towards the inner ear. An ototoxic agent is defined as a drug or other chemical substance that causes functional impairment or cellular damage in the inner ear, especially upon the end organs and neurons of hearing or balance, or the vestibulo-cochlear nerve.

The mechanisms of action of ototoxic substances may involve the entire organ, specific cells within the organ, components of specific cells or individual biochemical pathways. Drugs and other substances that alter hearing or equilibrium by acting primarily at the level of the brainstem or the central auditory pathways are considered to be neurotoxic and not strictly ototoxic (151, 370). In this document we will, however, consider also some substances for which the mode of action is primarily neurotoxic but the functional adverse effect is hearing loss.

Ototoxins are of interest in the work environment, not only because of their actions on the hearing system of man but also because they may interact with each other and with noise when exposure is combined (simultaneously or sequentially). It is well known that the effects of many drugs or agents when given concurrently cannot necessarily be predicted on the basis of their individual effects (264). In such instances, the damage incurred by agents acting together may exceed the simple summation of the damage each agent produces alone (166, 303). Since noise is the most common exposure that causes hearing loss in humans, special attention has been given to the combined exposure to noise and agents with ototoxic effects.

The ototoxicity of therapeutic drugs has been a concern in the health field for a long time. In comparison, only since the 1980s has the ototoxicity of chemicals found as contaminants in air, food or water, and in the workplace become a concern for health professionals and researchers.

Currently, the only hearing test required by the Organization for Economic Cooperation and Development (OECD) when a chemical is to enter the market is the qualitative assessment of the startle reflex (115 dB SPL click). This test is not sufficiently sensitive for the detection of ototoxicity (presented as an abstract) (235). For this reason, existing ototoxicity information is restricted to a limited number of substances.

Chemicals with confirmed ototoxic properties and with some significance for the work environment and therefore within the scope of the present document are listed in Table 2.

The classes of chemicals investigated as potential ototoxicants include organic solvents, heavy metals, nitriles, organotins, asphyxiants and pesticides. These chemicals have diverse structures suggesting a number of targets for injury within the auditory system and an array of possible underlying mechanisms (113).

Among the solvents, primarily the aromatic solvents have been found to be ototoxic. Some aliphatic solvents like *n*-hexane and *n*-heptane have been shown to affect the auditory system (34, 286, 287, 344) but in these cases the effect is connected to the neurotoxicity of these solvents. Also carbon disulphide is known to be a neurotoxicant that affects the central auditory system (251, 323, 324).

Table 2. Examples of substances confirmed to be ototoxic (36, 37, 253, 335, 338).

Class of medicinal drug	*Examples*
Aminoglycoside antibiotics	Streptomycin, dihydrostreptomycin, neomycin, amikacin, gentamicin, kanamycin, tobramycin, nentilmicin, sisomycin
Other antibiotics	Erythromycin, minocyclin
Chemotherapeutics	Cisplatin, carboplatin, mechloroethamine, vincristine, bleomycin, nitrogen mustard, vinblastine
Diuretics	Ethacrynic acid, furosemid, bumetanid, azoseamid, ozolinone
Malaria prophylaxes	Quinine, chloroquine
Non-steroidal anti-inflammatory drugs	Acetyl salicylic acid, ibuprofen, indomethacin, naproxen, phenylbutazone, sulindac
Antimicrobials	Chloramphenicol, colistin, erythromycin, minocycline, polymyxin B, vancomycin
Chelating agents	Deferoxamine
Arsenicals	Atoxyl, salvarsan
Class of chemical	*Examples*
Organic solvents	Styrene, toluene, *p*-xylene, ethylbenzene, chloro-benzene, trichloroethylene, *n*-hexane, *n*-heptane, carbon disulphide, solvent mixtures
Metals	Lead, mercury, organotins
Asphyxiants	Carbon monoxide, hydrogen cyanide, acrylonitrile, 3,3'-iminodipropionitrile
Other substances	Pesticides (organophosphates, paraquat, pyrethroids, hexachlorobenzene), polychlorinated biphenyls

4. Methods used to assess auditory effects

4.1 Audiometry

4.1.1 Pure-tone audiometry (PTA)

Pure-tone audiometry is a clinical test used to determine a person's hearing sensitivity at specific frequencies, i.e. the softest sound which can be perceived in a quiet environment. Most audiograms cover 0.125-8 kHz.

Pure tones are played to a person via earphones to right and left ears separately. The test results are summarised in a curve, in a frequency continuum, in an audiogram (Figure 4). The reason pure-tone thresholds form the core of the hearing test battery is that these tones are easily generated, calibrated and controlled. Additionally, pure-tone audiometry, if performed properly, has a very high intra-clinic and interclinic reliability (231). In several countries, workers who are exposed to noise levels above 85 dBA are required to have their hearing tested periodically by means of pure-tone air-conduction audiometry. Subjects must be tested in a room that meets the background noise requirements for audiometric testing environment. The equipment calibration records should be recent and available, and biologic calibration checks should also be performed everyday immediately before testing the subjects.

4.1.2 High-frequency audiometry

Pure-tone audiometry testing can be extended to include the frequencies of 10, 12.5, 14 and 16 kHz, which is known as high-frequency audiometry. This procedure has been suggested to be an early indicator of hearing deficits following the administration of ototoxic drugs (111).

4.1.3 Immittance audiometry

This is a routine clinical audiology test. It consists of a physical volume test, tympanometry, static compliance, contra and ipsilateral acoustic reflex testing, and contralateral acoustic reflex decay testing. The main objective in performing immittance audiometry and middle ear compliance is to obtain information on the type of hearing loss and the site of lesion.

4.1.4 Reflex modification audiometry (RMA)

RMA is used in experimental animals to determine sensory detection thresholds by finding the lowest intensity sensory stimuli which modifies the amplitude of the acoustic startle reflex (392). Within each test chamber, a cage is mounted on a coil to which a magnet is attached (through the centre of the wire coil). Ballistic vertical movements by the animals such as a startle response cause the magnet to move with the cage. This induces a voltage with the coil, which is proportional to cage velocity and, hence, to the amplitude of the startle response. The extent of such modification is related to the intensity of the initial low-intensity stimulus. A smooth function can be fitted illustrating the relationship between startle response amplitude and the intensity of the inhibiting stimulus. The resulting audiometric

curves closely approximate audiometric data obtained from traditional operant methods both in sensitivity and shape.

4.1.5 Behavioural audiometry (BA) or conditioned avoidance response (CAR)
This test can be used for multisensory stimuli. The animal is taught to pull or climb from the ceiling of the test chamber to avoid or escape a 1-mA shock on the grill floor (309). The aversive current is preceded by a pure-tone from the loudspeaker in the ceiling of the chamber. A response during the warning signal terminates the trial and is scored as a successful avoidance.

4.2 Otoacoustic emissions

Otoacoustic emissions are spontaneous or evoked acoustical signals that are produced by the cochlea and travel laterally out through the middle ear (190). Otoacoustic emission testing measures the reflection of sounds that are generated by the cochlear hair cells. These signals provide important objective information about the functional health of cochlear outer hair cells and can be analysed by placing a small microphone inside the ear canal.

Otoacoustic emissions facilitate the differentiation between sensory and neural hearing disorders. They can be measured by presenting a series of very brief sounds (clicks or tones) to the ear through a probe that is inserted in the outer portion of the ear canal. The probe contains a loudspeaker that generates clicks and a microphone that measures the resulting sounds that are produced in the cochlea and are then reflected back through the middle ear into the outer ear canal. The resulting sound that is picked up by the microphone is digitised and processed. If there is damage to the outer hair cells or problems with the eardrum or middle ear, the emissions will not be present. They are a sensitive measure of outer hair cell integrity and provide an indication of cochlear damage before hearing loss is observed. Transient (click) evoked otoacoustic emissions (TEOAE) and distortion product otoacoustic emissions (DPOAE) offer information on the status of the cochlea. The former provide an overview of cochlear function, while the latter provide frequency-specific data. Contralateral suppression of the TEOAE evaluates the auditory efferent function (239).

4.3 Central auditory processing tests

Tests that can be used to assess central auditory function fall into two categories, electrophysiological and behavioural tests.

4.3.1 Electrophysiological tests
Electrocochleography
Cochlear and auditory nerve electrical activity can be recorded from electrodes advanced through the tympanic membrane and placed on the otic capsule. This method allows assessment of cochlear and auditory nerve function independent of the patient's subjective response. Two electrical events are recorded from the

inner ear in response to sound: the cochlear microphonic (receptor) potential and the compound action potential of the auditory nerve. Distortion of the waveform of either of these potentials is an indication of inner ear disease. The *cochlear microphonic* is an alternating current signal recorded from the cochlea that exactly reproduces the auditory signal.

Generally, the results are reported as a ratio of the summating potential (SP) to the action potential (AP) (the SP/AP ratio), for which a ratio of 0.5 or greater is considered abnormal. The endocochlear potential (EP), a direct current resting potential in the scala media, is a constant positive potential in the endolymphatic space with respect to the surrounding tissues. This potential is present in all healthy cochleas and is not dependent upon the presence of auditory stimulation. The other three electrical potentials depend on the presence of sound. The vestibulocochlear nerve action potential is typical of other nerve responses. For a given nerve fibre, it has a discrete threshold of stimulus. The polarity and shape of the signal are identical from stimulus to stimulus, and it is an "all or none" phenomenon.

Auditory brainstem response (ABR)

The auditory brainstem response is an evoked potential test of auditory brainstem function in response to auditory stimuli, a brief click or tone beep transmitted from an acoustic transducer in the form of an insert earphone or headphone. The waveform response is detected by surface electrodes typically placed at the vertex of the scalp and ear lobes. The amplitude of the signal is averaged and charted against time. The waveform peaks are labelled I to VII. These waveforms normally occur within a 10-millisecond time period after a click stimulus presented at high intensities (70-90 dB normal hearing level).

Middle latency evoked functions

Middle latency-response testing is similar to the brainstem auditory evoked response but evaluates the auditory system central to the brainstem.

Late latency evoked functions

The cortical response audiometry (CRA) and the P300-potential are electrophysiological measures that test the central pathways of the auditory system.

4.3.2 Behavioural tests

While the electrophysiological tests provide information on the integrity of specific sites within the auditory system, behavioural tests measure the response of the entire auditory system and evaluate the hearing function. Behavioural tests are generally broken down into four subcategories, including monoaural low-redundancy speech tests, dichotic speech tests, temporal resolution or patterning tests, and binaural interaction tests.

Random gap detection test (RGDT)
This behavioural test of central auditory function is designed to measure an important aspect of audition called temporal resolution. A random gap detection task is one in which a short silent gap (inter-pulse interval) is inserted between a pair of stimuli and the listener reports whether the stimulus is heard as one or two.

Speech tests
The ability to understand speech is a very important and complex function of the human auditory system and is typically affected in varying degrees in people with cochlear and central auditory dysfunction. The most accurate assessment of this function is achieved with hearing tests that use speech material as stimuli. Speech tests, by evaluating speech discrimination, assist in the determination of the site of lesion. They are accomplished through the use of standardised recorded speech materials.

Northwestern University auditory test No. 6
This test uses lists of phonetically balanced monosyllabic words for assessing speech discrimination. The test result is expressed in percentage of words correctly identified and reflects the relationship of understanding to changes in intensity.

Dichotic digits test
The use of dichotic speech tests has proven effective in the evaluation of central auditory processing. This test consists of two digits presented simultaneously in each ear at a comfortable listening level, utilising a free-recall response mode. It has a reported high sensitivity and specificity for the detection of central auditory dysfunction and only requires approximately five minutes to administer and score.

5. Mechanisms for inner ear damage after exposure to different ototraumatic agents

The different ototraumatic agents considered in this document damage the auditory function by several different mechanisms. However, some common features can be found for the physical agent noise and some of the ototoxic chemicals. The most common finding in sensorineural hearing loss affecting the inner ear is the degeneration of the sensory hair cells in the cochlea (for details of inner ear anatomy, see Figure 1 in Section 3.1). In animal studies, both noise and solvent exposure have been shown to cause a loss of hair cells. A hypothesis is that the damage to the hair cells is caused by the formation of free radicals, so called reactive oxygen species (ROS) (see e.g. references (64, 152)). Other chemicals such as metals and pesticides may affect both the cochlea (331) and the central auditory pathways (89, 204, 293) depending on the substance. A schematic overview of the site of action for some chemicals is shown in Figure 5. More details will be given below.

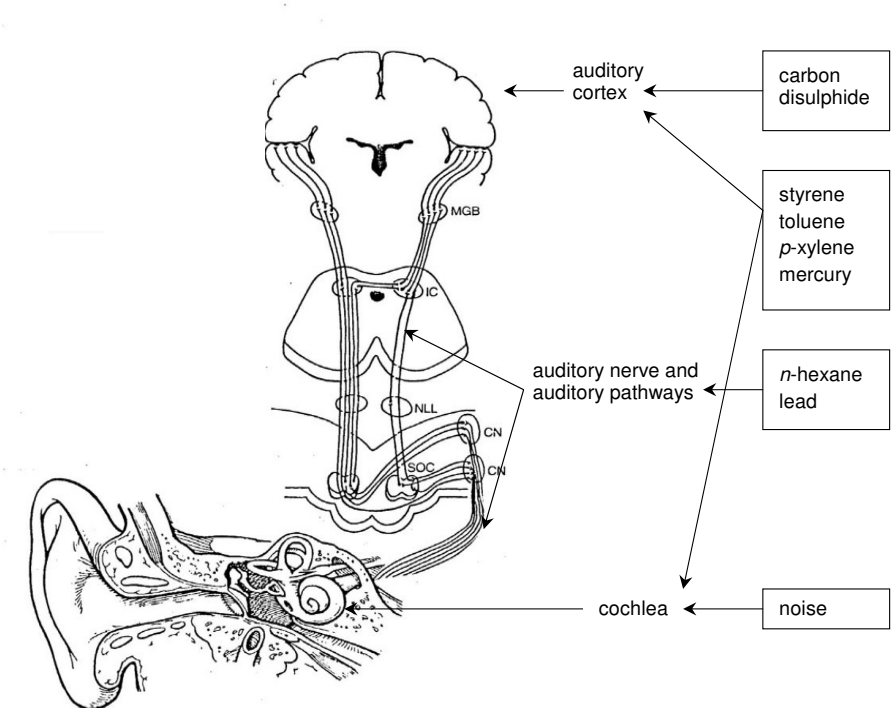

Figure 5. Schematic picture of the auditory system showing the possible site of action of some ototoxic chemicals. Figure adapted with permission from presentation by Mariola Śliwińska-Kowalska at the Transfer of Knowledge "NoiseHear" Meeting at the Nofer Institute of Occupational Medicine, Łódź, Poland, November 15-16, 2006.

Noise-induced hearing loss causes a degeneration of the sensory hair cells of the cochlea. The degenerative process starts within the outer hair cells and then continues to affect the inner hair cells and the supporting cells. The destruction may spread over the entire cochlea, leaving the basilar membrane naked (38). Morphologic studies have shown that the severity of hair cell damage and loss increases with the duration of the noise exposure (222).

Two different mechanisms, mechanical and metabolic, may cause this damage to the hair cells as supported by several studies (220, 283, 313). Mechanical injury occurs due to acoustic overstimulation of the stereocilia of the hair cells or, if the intensity of the noise is high enough, of the membranes of the inner ear (220). This overstimulation disrupts structures in the cells and kills the hair cells by necrosis or apoptosis (243, 263, 380).

Such damage to a cell causes a high level of metabolic activity and may initiate the formation of ROS (135). It has been shown that ROS form in the inner ear following noise exposure (152, 390) and appear to be involved in cell death (109,

152). It has also been shown that scavengers of ROS can reduce the effect of noise trauma on hearing (152, 215). Le Prell *et al* also reported that the formation of free radicals after noise trauma continued up to 10 days after cessation of the exposure (215), which could explain why the loss of hair cells gets worse also after exposure. Toxic insults on the cochlea have been shown to continue also after cessation of exposure to solvents (184).

There is solid evidence from experimental animal studies that exposure to solvents such as toluene, styrene and xylene produces cochlear lesions (48, 184, 310, 358). An example of loss of outer hair cells after exposure to toluene is seen in Figure 6. Clinical and occupational studies have linked exposures to a variety of solvents (e.g. styrene, solvent mixtures and jet fuels) also with disorders in the central auditory pathway (2, 137 , 147, 187, 214, 254, 272, 397, 398). Metals such as lead and mercury and organophosphate pesticides may affect both the cochlea (331, 333) and the central auditory pathways (89, 204, 205, 293) depending on the substance.

The outer hair cells are electromotile, i.e. the cells change their length in response to sound stimulation. This process is dependent on the calcium concentration within the hair cell. Thus, outer hair cells may be vulnerable to ototoxic agents that interfere with intracellular calcium regulation. *In vitro* studies with isolated outer hair cells exposed to toluene have shown dysmorphia and impaired regulation of intracellular levels of free calcium. Changes occurred rapidly at the low concentration of 100 µM toluene, a level predicted to occur in the brain of humans exposed to 80-100 ppm toluene in air (228).

Certain criteria have been shown to be necessary for the aromatic solvents to exhibit ototoxicity in the rat animal model. Gagnaire and Langlais studied 21 different aromatic solvents and found that only 8 (toluene, *para*-xylene, ethylbenzene, *n*-propylbenzene, styrene, α-methylstyrene, trans-β-methylstyrene and allylbenzene) caused loss of hair cells. Within those 8 solvents, the degree of hair cell loss differed. The degree of ototoxicity was not clearly related to the

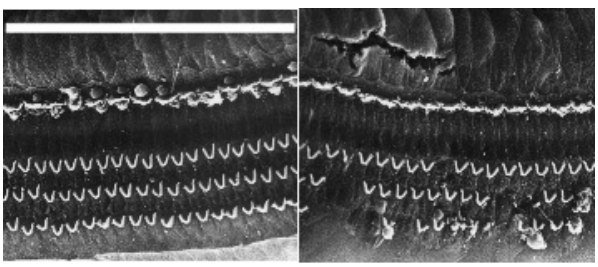

Figure 6. Scanning electron micrograph showing 3 rows of outer hair cells and 1 row of inner hair cells in the middle turn of the cochlea in a control rat (left) and in a rat exposed to toluene (1 000 ppm, 16 hours/day during 5 days).

octanol/water partition of the solvent but correlations between some structural properties and ototoxicity were observed. A single side-chain on the aromatic ring is essential. Only one solvent with two side-chains, *para*-xylene, was ototoxic. When the side-chain was branched no ototoxicity was found. Also the saturation and the number of carbon atoms in the side-chain are of importance. No more than three carbons must be present in the side-chain for ototoxicity to occur (139).

Studies investigating the differences between the three isomers of xylene (*ortho-, meta-* and *para*-xylene) have shown that only *p*-xylene is ototoxic (140). Both *o*- and *m*-xylene induce liver enzymes and are thereby eliminated faster from the body of rats. *p*-Xylene reaches a higher level in the blood and also gives rise to more potentially toxic intermediates than the other two isomers, which could explain why only *p*-xylene is ototoxic (238). However, Gagnaire *et al* showed that even when using a higher dosage and thereby obtaining the same blood and brain levels with *m*-xylene as with a known ototoxic dose of *p*-xylene, no ototoxic effect was observed after exposure to *m*-xylene. Therefore, the differences in metabolic rates probably do not explain the different ototoxic potentials of the xylene isomers (142). Instead, the presence of two methyl groups in the *para*-position on the aromatic ring may be necessary for the ototoxic properties of *p*-xylene (140).

Laboratory investigations appear to identify a common pattern of cochlear dysfunction and injury following solvent exposure. This pattern, produced by toluene, styrene, xylenes and trichloroethylene, involves impairment of outer hair cells that normally encode middle-frequency tones and are located in the middle turns of the cochlea (48, 74, 78). This tonotopicity of the cochlear damage is different from that induced by aminoglycoside antibiotics, which mainly affect the high-frequency tones. The pattern of damage is probably due to the intoxication route taken by the solvents to reach the organ of Corti as shown for styrene (48, 211).

In these studies, as well as in a recent study by Chen *et al*, it was shown that styrene reaches the hair cells in the cochlea from the blood via the stria vascularis (structures of the inner ear are shown in Figure 1, Section 3.1) and through the supporting cells (64). This explains why the third row of other hair cells is affected first, i.e. this row of outer hair cells is closer to the supporting cells.

The disorganisation of the membranous structures is thought to be the starting point for the cochlear injury induced by styrene. A corollary of the outer hair cells susceptibility is the progression of the trauma from the third to the first row of hair cells within the organ of Corti. This feature is likely related to the intoxication route taken by the solvents to reach the organ of Corti. It also explains why the ototoxic effect of styrene progresses beyond the cessation of styrene exposures to 700 ppm and above, i.e. organ exposure continues some time after cessation of air exposure and when the apoptotic cascade has been initiated, it takes some time before it turns off (48, 229, 230).

In rats, levels of solvents were measured in the blood, brain, auditory nerves, organ of Corti and in cerebrospinal and inner ear fluids after exposure to either toluene or styrene for one day. Solvents were detectable in the tissues but not in

the fluids, indicating that toluene and styrene are transported through the tissues of the organ of Corti rather than through the fluids of the inner ear (48).

Chen *et al* measured the concentration of styrene in different regions in the cochlea and found a higher solvent concentration in the middle region with lower levels in the apex and the basal turn, explaining the higher vulnerability in the middle-frequency region. The reason for the higher concentration in the middle region is not fully understood but it could be partly due to easier removal of solvents by diffusion to the perilymph in the basal turn of the cochlea, which is closer to the cochlear aqueduct (64).

Trichloroethylene has been shown to impair inner hair cell and spiral ganglion cell function through electrophysiological testing and cochlear histopathology. Loss of spiral ganglion cells was significant in the middle turn of the cochlea but not in the basal turn. The data suggested that the behaviourally determined loss in auditory function can be accounted for by a cochlear impairment and that the spiral ganglion cell may be a prominent target of this solvent (129).

Effects on the central auditory pathways after toluene exposure in rats have been further investigated in two recent studies. In these experiments, it was shown that toluene can inhibit the auditory efferent system by modifying the response of the protective acoustic reflexes from the efferent system originating from the olive complex in the brainstem. Toluene acted in these experiments in the same way as other known cholinergic receptor antagonists (45, 209). Maguin *et al* showed that toluene acts also on the regulation of acetylcholine release in muscles by blocking the voltage gated Ca^{2+} channels involved in the protective middle ear reflex exhibited by the stapedius muscle. This reflex is also mediated by efferent motorneurons emanating from the olive complex in the brainstem (237). These studies (45, 209, 237) all give an interesting insight into the mechanism of the interaction between solvents and noise. It is a probable hypothesis that when solvents cause the blocking of the protective middle ear reflex as well as disturb the efferent system, noise will be more damaging to the inner ear in the presence of solvent exposure.

Solvent-induced hearing loss is species dependent. The rat is sensitive to solvents, while the guinea pig and chinchilla seem unaffected. Davis *et al* reported no effects in the chinchilla auditory system following toluene exposure alone or combined with noise (84). The authors argued that the chinchilla liver was able to detoxify toluene. Hepatic microsomes from chinchillas, rats and humans were tested for their ability to convert toluene to the more water-soluble compound benzyl alcohol. Chinchillas had higher levels and activities of liver cytochrome P450 (CYP) enzymes than both rats and humans. Similar observations were reported by Lataye *et al* regarding the effects of toluene and styrene exposures in the rat and guinea pig (213). Lataye *et al* found that the styrene concentration in the blood of the rat was four times higher than the concentration in the blood of the guinea pig. The authors indicated that the difference in susceptibility between these species may be explained by: 1) the different amount of solvent transported by blood and capable of reaching the organ of Corti, 2) the difference in meta-

bolism, 3) the difference of glutathione within the sensory epithelium and 4) the morphological differences of the lateral membranes of the outer hair cells of the cochlea (213). Gagnaire *et al* investigated the difference in blood and brain levels of *p*-xylene between guinea pigs and rats. The blood level of *p*-xylene in the guinea pig was only half of that in the rat and the level in the brain reached only about 20-30 % of that in the rat. The rat also had four times slower elimination rate than the guinea pig (142). Thus, toxicokinetic factors may explain the species difference between rats and guinea pigs (54, 142). Solvent metabolism in humans is closer to that of the rat than to that of the guinea pig (213).

As mentioned above, several experimental studies have shown that noise exposure produces ROS in the inner ear (152, 390). Accumulating evidence links ROS to cochlear damage for both ototoxins and/or noise trauma (109, 193). This may also explain the interaction between noise and oxidising chemical agents like solvents and asphyxiants. It has been shown that combinations of non-damaging noise and oxidising chemical agents lead to oxidative stress that causes the death of hair cells in the inner ear (124, 125, 131, 297). A recent study by Chen *et al* produces evidence for apoptotic cell death by detecting activated caspase pathways in the outer hair cells after styrene exposure in rats (64).

6. Auditory effects of pharmaceuticals

Ototoxicity has been recognised since the 19th century. In 1884, it was reported that certain drugs such as quinine and acetyl salicylic acid could produce temporary hearing loss as well as dizziness and tinnitus (339). Drug ototoxicity was recognised as a problem in the 1940s when permanent damage to the vestibular and cochlear organs was reported in several patients treated with the newly discovered drug for treatment of tuberculosis, the aminoglycoside antibiotic streptomycin (155). Today there are many well-known ototoxic drugs used in clinical situations (143, 370). Groups of drugs and substances confirmed to be ototoxic are listed in Table 2 (Section 3.3). However, it is beyond the scope of this document to discuss the ototoxic features and risks of drugs. Most of them are used for treatment of serious health conditions after prescription, including antibiotics, chemotherapeutics, diuretics and malaria prophylaxes.

In the following section, some features of the common over-the-counter pharmaceutical acetyl salicylic acid are discussed, since it may interact with noise or solvents and thereby increase the risk of occupational hearing loss.

6.1 Acetyl salicylic acid

Acetyl salicylic acid or aspirin is one of the most commonly used drugs in the world, with effects on fever, pain and inflammation. This drug has many side-effects including irritation of the gastrointestinal system, dysfunction of kidneys and liver, allergies and hearing loss. There exists limited understanding of the mechanisms underlying these side-effects. Cazals has published a comprehensive

review about many aspects of the ototoxicity of acetyl salicylic acid (55) and his conclusions are summarised here.

The ototoxicity of acetyl salicylic acid in humans can be divided into loss of hearing, tinnitus and alterations of sound perception. The salient feature of the slight to moderate hearing loss connected with acetyl salicylic acid intake is that it is always reversible after the end of treatment. It is dose-dependent and correlates linearly to the plasma salicylate level. A slight hearing loss (10 dB) was observed at plasma levels of 50-100 mg/l salicylate corresponding to approximately 2 g acetyl salicylic acid/day in volunteers given slow release tablets for one week (85). The hearing loss can reach thresholds above 40-50 dB, which occurs around 300-500 mg salicylate/l plasma, corresponding to approximately 6-8 g acetyl salicylic acid/day (55). Several studies have demonstrated a large interindividual variability in the susceptibility to acetyl salicylic acid. Tinnitus is a common feature in acetyl salicylic acid induced auditory impairment especially after several days of drug intake. Tinnitus can be defined as a subjective perception of sound when no external sound source is present. Also tinnitus is reversible and correlates with the plasma level of salicylate. Acetyl salicylic acid has even been used to induce tinnitus in animal experiments. In these experiments, behavioural methods have been used to quantify the loudness of the tinnitus-tone (180, 181). In humans, the alterations of the perceptions of sounds after acetyl salicylic acid intake include loss of speech discrimination, change in frequency filtering and temporal detection, as well as hypersensitivity to noise-induced temporary elevation of thresholds. The mechanism of the effects of acetyl salicylic acid on the auditory system relates to the outer hair cells and their motility, the cochlear blood flow, and the spontaneous activity in the cochlear nerve (55).

In animal studies, interactions between acetyl salicylic acid and noise exposure have been shown, but limited evidence supports permanent hearing loss or loss of hair cells after combined exposure. One study in rats showed that acetyl salicylic acid may increase the severity of the permanent hearing loss caused by toluene (182).

7. Auditory effects of organic solvents

Organic solvent ototoxicity was suggested already in the 1960s (216) but was not clearly demonstrated until the 1980s. In a review paper that briefly discussed five occupational studies and four case reports, it was observed that the incidence of sensorineural hearing loss was higher than expected in noise-exposed workers who were also exposed to solvents (23). An ototraumatic interaction between noise and organic solvents was suggested and its biological plausibility discussed. Since organic solvents are known for their neurotoxic effects in both the central and the peripheral nervous system, it was argued that solvents might injure the sensory cells and peripheral endings in the cochlea. It was further hypothesised that, since solvent-related effects had been detected in the brain, a more central component on the auditory disorders could also be expected.

The aromatic solvents of the alkylbenzene family (e.g. toluene, ethylbenzene and xylene) are the largest group among the solvents that have been found to affect the auditory system (Table 2). The relative ototoxicity varies among the aromatic solvents. A tentative ranking of decreasing ototoxocity for 8 aromatic solvents based on histological hair cell losses was proposed as: allylbenzene > ethylbenzene, styrene > *n*-propylbenzene > *p*-xylene ≥ toluene, α-methylstyrene, trans-β-methylstyrene. Of the xylene isomers, *p*-xylene showed ototoxic effects whereas *o*-xylene and *m*-xylene did not (139, 238). Benzene itself is not ototoxic. Aliphatic solvents like *n*-hexane and *n*-heptane as well as carbon disulphide are all known neurotoxic substances that can affect the auditory system but their effects are probably due to effects on neurons in the central nervous system (251, 286, 287, 344).

In animal experiments as well as in human studies, the ototoxic effect after inhalation of organic solvents has been established using many different methods. In animals, the most sensitive method to discover the ototoxic effect of solvents is morphological studies detecting the loss of hair cells in the cochlea as shown after toluene and styrene exposure in rats (see e.g. references (64, 184, 208, 210, 229, 268)). Detailed information on the different methods used to assess the auditory function in man and animals is found in Chapter 4.

The auditory system of the guinea pig is not as sensitive to the ototoxic effects of solvents as that of the rat. This has been shown in experiments using styrene and xylene exposure of guinea pigs (114, 142, 213). Differences in solvent metabolism are likely to explain the differences in susceptibility between species (142, 213). As solvent metabolism in humans is more similar to that of rats, the latter is the best animal model for studying the ototoxic effects of solvents.

7.1 Styrene

7.1.1 General

Styrene (vinylbenzene) is a colourless to yellow, volatile liquid with a sweet sharp odour (192, 377).

Originally, styrene was used primarily in the synthetic rubber industry. It is currently used as an intermediate chemical for polymers in making plastics, resins, coatings and paints. Styrene is obtained from crude oil or liquefied petroleum gas and produced throughout the world in large quantities (192).

The most significant occupational exposures to styrene occur in the manufacturing of fibreglass-reinforced polyester products, especially in plants involved with the fabrication of reinforced plastics and composites including boat producers. In addition, exposures to styrene may occur during the use of miscellaneous products such as floor waxes and polishes, paints, adhesives, metal cleaners and varnishes (245, 377).

The major route of exposure is via the respiratory system. The absorption of styrene vapour by skin is negligible compared to the uptake via the lungs. Liquid styrene may be absorbed through the skin to a limited extent (4). In humans, some 90-97 % of absorbed styrene is eliminated as the urinary metabolites mandelic acid

(MA) and phenylglyoxylic acid (PGA) with only a small fraction accounted for as parent compound in expired air or urine (318).

The neurotoxic properties of styrene including effects on colour vision (65, 250, 265) represent the main health hazards that are recognised in humans. Also genotoxic effects have been reported (169).

7.1.2 Effects in animals

7.1.2.1 Styrene alone

In several animal experiments, the effects of styrene alone on the auditory system have been investigated (Table 3). These studies show that styrene exposure causes a permanent and progressive damage to the auditory system of the rat.

Concentration and duration of styrene exposure were shown to influence the ototoxic effects in rats (48, 64, 229). In most of the experiments, animals were exposed to styrene by inhalation. However, intraperitoneal injections (114, 378) and gavage (64, 139) were also used in some studies. The results obtained by various modes of administration do not differ significantly because the solvent reach the cochlea whatever route is used (47).

In animal experiments, the ototoxic effect of styrene has been established using electrophysiological methods (showing a permanent loss of auditory sensitivity) and by morphological examination of the cochlea (showing loss of outer hair cells).

The histological findings demonstrate that the supporting cells are the first targets of the solvent. Then, the outer hair cells of the third row are disrupted, followed successively by the cells from the second and first rows from the basal (20 kHz) to the upper turn (4 kHz) of the cochlea. The ototoxic effects of styrene in rats progressed beyond the cessation of exposure to 700 ppm and above (48, 208, 298).

In rats, inhalation of 600 ppm styrene (12 hours/day, 5 days/week for 4 weeks) caused hearing loss and a loss of outer hair cells that amounted to 50-100 %. Animals exposed to 300 ppm did not display such effects (268).

Lataye *et al* exposed rats to 300-1 000 ppm styrene (6 hours/day, 5 days/weeks for 4 weeks) and found higher concentrations of styrene in the blood when rats were forced to be active during the exposure. Consequently, a loss of outer hair cells occurred at the lower exposure level of 300 ppm in active rats when compared to sedentary rats that displayed a similar effect at 500 ppm. These results show that the ototoxic effect of styrene is affected by physical activity (210).

The age of the rats also influence the degree of hair cell loss in the cochlea. A significant loss of hair cells was seen in young rats (3 months of age) exposed to 700 ppm styrene (6 hours/day, 5 days/week for 4 weeks) despite the fact that almost no hearing loss could be measured by auditory brainstem response. In comparison, aged rats (24-26 months of age) displayed less outer hair cell loss than the young rats but more hearing loss, which might be due to aging and not only to styrene exposure (49). In a follow up study, 14-week old rats (corresponding to young adults) lost more outer hair cells and had more hearing loss compared to

21-week old rats, suggesting a critical sensitive period around the age of 3 months for rats exposed to solvents (212).

Styrene has been shown to be a more potent ototoxicant than toluene (47, 139, 229). Permanent auditory threshold shifts in rats occurred with a styrene dose 2.4 times lower than that of toluene. The sequence and location of histopathological trauma along the organ of Corti was compared between styrene and toluene. The same magnitude of outer hair cell loss was observed at 650 ppm styrene and 1 500 ppm toluene and also, but more severe, at 850 ppm styrene and 1 750 ppm toluene (all exposures 6 hours/day, 5 days/week for 4 weeks) (229).

In a recent study, Chen *et al* investigated the dose-response relationship after exposure to styrene by gavage to different doses (0-800 mg/kg body weight, 5 days/week for 3 weeks). The noise level was around 45 dBA. A dose-dependent increase in hearing loss was seen from 300 mg/kg. Also the loss of outer hair cells increased in a dose-dependent manner with small losses (5 %) present already at 200 mg/kg. The loss of outer hair cells started after 7 days of exposure and the magnitude grew with increasing time (investigated only at 800 mg/kg). The styrene levels in blood were directly proportional to the oral dose given (2-3 % of the oral gavage dose on weight basis). The level of styrene in blood after the oral dose of 800 mg/kg was approximately 20 µg/g, measured after one dose as well as after 6 days of dosing (64). This corresponded to the styrene blood levels after inhalation exposure to 1 000 ppm (22.8 µg/g, 6 hours/day for 5 days) (64, 213). Thus, linear extrapolation suggests that an oral dose of 200 mg/kg corresponds to inhalation exposure at approximately 250 ppm. The magnitude of outer hair cell loss at 800 mg/kg was the same as that observed following the exposure to 850 ppm for 4 weeks described above (229). The styrene blood levels measured in rats exposed to the lowest styrene dose (200 mg/kg) in the study by Chen *et al* (64) were only 3 times higher than blood levels of styrene found in workers (248).

Prolonged middle latency auditory potentials and damage to central nervous system structures were found after intraperitoneal injection of high daily doses (800 mg/kg body weight) of styrene to rats during two weeks indicating also a central nervous effect on the auditory system (378).

7.1.2.2 Styrene combined with noise

Some experiments have revealed that noise interacts with styrene in a synergistic manner (Table 4) (208, 210, 269).

Combined exposure to 600 ppm styrene and noise at 100-105 dB SPL (12 hours/day, 5 days/week for 4 weeks) caused a synergistic effect in rats, shown by hearing loss and outer hair cell loss. At 300 ppm, no interaction was observed (269).

Exposure to 400 ppm styrene in combination with noise at 86.2 dB SPL (= 85 dB Leq8h) (6 hours/day, 5 days/week for 4 weeks) resulted in a greater loss of outer hair cells than exposure to styrene alone in rats forced to be active. Noise only exposure did not induce any loss of outer hair cells at this exposure level (210).

7.1.2.3 Styrene combined with other agents

Ethanol consumption, which by itself did not affect the auditory function in rats when administered by gavage once a day (4 g/kg body weight, 5 days/week for 4 weeks), has been shown to potentiate the effects of styrene (750 ppm, 6 hours/day, 5 days/week for 4 weeks) on the auditory system. Styrene alone caused a permanent hearing loss of about 10 dB at 2 and 16-20 kHz as shown by auditory brainstem response and a corresponding loss of outer hair cells. The combined exposure to styrene and ethanol caused a greater hearing loss (20-30 dB) over a wider frequency range (2-24 kHz) together with increased loss of outer hair cells. Rats simultaneously exposed to styrene and ethanol had increased CYP enzyme activity in the liver compared to rats exposed to styrene alone (Table 4) (230).

Trichloroethylene did not show synergistic or antagonistic interaction with styrene among groups of rats who received iso-effective concentrations of solvent pairs (8 hours/day for 5 days). Decreased auditory brainstem responses indicative of hearing loss correlated with blood levels of total solvent as predicted by a linear dose-addition model (Table 4) (329).

7.1.3 Observations in man

7.1.3.1 Styrene alone

Several occupational studies have been conducted regarding the effects of styrene on the auditory system as well as on the interaction of styrene with noise (Table 5).

No studies exist in which workers were exposed to styrene alone in a totally quiet environment. However, in several studies, groups of workers were exposed to styrene at noise levels below 85 dBA. These studies are discussed below.

7.1.3.2 Styrene combined with noise and other agents

In an early study, routine audiometric results of workers exposed to styrene in a plastic boat plant were evaluated. The plant records indicated that exposures had ranged from 5.6 to 24 ppm although rare peaks above 70 ppm did occur. Also acetone exposure occurred at the plant but no other solvent exposure. Noise levels were not measured but were estimated to be below 85 dB. Audiometric records did not indicate hearing losses resulting from causes other than exposure to noise. Seven of 18 workers, however, displayed abnormal results in central auditory system testing. In addition, 16 of the workers showed abnormal results in tests involving the vestibular system (271).

A similar study in workers exposed to 3-92 ppm styrene was reported by Calabrese *et al* who found no effects when measuring pure-tone audiometry but concluded that 17 out of 20 workers had deficient vestibular reflexes (43).

Muijser *et al* compared two groups exposed to styrene, directly (mean 32 ppm) and indirectly (14 ppm). Individual exposures did at times reach up to 164 ppm in the directly exposed group. Noise levels were generally low, 66-70 dBA, but occasionally up to 104 dB. A significant difference in hearing thresholds at the high frequencies was noted between the groups (261).

Table 3. Auditory effects in animals exposed to styrene (STY) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
STY level (ppm)	Regimen				
0, 100, 300, 600	Inhalation: 12 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Hearing loss at 8 kHz and OHC loss at 600 ppm. No effect at 300 ppm.	(268)
0, 200, 300, 400, 800 mg/kg bw or 800 mg/kg bw (800 mg/kg ≈ 1 000 ppm) [a]	Gavage: 5 d/w, 3 w or 3 d, 5 d, 7 d, 9 d, 5 d/w, 3 w	Adult rats	ABR ME	Dose-dependent threshold shifts and OHC loss. Slight OHC loss (5 %) at 200 mg/kg (≈ 250 ppm) (LOAEL) and hearing loss at 300 mg/kg. Supporting cells showed early signs of apoptosis. The OHC loss was time-dependent with increasing OHC loss starting after 7 d of exposure to 800 mg/kg.	(64)
Active rats: 0, 300, 400, 500, 600 Sedentary rats: 0, 500, 650, 850, 1 000	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats	ABR ME	Dose-dependent hearing loss and OHC loss. Slight OHC loss at 300 ppm (5 %) in active rats (LOAEL) and at 500 ppm (3 %) in sedentary rats. Same amount of hearing loss by lower concentrations in active rats compared to sedentary rats.	(210)
0, 500, 650, 850, 1 000, 1 500	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Dose-dependent hearing loss and OHC loss. OHC loss at and above 650 ppm and hearing loss at 12-24 kHz at 850 ppm. Higher exposures affected all frequencies. No effect at 500 ppm.	(229)
0, 500, 1 000, 1 500, 2 000, 2 500	Inhalation: 8 h/d, 5 d	Young rats	ABR at 16 kHz only	Diminished amplitudes of ABR ≥ 1 000 ppm. No effect at 500 ppm.	(329)
0, 400, 800 mg/kg bw	Ip. injection: 2 w daily	Adult rats	MAEP ME of brain	Longer latencies and interpeak intervals in MAEP and damaged structures in the CNS at 800 mg/kg. Some effects at 400 mg/kg.	(378)
0, 500, 1 200 or 0, 2 × 0.75 ml	Inhalation: 7 h or ip. injection	Guinea pigs	CAP CM	No difference between exposed and control animals. Guinea pig not a good model for solvent experiments.	(114)

Table 3. Auditory effects in animals exposed to styrene (STY) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
STY level (ppm)	Regimen				
0, 650, 750	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats	ABR DPOAE ME	OHC loss at and above 650 ppm and hearing loss at 750 ppm. DPOAE appeared as sensitive to STY as ABR.	(295)
0, 700	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats	ABR ME	Loss of OHC up to 63 % in the 3rd row. Hearing loss at 10-24 kHz.	(298)
0, 700	Inhalation: 6 h/d, 5 d/w, 4 w	Young (14 w) and aged (24-26 m) rats	ABR ME	Aged rats had significantly less OHC loss than young animals but more hearing loss. Age affected STY-induced threshold shift and OHC loss.	(49)
0, 700	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats 14 and 21 w, and 21 w of different weight	ABR ME	14-week old rats had more hearing loss and OHC loss than 21-week old rats. Age affected STY-induced threshold shift and hair cell loss. Rats of same age but different weights had the same sensitivity to STY.	(212, 296)
0, 750	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Hearing loss at 16-20 kHz. OHC loss: row 3 > row 2 > row 1.	(208, 230)
0, 750, 1 000, 1 500	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Hearing loss at 12-16 kHz and OHC loss at all levels. Two different intoxication routes may exist within the cochlea.	(211)
0, 800	Inhalation: 14 h/d, 5 d/w, 3 w	Young rats	ABR ME	Hearing loss at 8-16 and 30 kHz; decreased ABR amplitude and increased latency. Deficit restricted to mid-frequencies. OHC loss.	(391)
0, 800, 1 000, 1 200	Inhalation: 14 h/d, 7 d/w, 3 w	Young rats	ABR BA CAR	Elevated BA and/or ABR thresholds at all levels.	(306)

Table 3. Auditory effects in animals exposed to styrene (STY) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
STY level (ppm)	Regimen				
0, 1 000	Inhalation: 6 h/d, 5 d/w, 1- 4 w	Adult rats	ABR ME	Hearing loss at 16-20 kHz, persistent and stable from 1st to 4th week. OHC loss: row 3 > row 2 > row 1. The effect progressed beyond cessation of exposure. Disorganisation of membranous structures is probably the starting point for STY-induced cochlear injury.	(48)
0, 1 000	Inhalation: 6 h/d, 5 d	Adult rats, guinea pigs	DPOAE ME	Hearing loss at 5-16 kHz and OHC loss: row 3 > row 2 > row 1 in rats but not in guinea pigs. Blood STY concentrations 4-fold higher in rats than in guinea pigs.	(213)
0, 1 600	Inhalation: 8 h/d, 5 d	Adult rats	RMA	Increased RMA thresholds for the mid-frequency tones (8 and 16 kHz).	(78)
0, 1 700	Inhalation: 6 h + 4 h next day	Adult rats	-	STY detected in blood, brain, auditory nerve and cochlea but not in cerebrospinal or inner ear fluids, indicating that styrene-induced hearing loss is caused by tissue intoxication rather than fluid contamination.	(47)
882 mg/kg bw (8.47 mmol/kg bw)	Gavage: 5 d/w, 2 w	Adult rats	ME	Almost complete OHC loss in the mid-frequency region of the cochlea: row 3 > row 2 > row 1.	(139)

[a] 800 mg/kg corresponds approximately to 1 000 ppm. Estimated by comparing blood levels of styrene (20.7 µg/g and 22.8 µg/g) after administration of 800 mg/kg bw by gavage for 6 d with those after inhalation exposure of 1 000 ppm, 6 h/d for 5 d.

ABR: auditory brainstem response, BA: behavioural audiometry, bw: body weight, CAP: compound action potential, CAR: conditioned avoidance response, CM: cochlear microphonics, CNS: central nervous system, DPOAE: distortion product otoacoustic emissions, ip.: intraperitoneal, LOAEL: lowest observed adverse effect level, MAEP: middle latency auditory evoked potentials, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, RMA: reflex modification audiometry, STY: styrene.

Table 4. Auditory effects in animals of combined exposure to styrene (STY) and other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen				
0, 100, 300, 600 STY	100-105 dB SPL	Inhalation and N: 12 h/d, 5 d/w, 4 w	Adult rats	ABR ME	STY+N: Slight hearing loss at 100 and 300 ppm but not different from N. Synergistic effects at 600 ppm shown by hearing loss and OHC loss. NOAEL at 300 ppm with noise.	(269)
0, 400 STY	85 dB Leq8h OBN at 8 kHz (86.2 dB SPL)	Inhalation and N: 6 h/d, 5 d/w, 4 w	Active young rats	ABR ME	N: Hearing loss but no OHC loss. STY: OHC loss. STY+N: Same hearing loss as after N, greater OHC loss than STY (synergism). LOAEL at 400 ppm both with and without N.	(210)
0, 500, 1 200 STY	95 dBA	Inhalation and N: 7 h	Guinea pigs	CAP CM	STY: No effect. N: Hearing loss. STY+N: No potentiation by STY. Guinea pig not a good model for solvent experiments.	(114)
0, 750 STY	97 dB OBN at 8 kHz	Inhalation and N: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	STY: Hearing loss at 16-20 kHz and OHC loss: row 3 > row 2 > row 1. N: Hearing loss at 8-20 kHz and OHC loss: row 1 > row 2 > row 3. STY+N: Synergistic effects seen as increased hearing loss (6-12 kHz) and larger OHC loss.	(208)
0, 750 STY+ 4 g/kg bw EtOH	No N	Inhalation: 6 h/d, 5 d/w, 4w EtOH gavage: 5 d/w, 4 w	Adult rats	ABR ME	STY: Hearing loss at 16-20 kHz and OHC loss. EtOH: No effect. STY+EtOH: Potentiation of the effects of STY by EtOH (greater hearing loss at 2-24 kHz and increased OHC loss). EtOH alters STY metabolism by increasing the liver CYP activity.	(230)
STY+TCE iso-effective conc.: 1 000 + 0 750 + 750 500 + 1 500 250 + 2 250 0 + 3 000	No N	Inhalation: 8 h/d, 5 d	Young rats	ABR at 16 kHz only	Combined exposure to iso-effective concentrations of both solvents caused additive effects on the diminished amplitudes of ABR.	(329)

ABR: auditory brainstem response, bw: body weight, CAP: compound action potential, CM: cochlear microphonics, CYP: cytochrome P450, EtOH: ethanol, Leq8h: equivalent level of noise during 8 hours, LOAEL: lowest observed adverse effect level, ME: morphological examination, N: noise, NOAEL: no observed adverse effect level, OBN: octave band noise, OHC: outer hair cell, SPL: sound pressure level, STY: styrene, TCE: trichloroethylene.

Styrene and noise exposures were assessed for 299 workers in the reinforced fibre industry in Canada. TWA styrene exposures for directly and indirectly exposed were 25 ppm and 8 ppm, respectively, while noise exposures were similar in the groups, i.e. 88 and 90 dBA. The association between noise exposure (based on an estimated life-time noise dose) and hearing loss was significant. Styrene exposure approached significance for hearing loss only at specific frequencies (4 and 6 kHz) in the left but not in the right ear. Noise and styrene exposures were highly correlated. Both noise exposure and age were found to be confounding factors regarding the possible hearing loss caused by styrene and might shadow the styrene effect (336).

More recently, the effects of styrene were investigated in male workers exposed in factories producing plastic buttons or bathtubs (258, 259). In the first study, workers whose noise exposures exceeded 85 dBA were excluded from the study population. Participants were exposed to a mixture of solvents, mainly styrene and toluene. Of the 93 participants, only 6 were exposed to levels of styrene that exceeded 50 ppm and 2 were exposed to toluene levels exceeding 50 ppm (258). In the second study, the 48 study participants were divided into 3 subgroups based on exposure: an unexposed control group, a group exposed to noise levels of 82-86 dBA, and a group exposed to low average levels of both styrene (22 ppm, range 3.7-46) and noise (69-76 dBA) (259). No effects of the solvents were detected by routine audiometric testing up to 8 kHz. In both investigations, the hearing as assessed by high-frequency audiometry (10-16 kHz) was reduced in workers exposed to styrene for 5 years or more, although both noise levels and styrene concentration in air were within limits recommended by several international agencies. Hearing impairment was associated with styrene concentrations in air and MA (biomarker of styrene) concentrations in urine. No effects of other solvent exposures were detected (258, 259).

The association between MA and hearing loss was also observed in a cross-sectional study conducted in Sweden, which aimed to investigate the effects of occupational exposure to low levels of styrene and noise (187, 256). Styrene measurements were conducted for all exposed workers by personal air samples and biological monitoring of MA in urine. Also historical and life-time exposures to styrene were estimated using company records. For about 60 % of the participants in the groups exposed to noise (with or without styrene), the noise levels were above 85 dB Leq measured outside ear protection. The range of noise exposure was similar in these two groups (75-116 dBA). Styrene exposures were low, averaging 3.5 ppm, with a maximum level of 22 ppm (8-hour values) in the styrene only group and 2.8 ppm in the group exposed also to noise. Workers exposed to styrene (with and without noise) had significantly worse pure-tone thresholds at 2, 3, 4 and 6 kHz when compared to noise-only exposed or non-exposed workers. From the numerous variables that were analysed for their contribution to the development of hearing loss, only age, current noise exposure and MA levels were significant in the final multiple logistic regression model. The odds ratio (OR) estimates for hearing loss were 1.18 times greater for each dB of current noise

exposure (95 % confidence interval (CI) 1.01-1.34), 1.19 times greater for each year of age (95 % CI 1.11-1.28) and 2.44 times greater for each increment of 1 mmol (152 mg) of MA/g creatinine (95 % CI 1.01-5.88). At the NIOSH recommended exposure limit (REL) of 300 mg of MA/g creatinine (~19 ppm in air), the OR for hearing loss would be 4.88 (2 × 2.44 mg). Testing for interaction between noise and styrene exposure was not significant, suggesting an additive effect between the two agents (256). Interaction between agents certainly depends on the exposure levels of the studied populations. The audiometric results were later compared to a Swedish database of an otologically unscreened, non-occupationally noise-exposed population (187). The audiological test battery included pure-tone audiometry, distortion product otoacoustic emissions, psychoacoustic modulation transfer function, interrupted speech, speech recognition in noise, and cortical response audiometry. Workers exposed to both noise (> 85 dBA) and styrene and to styrene alone (noise < 85 dBA) had significantly poorer pure-tone thresholds in the frequency range of 3-8 kHz than controls, noise-only exposed workers, and those listed in the Swedish age-specific database. Even though abnormalities were noted on distortion product otoacoustic emissions and cortical response audiometry testing, the interrupted speech and speech recognition in noise tests were the more sensitive tests for styrene effects (187).

Śliwińska-Kowalska *et al* evaluated the effects of occupational exposure to styrene and combined exposure to styrene and noise on hearing (346, 347). The study group, 290 yacht and plastic factory workers, was exposed to a mixture of solvents having styrene as its main compound. The mean of individual work-life average styrene exposure levels was 14 ppm (range 0.8-71) and the mean noise level 82 dBA (range 71-93). The reference group, totalling 223 subjects, included white collar workers exposed neither to solvent nor noise (n = 157) and metal factory workers exposed exclusively to noise (n = 66). The mean noise level in the noise-only exposed group was 89 dBA and in the non-exposed controls 73 dBA. Hearing loss was observed in 63 % of the styrene exposed workers and in 42 % of the referents. The OR for hearing loss in the styrene exposed group was 3.9 (95 % CI 2.4-6.2, adjusted for age, gender, current occupational exposure to noise and exposure to noise in past). The mean hearing thresholds differed significantly from the reference group in a wide range of frequencies from 1 to 8 kHz. Depending on type of exposure, the styrene exposed group was divided into 4 subgroups: exposed to styrene only (n = 194), styrene and noise (n = 56), styrene and toluene (n = 26), and styrene, toluene and noise (n = 14). Hearing loss was observed in 79 % of the workers exposed to styrene, toluene and noise, in 77 % of the styrene and noise group, in 77 % of the styrene and toluene group, in 56 % of the styrene only group, in 63 % of the noise only exposed group and in 34 % of the unexposed group. The ORs for hearing loss adjusted for age and gender were: 5.2 for the styrene only group, 3.4 for the noise only group, 10.9 the for styrene and noise group, 13.1 for the styrene and toluene group and 21.5 for the styrene, toluene and noise group (all significant when compared to the unexposed group). A significant increase in hearing thresholds was found in all subgroups within the frequency range 2-8 kHz

as compared to the unexposed group. The highest hearing threshold was found in the group exposed to styrene and noise even if only an additive effect between styrene and noise could be statistically verified (346, 347).

In a smaller study of 16 workers in a boat manufacturing plant in Germany, no consistent correlations were reported between hearing ability and the biological exposure measurements of styrene. The exposure level was assessed by biological exposure index using the sum of MA+PGA in urine. The mean value was 656 mg/g creatinine (standard deviation (SD) 639, range 72-2 213) for the laminators and 130 mg/g creatinine (SD 129, range 25-478) for the controls (161). This corresponds to approximately 22 ppm styrene in the air for the laminators and 4.3 ppm for the controls assuming that 20 ppm corresponds to 600 mg/g creatinine (88). Noise levels were not reported.

This study was recently followed by a larger study by Triebig *et al* (374) investigating workers from the same boat manufacturing plant as in the study above (161). Workers currently exposed to noise above 85 dBA were excluded. Hearing, including high-frequency thresholds, was assessed with pure-tone audiometry between 0.125 and 16 kHz and with transient evoked otoacoustic emissions (TEOAE). The exposure levels of styrene were assessed by biological monitoring using MA+PGA in urine as well as styrene levels in the blood. Also historical and life-time exposures to styrene were estimated using company records. A total of 248 workers were divided in three different groups with low, medium or high current exposure to styrene. The mean current MA+PGA urinary values for the three exposed groups were 51 (SD 27), 229 (SD 103) and 970 (SD 410) mg/g creatinine. According to the authors' transformation of MA+PGA urinary levels to styrene air levels, these values would correspond to approximately 2-3, 8-15 and 40-50 ppm styrene in air, respectively. Extensive statistical analyses were made regarding the relationship between hearing thresholds and both current and life-time exposure levels. No differences between the three exposed groups were found in thresholds of pure-tone audiometry or in the results of TEOAE. However, elevated thresholds at some frequencies (> 25 dB, 3-6 kHz) and an increased risk for hearing loss were found in a subgroup (n = 17) exposed to high levels of styrene (30-50 ppm, with levels above 50 ppm in the past) during 10-26 years as compared to a group (n = 34) exposed to low levels for a shorter time (2-16 years). The OR was 7.5 (95 % CI 1.1-51.4) adjusted for education, age, alcohol intake, tenure and mother tongue. Air levels were estimated from the life-time average MA+PGA urinary value of 660 mg/creatinine (SD 613). The corresponding value for the low-exposure group was 200 mg MA+PGA/g creatinine (SD 171) (374).

Mascagni *et al* measured pure-tone audiometry in 32 workers exposed to styrene in a fibreglass reinforced plastic boat manufacturing industry in Italy with an average exposure duration of 7 years. Hearing thresholds were compared to 60 unexposed control subjects. Styrene exposure was measured using biological monitoring of MA+PGA in urine. The mean value for the exposed group was 149 mg/g creatinine (SD 80, range 20-410) (240). This corresponds to approximately 5 ppm styrene (88). The noise levels were measured to 73 dBA (Leq). Twenty-four

of the exposed workers were compared to age-matched controls and had slightly but significantly ($p < 0.05$) higher thresholds at all frequencies (except at 8 kHz on the right ear) (240). The study confirms the results from earlier studies with larger population that styrene alone can cause a slightly elevated hearing threshold.

7.1.4 Conclusion on styrene

The ototoxic effects of styrene in animals have only been demonstrated in rats. Styrene does not cause hearing loss in guinea pigs (114, 213).

Based on available rat studies, the LOAEL for styrene alone is 200 mg/kg body weight by gavage (64). This corresponds to approximately 250 ppm by inhalation (for details, see Section 7.1.2.1). In rat inhalation studies, 300 ppm (4 weeks) was identified as a no effect level, both with and without simultaneous noise exposure (100 dB SPL) (268, 269). However, in rats forced to be active, a LOAEL of 300 ppm styrene alone was obtained (210). Thus, no NOAEL for styrene alone can be identified in animals. Synergistic interaction between styrene and noise was manifested only at concentrations above the LOAEL for styrene alone, i.e. at and above 400 ppm (208, 210, 269).

In occupational studies, current levels of styrene averaged approximately 3.5-50 ppm. The lowest current average exposures among workers exhibiting significant hearing loss when compared to non-exposed controls were 3.5-22 ppm (187, 240, 256, 258, 259). In these studies, the styrene exposed groups were exposed to noise below 85 dBA at the time of study. However, exposure to higher styrene and noise levels in the past, as well as peaks of high concentration in the present, is likely and may have contributed to the effect. One study demonstrated a significant positive correlation between the average work-life styrene concentration of 14 ppm and hearing loss (346, 347). In another study, neither life-time (average 16 ppm) nor current air styrene (3.5 ppm) levels were associated with hearing loss, whereas current urinary MA levels were positively correlated with hearing thresholds (256).

The influence of long-term exposure was shown in the study by Triebig *et al*, which demonstrated auditory effects in workers estimated to have been exposed to 30-50 ppm for at least 10 years with levels above 50 ppm in the past (374).

In humans, the type of interaction taking place between noise and styrene exposure is not yet clear.

International 8-hour occupational exposure limits (OELs) for styrene vary from 20 to 100 ppm (Appendix 1). The main concerns are neurotoxicity, genotoxicity and potential carcinogenic effects (250).

Table 5. Occupational studies on auditory effects of styrene (STY) exposure, tested with pure-tone audiometry, unless otherwise stated.

STY levels (ppm) and exposure duration, mean ± SD, (range)	Noise (N)	No. of exposed and controls	Results and comments	Reference
Current exposure: 3.5 (0.05-22) (STY) 2.8 (0.007-12) (STY+N) Average work-life exposure: 18 (STY) 14 (STY+N) Duration: 17 (1-39) yrs (STY) 15 (2-37) yrs (STY+N)	≤ 84 dBA (STY) 89 dBA TWA (STY+N) 86 dBA TWA (N)	65 STY 89 STY+N 78 N 81 controls	STY and STY+N exposed had worse PTA and lower scores in speech tests than N exposed and controls. Biological exposure measure of STY (MA in urine) associated with hearing loss.	(187, 256)
Current exposure [a]: ~ 5 (0.7-14) Duration: 7 ± 6.2 yrs	73 dBA	32 STY 60 controls, age-matched	Slightly but significantly ($p < 0.05$) higher hearing thresholds at all frequencies (except at 8 kHz on the right ear) compared to controls.	(240)
Current exposure: 8 (0.1-92) (6 subjects > 50 ppm) Duration: 9.4 ± 8.9 yrs	< 85 dB	44 STY 49 STY in mixtures 33 controls	No effect in PTA. Poorer detection of high-frequency tones in STY workers exposed to > 16 ppm for > 5 yrs (n = 54). A dose-dependent deterioration which correlated to STY in air and biological exposure measures of STY (MA in urine). Co-exposure to TOL, methanol and/ or acetone but also to XYL, methyl and butyl acetate.	(258)
Average work-life exposure: 14 ± 9.3 (STY) 8 ± 6.1 (STY+N) 38 ± 13 (STY+TOL) 1.6 ± 1.4 (STY+TOL+N) 14 ± 12 (0.8-71) (all STY groups)	80 dBA (STY) 89 dBA (STY+N) 80 dBA (STY+TOL) 86 dBA (STY+TOL+N) 89 dBA (N) 73 dBA (controls)	194 STY 56 STY+N 26 STY+TOL 14 STY+TOL+N 66 N 157 controls	4-fold increase in the odds (OR 3.9, 95 % CI 2.4-6.2) of developing hearing loss related to STY (all STY groups compared to N+controls). 2-3-fold increase of OR in STY+N exposed compared to STY group and N group. A positive linear relationship existed between an averaged work-life exposure to STY and hearing loss at 6 and 8 kHz.	(346, 347)
Current exposure: 22 (3.7-46) Duration: 5.4 yrs	69-76 dBA (STY) 82-86 dBA (N) 58-62 dB (controls)	19 STY 18 N 11 controls	No effect in PTA. STY exposed had poorer detection of high-frequency tones than N exposed or controls. N exposures not equivalent. Co-exposure to methanol and methyl acetate.	(259)

Table 5. Occupational studies on auditory effects of styrene (STY) exposure, tested with pure-tone audiometry, unless otherwise stated.

STY levels (ppm) and exposure duration, mean ± SD, (range)	Noise (N)	No. of exposed and controls	Results and comments	Reference
Historic exposure: (5.6-24), peaks > 70 Duration: 11 (6-15) yrs	Estimated < 85 dBA TWA	18 STY Clinical reference group	7 workers showed disturbances of central hearing pathways. 16 workers showed abnormalities in vestibular tests.	(271)
Current exposure [a]: 22 (2.4-74) Duration: 7.5 ± 5 yrs	Not given	16 STY 16 controls	Isolated correlations between STY and hearing loss but no consistent relationships between impaired hearing and biological exposure measure (MA+PGA in urine).	(161)
Current exposure [a]: ~ 2-3 (low STY) ~ 8-15 (medium STY) ~ 40-50 (high STY) Duration: 6 (1-26) yrs	75-83 dBA (STY) 70-85 dBA (controls) > 85 dBA excluded	99 low STY 118 medium STY 31 high STY	Increased risk (OR 7.5, 95 % CI 1.1-51.4) for hearing loss (> 25 dB at 3-6 kHz) in a subgroup of workers (n = 17) exposed to high STY levels (30-50 ppm with levels above 50 ppm in the past [a]) at least 10 years compared to workers exposed to low STY levels for a shorter time. No other relationships between impaired hearing and biological exposure measures (MA+PGA in urine and STY in blood).	(374)
Current exposure: 25 ± 22 (dir STY) 8 ± 11 (indir STY)	88 dBA (dir STY) 90 dBA (indir STY) 80 dBA (controls)	170 dir STY 86 indir STY 43 controls	N and STY exposures highly correlated. Age and N exposures significantly associated with hearing loss.	(336)
Current exposure: 32 ± 17 (dir STY) 14 ± 7 (indir STY) Duration: 8.6 (0.1-24) yrs	66-70 dBA, occasionally up to 104 dB (STY) 80-85 dBA (controls)	31 dir STY 28 indir STY 88 controls	Difference in hearing thresholds at 8 kHz between the two STY-groups. N exposures qualitatively different between groups.	(261)
Current exposure: 35 (3-92) Duration: 7.6 (2-23) yrs	Not given	20 STY	No effects in PTA. 17 workers showed abnormalities in vestibular tests.	(43)

[a] Air levels estimated from MA+PGA in urine.

CI: confidence interval, dir: directly exposed, indir: indirectly exposed, MA: mandelic acid, N: noise, OR: odds ratio, PGA: phenylglyoxylic acid, PTA: pure-tone audiometry, SD: standard deviation, STY: styrene, TOL: toluene, TWA: time-weighted average, XYL: xylene.

7.2 Toluene

7.2.1 General

Toluene or methylbenzene is a colourless, flammable and explosive liquid with an aromatic odour (399).

Toluene is a commercially important intermediate chemical produced throughout the world in large quantities. It is used as a solvent carrier in paints, thinners, adhesives and inks, and is added to gasoline to increase the octane number. The vast use makes toluene present in many industrial settings (399).

Numerous groups of individuals are occupationally exposed to toluene. Occupations in which exposure to toluene may occur include production, handling and use of toluene and toluene containing products, e.g. chemical laboratory workers, gasoline blenders, lacquer workers, paint and paint thinner makers, petrochemical workers, maintenance workers, painters and printers (168, 399).

The major route of exposure is via the respiratory system. In human studies, the uptake of toluene has been estimated to be 40-60 % of the total amount inhaled (171). Physical work increases the respiratory uptake. Liquid toluene may be absorbed through the skin to a limited extent. Toluene is partly excreted via exhaled air but 80-90 % of the absorbed toluene is biotransformed and excreted by the kidneys mainly as hippuric acid (399).

7.2.2 Effects in animals

7.2.2.1 Toluene alone

Several animal studies have shown effects of toluene on the auditory system (Table 6).

Toluene causes permanent auditory dysfunction in rats. No permanent effects on hearing have been shown in chinchillas (84) or guinea pigs (244). In mice, toluene increased the progression of age-related hearing loss in a strain (C57BL/6J) with a genetic predisposition for presbyacusis (hearing loss as part of the aging process) but not in normal (CBA/Ca) mice (218).

Effects of toluene on the auditory system of the rat were first reported by Pryor *et al* in a study addressing neurobehavioural effects of toluene (308). Effects on the auditory system in this and several other studies were shown at high exposure concentrations (at and above 1 000 ppm, 14-21 hours/day, 5-7 days/week for 2-14 weeks) (182, 185, 186, 287, 288, 305, 307, 308, 310, 326). Light microscopy or scanning electron microscopy examination of the cochlea confirmed the initial findings and revealed a progressive severe loss of hair cells (184). The morphological appearance of toluene-induced cochlear damage has since been documented in other studies (46, 206, 358). Later studies revealed that a shorter daily exposure time (6 hours) caused no effect on the auditory brainstem response at 1 000 ppm exposure (39, 229).

Considerably lower toluene concentrations were able to acutely produce equivalent auditory dysfunction in the guinea pig (244) as compared to published data in the rat (46, 183). Concentrations as low as 250 ppm toluene (8 hours/day, 5 days/week for 1-4 weeks) temporarily disrupted auditory function in the guinea

pig, and 500 and 1 000 ppm toluene produced greater acute dysfunction. However, no permanent hearing loss developed in the guinea pigs and hair cell death was not observed (244).

Effects of prenatal exposure to toluene and hearing loss in the offspring were reported by Hougaard *et al*. Pregnant rats were exposed to 1 800 ppm toluene, 6 hours/day during gestation days 7-20. Body weights of exposed offspring were lower until day 10 after parturition. A behavioural test battery revealed some cognitive effects on learning and memory, most marked in female offspring. The hearing function of the offspring, tested with auditory brainstem response 3 months after exposure, showed a slight tendency towards hearing loss in the male offspring, significant from controls only at 8 kHz (162).

7.2.2.2 Toluene combined with noise

Several animal studies have investigated the interactions between toluene exposure and noise on the auditory system (Table 7).

The most serious effect was observed in a study of sequential exposure to toluene (1 000 ppm, 16 hours/day, 5 days/week for 2 weeks) and noise (100 dB Leq8h, 10 hours/day, 7 days/week for 4 weeks) in rats. Toluene exposure followed by noise caused a greater loss (synergism) of auditory sensitivity than toluene or noise alone (186). Additionally, Lataye and Campo observed that simultaneous exposure to toluene (2 000 ppm, 6 hours/day, 5 days/week for 4 weeks) and noise (92 dB OBN at 8 kHz), which each alone resulted in hearing loss, generated an auditory deficit that exceeded the summated losses for successive exposure to toluene and noise (206). Brandt-Lassen *et al* observed interaction on auditory threshold shifts in rats between toluene exposure at 1 000, 1 500 and 2 000 ppm (6 hours/day for 10 days) and noise exposure (96 dB SPL, 2 hours following the daily toluene exposure). In the same study, exposure to 500 ppm toluene plus noise resulted only in a threshold shift similar to that found after noise only exposure (39).

Impulse noise is known to cause more hearing loss than wide band noise. In a study by Lund and Kristiansen, combined exposure to toluene (500, 1 000 or 1 500 ppm, 6 hours/day for 10 days) and either impulse noise or wide band noise (both equivalent to 90.8 dB Leq8h) showed that toluene (1 500 ppm) potentiated the noise-induced hearing loss. The combination of toluene and impulse noise caused a larger hearing loss than wide band noise and toluene (234). The possible mechanisms underlying these interactions are discussed in Chapter 5.

Lund and Kristiansen also performed a long-term experiment with low doses of toluene (100, 200 and 500 ppm, 6 hours/day, 5 days/week for 90 days), known to not cause a hearing loss, combined with steady state noise equivalent to 87 dB Leq8h. The rats exposed to 100 or 200 ppm toluene and noise had a tendency towards less hearing loss than the rats exposed to noise alone (234).

7.2.2.3 Toluene combined with other agents

Interactions between toluene exposure and intake of alcohol or acetyl salicylic acid or exposure to other solvents (with and without noise) have been reported (Table 7).

Combined exposure to toluene and *n*-hexane or to iso-effective concentrations of toluene and trichloroethylene or toluene and chlorobenzene caused additive effects on the auditory system in rats (305, 328). One study showed synergistic effects of toluene and *n*-hexane 3 months after exposure to high levels of both solvents (1 000 + 1 000 ppm, 21 hours/day, 7 days/week for 28 days) (287).

Ethanol alone (6 % in drinking water for 2 weeks or 4 g/kg body weight by gavage for 4 weeks) did not cause hearing loss but potentiated the ototoxic effects of toluene (1 000 ppm for 2 weeks and 1 750 ppm for 4 weeks, respectively) (50, 312). However, in another study on rats, ethanol (6 % in drinking water for 8 weeks) reduced the effect of toluene (1 000 ppm for 8 weeks) (288). A synergistic interaction between acetyl salicylic acid and toluene was shown by Johnson. Rats were given high daily doses (100 mg/kg body weight) of acetyl salicylic acid by gavage and were simultaneously exposed to 1 000 ppm toluene for 10 days. Acetyl salicylic acid alone did not cause hearing loss but potentiated the permanent ototoxic effect caused by toluene (182). These results might be of interest since ethanol is commonly used and pain killers of the acetyl salicylic acid type are likely to be used by workers exposed to toluene although in lower doses.

Recent studies have demonstrated that adding several factors, e.g. another solvent and noise or carbon monoxide and impact noise, much reduce the lowest level of toluene exposure needed to elicit an auditory damage (133, 233). Synergistic interaction was seen in rats exposed to 400 ppm toluene and 660 ppm ethylbenzene together with 93 dB noise (6 hours/day for 10 days) (133). Exposure 6 hours/day for 10 days to toluene (500 or 1 000 ppm) together with carbon monoxide (300 or 500 ppm) and impulse noise (84 dB SPL noise with 75 % impulses at 4-20 kHz) caused a synergistic interaction, which increased with increasing doses of both carbon monoxide and toluene. Combined exposure to toluene and impulse noise did not cause interaction at the tested levels (233).

7.2.3 Observations in man

7.2.3.1 Toluene alone

No studies were identified.

7.2.3.2 Toluene combined with noise

Several occupational studies have been conducted regarding the effects of toluene on the auditory system and the interaction between toluene and noise (Table 8).

A group of rotogravure printers with normal hearing ability and exposed to an average of 97 ppm toluene for 12-14 years showed alterations of brainstem auditory evoked responses (2). Thus, auditory nervous system modifications were demonstrated before clinical signs of chronic exposure to toluene appeared.

Table 6. Auditory effects in animals exposed to toluene (TOL) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
TOL level (ppm)	Regimen				
0, 250, 500, 1 000	Inhalation: 8 h/d, 5 d/w, 1- 4 w	Adult guinea pigs	DPOAE ME	Temporary disruption of auditory function at all doses, with dose-related results. No permanent threshold shift. Reduced enzyme (SDH) activity in mid-frequency region of the cochlea.	(244)
0, 400, 700, 1 000 0, 1 500 0, 2 000 0, 4 000	Inhalation: 14 h/d, 16 w 14 h/d, 3 d 8 h/d, 1 or 3 d 4 h	Adult rats	CAR BA ABR	No hearing loss at 700 ppm for 16 w (NOAEL) or at 2 000-4 000 ppm for ≤ 8 h. Hearing loss at 1 000 ppm for 16 w (observed after 2 w) (LOAEL) and at 1 500-2 000 ppm for 3 d.	(311)
0, 500, 1 000, 1 500, 2 000	Inhalation: 6 h/d, 10 d	Young rats	ABR	Mid-frequency hearing loss at 1 500 and 2 000 ppm. No effect at 1 000 ppm.	(39)
0, 900, 1 200, 1 400	Inhalation: 14 h/d, 7 d/w, 4-14 w	Weanling and young rats	CAR BA ABR	Impaired CAR responses or elevated ABR at and above 1 200 ppm. NOAEL at 900 ppm with CAR.	(307, 308, 326)
0, 1 000	Inhalation: 12 h/d, 7 d	Young and adult mice	ABR	Increased progression of age-related hearing loss (mid-frequency) in predispositioned (C57BL/6J) mice but not in normal (CBA/Ca) mice. Influence of strain and age shown.	(218)
0, 1 000	Inhalation: 16 h/d, 5 or 7 d/w, 2 w	Young rats	ABR	Hearing loss at 12.5-20 kHz.	(182, 185, 186)
0, 1 000	Inhalation: 21 h/d, 7 d/w, 4 w	Adult rats	ABR	Hearing loss shown by click-evoked non-frequency specific ABR.	(287)
0, 1 000	Inhalation: 21 h/d, 5 d/w, 8 w	Adult rats	ABR	Hearing loss at 6-20 kHz.	(288)

Table 6. Auditory effects in animals exposed to toluene (TOL) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
TOL level (ppm)	Regimen				
0, 1 000, 1 250, 1 500, 1 750, 2 000	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Dose-dependent increase in hearing loss and OHC loss. Severe OHC loss (row 3) at 1 000 and 1 500 ppm. Slight hearing loss (4 dB) at 1 500 ppm at 20 kHz. Higher exposures affected more frequencies.	(46, 229)
0, 1 000, 1 500, 2 000	Inhalation: 10 or 30 min before and during 60-min test	Adult rats	BA	Acute effect of response during exposure. No permanent effect.	(42)
0, 1 200	Inhalation: 14 h/d, 7 d/w, 5 w	Weanling and young rats	CAR BA ABR ME	Hearing loss at 12-16 kHz. OHC loss in basal turn of cochlea (i.e. cochlear origin shown). Greater hearing loss in weanling rats.	(310)
0, 1 200	Inhalation: 14 h/d, 7 d/w, 9 w	Weanling rats	CAR ABR	Impaired CAR responses and elevated ABR threshold.	(305)
0, 1 400	Inhalation: 16 h/d, 3-8 d	Adult rats	ABR DPOAE ME	Hearing loss at 2-20 kHz, DPOAE amplitudes lowered. OHC loss. Progressive hearing loss after end of exposure.	(183, 184)
0, 1 750	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Hearing loss at 12-20 kHz and OHC loss.	(50)
0, 1 750	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	CM ME	Hearing loss at 2-4 kHz and 12-16 kHz. No effect at other frequencies (tested range: 2-32 kHz). Correlated OHC loss.	(207)
0, 1 800	Inhalation: 6 h/d, GD 7-20	Adult rats	ABR BA	Hearing loss at 8 kHz in male offspring. Cognitive effects on learning and memory, most marked in female offspring.	(162)
0, 2 000	Inhalation: 8-12 h/d, 10 d	Adult chinchillas and rats	ABR	Hearing loss in rats but not in chinchillas. Chinchillas had higher levels and activities of CYP enzymes in the liver.	(84)

Table 6. Auditory effects in animals exposed to toluene (TOL) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
TOL level (ppm)	Regimen				
0, 2 000	Inhalation: 8 h/d, 7 d/w, 2 w	Weanling rats	CAR BA	Hearing loss shown by CAR and BA.	(312)
0, 2 000	Inhalation: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	Hearing loss at 8-16 kHz and OHC loss.	(206)
0, 2 500	Inhalation: 8 h/d, 5 d	Young rats	RMA	Increased RMA thresholds for the mid-frequency tones (8 and 16 kHz).	(78)
0, 2 600, 2 800	Inhalation: 8 h/d, 5 d	Young rats	ABR, at 16 kHz only	Diminished amplitudes of ABR.	(328)
0, 8 000	Inhalation, intermittent: 4 times/d, 13 w	Adult rats	ABR	Mid-frequency hearing loss at 10 kHz. Less effect at 30 kHz.	(241)
0, 1 ml/kg bw	Gavage: 1 time/d, 21 d	Adult rats	ABR ME	Hearing loss at 2-8 Hz. OHC loss. Cochlear origin confirmed.	(358)
780 mg/kg bw (8.47 mmol/kg bw)	Gavage: 5 d/w, 2 w	Adult rats	ME	OHC loss in the mid-frequency region of the cochlea: row 3 > row 2 > row 1.	(139)
0, 1.5, 1.7 g/kg bw	Sc. injection: 2 times/d, 7 d	Weanling rats	CAR	Dose-related hearing loss. Ototoxicity shown by injection.	(304)

ABR: auditory brainstem response, BA: behavioural audiometry, CAR: conditioned avoidance response, CM: cochlear microphonics, CYP: cytochrome P450, DPOAE: distortion product otoacoustic emissions, GD: gestation days, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, RMA: reflex modification audiometry, sc.: subcutaneous, SDH: succinate dehydrogenase, TOL: toluene.

Table 7. Auditory effects in animals of combined exposure to toluene (TOL) and other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen				
Toluene and noise						
0, 100, 200, 500 TOL	90 dB SPL, steady WBN 4-20 kHz (= 87 dB Leq8h)	Inhalation: 6 h/d, 5 d/w, 90 d N: 4 h/d, 5 d/w, 90 d	Adult rats	ABR DPOAE	N: Hearing loss at 10-16 kHz. TOL+N: Tendency to less hearing loss than by N at 100 or 200 ppm. Same hearing loss as N at 500 ppm. NOAEL at 500 ppm with N.	(234)
0, 500, 1 000, 1 500 TOL	92 dB SPL, steady WBN 4-24 kHz or 92 dB SPL, impulse N 4-24 kHz, both = 90.8 dB Leq8h	Inhalation and N: 6 h/d, 10 d	Adult rats	ABR DPOAE	N: Impulse N caused worse hearing loss than WBN. TOL+either N: No interaction at 500 or 1 000 ppm. Synergistic interaction at 1 500 ppm. Hearing loss after TOL+impulse N much worse than after TOL+WBN.	(234)
0, 500, 1 000, 1 500, 2 000 TOL	96 dB SPL (≈ 90 dB Leq8h)	Inhalation: 6 h/d, 10 d N: 2 h after the daily TOL exposure, 10 d	Young rats	ABR	TOL: Mid-frequency hearing loss at 1 500 and 2 000 ppm. TOL+N: Same hearing loss as N at 500 ppm, small interaction at 1 000 ppm, clear synergistic interaction at ≥ 1 500 ppm. NOAEL at 1 000 ppm without N and at 500 ppm with N. LOAEL at 1 500 ppm without N and at 1 000 ppm with N.	(39)
0, 1 000 TOL	100 dB Leq8h, before or after TOL	Inhalation: 16 h/d, 5 or 7 d/w, 2 w N: 10 h/d, 7 d/w, 4 w Sequential exposure	Young rats	ABR	N: Hearing loss at 16-20 kHz. TOL: Hearing loss at 12.5-20 kHz. TOL+N: Synergistic effect of TOL followed by N but only additive effect by reverse order (N+TOL).	(185, 186)

Table 7. Auditory effects in animals of combined exposure to toluene (TOL) and other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen				
0, 2 000 TOL	92 dB OBN at 8 kHz	Inhalation and N: 6 h/d, 5 d/w, 4 w	Adult rats	ABR ME	N: Hearing loss at 8-12 kHz and OHC loss. TOL: Hearing loss at 8-16 kHz and OHC loss. TOL+N: Synergistic interaction (both hearing loss and OHC loss). Cochlear damage from N (injured stereocilia) different from TOL (OHC loss).	(206)
0, 2 000 TOL	95 dB OBN at 0.5 kHz	Inhalation and N: 8-12 h/d, 10 d	Chin-chillas	ABR	TOL: No hearing loss. N: hearing loss. TOL+N: No interaction found. Chinchillas less sensitive than rats.	(84)
Toluene and noise and other agents						
400 TOL+ 660 EBZ	95 dB or 93 dB OBN at 8 kHz	Inhalation: 6 h/d, 5 d or 10 d N: 6 h/d, 5 d (95 dB) or 6 h/d, 10 d (93 dB)	Adult rats	CAP DPOAE ME	N: Small reversible effects on DPOAE, no effect on CAP and small OHC loss. TOL+EBZ: No effect on DPOAE and CAP, no OHC loss. TOL+EBZ+either N: Permanent effects on DPOAE, significant effect on CAP and larger OHC loss than N alone (synergism).	(133)
0, 500, 1 000 TOL + 0, 300, 500 CO	84 dB SPL, 75 % impulses 4-20 kHz	Inhalation and N: 6 h/d, 10 d	Adult rats	ABR DPOAE	N: Hearing loss at 8-14 kHz. TOL+N: Same hearing loss as N. CO+N: Dose-dependent increase in hearing loss with a synergistic interaction with 500 ppm CO. TOL+CO+N: TOL caused increased hearing loss as compared to CO+N, synergistic interaction that increased with concentrations of both CO and TOL. Worst hearing loss after 1 000 ppm TOL+500 ppm CO+N.	(233)

Table 7. Auditory effects in animals of combined exposure to toluene (TOL) and other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen				
Toluene and other solvents						
1 000 TOL+ 1 000 *n*-HEX	No N	Inhalation: 21 h/d, 7 d/w, 28 d	Adult rats	ABR	TOL: Hearing loss. *n*-HEX: No hearing loss. *n*-HEX+TOL: Potentiated hearing loss compared to TOL.	(287)
1 200 TOL+ 4 000 *n*-HEX	No N	Inhalation: 14 h/d, 7 d/w, 9 w	Weanling rats	CAR ABR-latency	TOL: Hearing loss. *n*-HEX: No hearing loss but slower ABR latencies. *n*-HEX+TOL: No interaction but additive effect on CAR.	(305)
TOL+TCE iso-effective conc.: 2 600 + 0 1 950 + 700 1 300 + 1 400 650 + 2 100 0 + 2 800	No N	Inhalation: 8 h/d, 5 d	Young rats	ABR at 16 kHz only	Additive effects on the diminished amplitudes of ABR.	(328)
TOL+CBZ iso-effective conc.: 2 800 + 0 2 100 + 500 1 400 + 1 000 700 + 1 500 0 + 2 000	No N	Inhalation: 8 h/d, 5 d	Young rats	ABR at 16 kHz only	Additive effects on the diminished amplitudes of ABR.	(328)

Table 7. Auditory effects in animals of combined exposure to toluene (TOL) and other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen				
Toluene and ethanol						
1 000 TOL+ 6 % EtOH in drinking water	No N	Inhalation: 8 h/d, 7 d/w, 2 w EtOH in water: 2 w, forced consumption	Wean-ling rats	CAR BA	TOL: Hearing loss. EtOH: No hearing loss. TOL+EtOH: Trend towards potentiation of the effects of TOL.	(312)
1 000 TOL+ 6 % EtOH in drinking water	No N	Inhalation: 21 h/d, 5 d/w, 8 w EtOH in water: 8 w	Adult rats	ABR	TOL: Hearing loss at 6-20 kHz. EtOH: No hearing loss. TOL+EtOH: Less severe hearing loss (antagonism), increased EtOH consumption.	(288)
1 750 TOL+ 4g/kg bw EtOH	No N	Inhalation: 6 h/d, 5 d/w, 4 w EtOH by gavage: 1 time/d, 4 w	Adult rats	ABR ME	TOL: Hearing loss at 8-16 kHz and OHC loss. EtOH: No hearing loss and no OHC loss. TOL+EtOH: Potentiation of the effects of TOL (OHC loss) by EtOH. EtOH altered TOL metabolism.	(50)
Toluene and drugs						
1 000 TOL+ 100 mg ASA/kg bw	No N	Inhalation: 16 h/d, 10 d ASA by gavage: 1 time/d, 10 d	Adult rats	ABR	TOL: Hearing loss at 12.5-20 kHz. ASA: No hearing loss. TOL+ASA: Potentiation of effects of TOL by ASA.	(182)

ABR: auditory brainstem response, ASA: acetyl salicylic acid, BA: behavioural audiometry, CAP: cochlear action potential, CAR: conditioned avoidance response, CBZ: chlorobenzene, CO: carbon monoxide, DPOAE: distortion product otoacoustic emissions, EBZ: ethylbenzene, EtOH: ethanol, HEX: hexane, Leq8h: equivalent level of noise during 8 hours, LOAEL: lowest observed adverse effect level, ME: morphological examination, N: noise, NOAEL: no observed adverse effect level, OBN: octave band noise, OHC: outer hair cell, SPL: sound pressure level, TCE: trichloroethylene, TOL: toluene, WBN: wide band noise.

Similar results were reported by Vrca *et al*. Printing workers exposed to low concentrations of toluene for an average of 21 years were compared to controls. The current exposure to toluene in both groups was evaluated by measuring the concentration of toluene in peripheral blood and the concentrations of hippuric acid and *ortho*-cresol (biomarkers of toluene exposure) in urine. The mean value for toluene in blood was 36 µg/l (SD 25, range 0.2-94) for the exposed and 0.96 (SD 3.7, range 0.0-19) for the controls (384). This corresponds to approximately 34 ppm toluene in the air for the printers (160). Decrease in amplitudes, prolongation of latencies and increased interpeak intervals of the auditory brainstem responses were shown after chronic exposure to these low concentrations of toluene (384).

In a cross-sectional study, workers from rotogravure printing and paint manufacturing industries had their hearing function tested by pure-tone audiometry, impedance audiometry and stapedius reflex testing (reflex threshold and decay) (254). Hearing loss was compared among workers exposed to both noise (88-98 dBA) and toluene (75-365 ppm), workers exposed solely to noise (88-97 dBA), or solely to a mixture of solvents in which toluene was the major component, and workers exposed to neither. Compared to the unexposed control group, the adjusted relative risks for hearing loss were 4.1 (95 % CI 1.4-12.2) for the noise group, 5.0 (95 % CI 1.5-17.5) for the solvent-mixture group, and 10.9 (95 % CI 4.1-28.9) for the combined noise and toluene group. The results from the acoustic reflex measurements suggested a predominantly non-cochlear site of the damage, since the percentage of workers with acoustic reflex decay was largest in the group exposed to noise and toluene. Also, the decay was largest after contralateral stimulation, suggesting a lower brainstem disorder. A peripheral component of the observed hearing loss was, however, not excluded (254).

Another study involved 124 rotogravure printing workers exposed to various levels of noise and an organic solvent mixture of toluene, ethyl acetate and ethanol (257). Toluene exposures were below 50 ppm for 109 of the 124 studied workers. Forty-nine per cent of the workers exhibited hearing loss. Among the numerous variables that were analysed for their contribution to the development of hearing loss, only age and hippuric acid met the significance level criterion in the final multiple logistic regression model. The OR estimates for hearing loss were 1.07 for each increment of 1 year of age (95 % CI 1.03-1.11, $p = 0.0003$) and 1.76 for each g hippuric acid/g creatinine (95 % CI 1.00-2.98, $p = 0.0338$). Since low hippuric acid levels were observed in the majority of the studied group with no or little occupational exposure to toluene, this marker provided reliable information on occupational exposure. At the hippuric acid limit level of 1.6 g/g creatinine recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) (7), the OR for hearing loss can be calculated to 2.8 (OR 1.76 × 1.6 g hippuric acid/g creatinine). Moreover, results of the acoustic reflex decay test suggested that there might be retrocochlear or central auditory pathway involvement in some of the hearing disorders observed. The workers in this study had a

relatively short history of noise exposure and had presumably not been exposed long enough for noise effects to develop (257).

The ototoxicity of occupational exposure to toluene below 50 ppm was investigated in a longitudinal study over 5 years with four repeated examinations of workers from rotogravure printing plants (342). Past life-time weighted average exposures to toluene and noise were determined from individual work histories and were 10 ppm in the low-exposure group and 45 ppm in the high-exposure group, with noise levels of 82 dBA in both groups. Recent averaged individual exposure was 3 ppm in the low-exposure group and 26 ppm in the high-exposure group with noise levels of 82 and 81 dBA, respectively. Repeated measurement analyses (grouping factors: toluene intensity, exposure duration and noise intensity) and logistic regressions did not reveal significant effects of toluene level, exposure duration or interactions between toluene and noise. Hippuric acid levels elevated the OR for high frequency hearing loss to 1.28 (95 % CI 0.75-2.18) but this association was not significant. Stratification according to noise intensity alone (79 ± 3 versus 84 ± 1 dBA) was significantly associated with increased auditory thresholds. The authors of this study suggested that the threshold level for developing a hearing loss as a result of occupational toluene exposure might be above 50 ppm (342).

Chang *et al* investigated hearing loss among workers exposed to both toluene and noise in an adhesive material manufacturing plant (57). Fifty-eight workers were exposed to both toluene and noise (79-87 dBA) and were compared to 58 workers exposed to noise only (83-90 dBA) and to a control group of 58 persons (68-73 dBA). Seven breathing-zone air samples were collected in each of the three divisions with toluene exposure with average levels of 33, 108 and 165 ppm and corresponding average noise levels of 83, 84 and 84 dBA. The toluene levels were also given as a cumulative exposure index in year-ppm, calculated as the average toluene level in each division multiplied by the years of employment. The prevalence of hearing loss (≥ 25 dB) was higher in the toluene plus noise group (86 %) than in the noise-only group (45 %) or the controls (5 %). Multivariate logistic regression analysis to evaluate dose-response effects based on cumulative exposure index showed that the OR for hearing loss was 6 times higher for the toluene plus noise group compared to the noise alone group (57).

In a cross-sectional study conducted in the same printing company as by Morata *et al* (254), Bernardi examined 140 workers (31). Participants were subdivided in controls and exposed groups according to exposures to noise alone or combined with toluene. Participants' age ranged from 18 to 48 years and to be included in the study, participants had to have normal hearing (all pure-tone audiometric thresholds < 25 dBHL, type A tympanogram and presence of acoustic reflexes). TEOAE testing and their suppression by continuous contralateral white noise were used to evaluate the effects of toluene exposure. Absence of TEOAE response was slightly higher in the group exposed to both toluene and noise (64 %) compared to the noise alone group (62 %), which both by far exceeded the non-exposed group (27 %). The absence of the contralateral suppression of the TEOAE was much

more prevalent among the workers exposed to both agents (49 %) than in the noise exposed (17 %) or non-exposed group (7 %). The OR for absence of contralateral suppression was estimated to be 12 (95 % CI 3.1-43.5) compared to non-exposed workers (31). These results suggest a retrocochlear effect of toluene.

7.2.3.3 Toluene combined with other agents

Several of the occupational studies investigating the auditory effects of solvents were performed in populations simultaneously exposed to many different solvents in their workplaces. In some of these studies, toluene was one of the main components. The effects of mixed solvent exposures are reported in Section 7.8 and in Table 20.

7.2.4 Conclusion on toluene

The ototoxic effects of toluene in animals have been clearly demonstrated in rats. Toluene exposure caused temporary disruption of auditory functions in guinea pigs (244) and hearing loss in a strain of mice genetically predisposed for hearing loss (218).

In adult rats, the NOAEL for toluene is 700 ppm (311) and 500 ppm when combined with noise (39, 234). The LOAEL is 1 000 ppm both with and without noise exposure (39, 46, 182, 185, 186, 229, 311). In *weanling* rats, the NOAEL for toluene alone is 900 ppm (308). Synergistic interaction between toluene and noise was manifested at the same concentration as the LOAEL for toluene alone, i.e. at and above 1 000 ppm (39, 186).

In some occupational studies, ototoxic effects from toluene were associated with current exposure levels of approximately 10-50 ppm (31, 257, 384). Historic toluene and/or noise exposure levels were not well characterised and some groups of workers were co-exposed to other solvents. It is likely that participants were exposed to higher concentrations in the past, as well as to peaks of high exposure in the present that could explain the observed effects. In one study, however, chronic exposure (12-14 years) to an average of 97 ppm caused hearing loss but noise levels were not given (2). No auditory effects were observed in a study in which both current and historic levels of toluene (up to 50 ppm) and noise were low (342).

International 8-hour OELs for toluene vary from 20 to 200 ppm (Appendix 1). The ACGIH threshold limit value is set to protect from subclinical changes in blue-yellow colour vision and the potential for spontaneous abortion in female workers (6).

Table 8. Occupational studies on auditory effects of toluene (TOL) exposure, tested with pure-tone audiometry, unless otherwise stated.

TOL level (ppm) and exposure duration, mean ± SD, range	Noise (N)	No. of exposed and controls	Results and comments	Reference
Current exposure: 26 ± 20 (high) 3 ± 3 (low) Life-time weighted average: 45 ± 17 (high) 10 ± 7 (low)	 81 ± 4 dBA 82 ± 4 dBA 82 ± 7 dBA 82 ± 4 dBA	 106 high TOL 86 low TOL	Repeated measures during 5 years. No association between hearing loss and TOL exposure levels or duration. NOAEL estimated by the authors to 50 ppm.	(342)
Current exposure: 9-37 Duration: 12 (2-24) yrs (TOL+N) 6 (3-15) yrs (N)	88-98 dBA, (TOL+N and N)	50 TOL+N 50 N 40 controls	Workers without signs of hearing loss selected. > 60 % of TOL+N and of N had no response in TEOAE compared to 27 % of controls. 49 % of TOL+N had no contralateral inhibition in TEOAE compared to 17 % of N and 7 % of controls. OR for absence of contralateral inhibition: 12 (95 % CI 3.1-43.5). The results suggest a retrocochlear effect of TOL.	(31)
Current exposure: ~ 34 (range 2-89), estimated from TOL in blood (160) Duration: 21.4 (4-30) yrs	Not given	49 TOL 59 controls	Decrease in amplitudes, prolongation of latencies and increased interpeak intervals of the ABR.	(384)
Current exposure: 0.04-244 (range) ≤ 50 for 109 of 124 workers Duration: 7.7 (1-25) yrs	71-93 dBA	124 TOL (in mixture)+N Mixture incl. ethyl acetate and ethanol)	49 % of the workers had hearing loss. Age and urinary hippuric acid (biological marker for TOL) were significantly correlated to the hearing loss.	(257)

Table 8. Occupational studies on auditory effects of toluene (TOL) exposure, tested with pure-tone audiometry, unless otherwise stated.

TOL level (ppm) and exposure duration, mean ± SD, range	Noise (N)	No. of exposed and controls	Results and comments	Reference
97 (average measured for 12-14 yrs while employed)	Not given	40 TOL 40 controls	Workers without signs of hearing loss chosen from 300. Effects of ABR showed alteration in the responses for all waves. Sign of affected auditory system before manifested hearing loss.	(2)
Current exposure: 10-70 (range) (TOL in mixture) 75-365 (range of means) (TOL+N) Historic exposure: 140-600 (range of means) (TOL+N). Peak at 1 860 ppm Duration: 5.6 ± 3.7 yrs (TOL) 8.1 ± 6.2 yrs (TOL+N)	< 85 dBA (TOL in mixture) 88-98 dBA (TOL+N) 88-97 dBA (N)	39 TOL (in mixture incl. XYL) 51 TOL+N 50 N 50 controls	Increased risk of hearing loss in exposed: N: RR 4.1 (95 % CI 1.4-12.2). TOL+N: RR 10.9 (95 % CI 4.1-28.9). TOL (in mixture): RR 5.0 (95 % CI 1.5-17.5).	(254)
Current exposure: 33, 108 and 165 (TOL+N, 3 areas) Duration: 12.3 ± 8.8 yrs	79-87 dBA (TOL+N) 83-90 dBA (N)	58 TOL+N (all 3 areas) 58 N 58 controls	Prevalence of hearing loss (≥ 25 dB): 86 % in TOL+N, 45 % in N and 5 % in controls. Similar prevalences in the 3 different areas. Multivariate logistic regression based on CEI showed that TOL+N had a 6 times higher estimated risk of ≥ 25 dB hearing loss than N exposed. No dose-response relationship.	(57)

ABR: auditory brainstem response, CEI: cumulative exposure index, CI: confidence interval, N: noise, NOAEL: no observed adverse effect level, OR: odds ratio, RR: relative risk, SD: standard deviation, TEOAE: transient evoked otoacoustic emissions, TOL: toluene, XYL: xylene.

7.3 Xylenes

7.3.1. General

Xylene (or dimethylbenzene) exists in three isomeric forms: *ortho*, *meta* and *para*. At room temperature, the xylenes are colourless liquids with an aromatic odour. After inhalation exposure the retention in the lungs is about 60 % of the inhaled dose. More than 90 % is biotransformed to methylhippuric acid, which is excreted in urine (173).

Xylene is produced from coal tar or by the aromatisation of petroleum hydrocarbons. Xylene is marketed principally as a mixture of the *o*-, *m*- and *p*-isomers, generally denoted "mixed xylenes". The individual isomers are also available commercially. Most commercial mixed xylenes also contain ethylbenzene (170, 173, 192).

The main use of xylene is as a constituent in motor and aviation fuel but it is also used as a solvent in the paint, printing, rubber and leather industries (173, 279).

7.3.2 Effects in animals

7.3.2.1 Xylene alone

Several studies have investigated the effects of xylene on the auditory system in rats (Table 9). In the earlier studies, rats were exposed to mixed xylenes containing all three isomers in different often unstated proportions. Mixed xylene (10 % *o*-, 80 % *m*- and 10 % *p*-xylene, ethylbenzene content not mentioned) affected the auditory sensitivity after exposure to 1 450 ppm (8 hours/day for 3 days) or 800 ppm (14 hours/day, 7 days/week for 6 weeks), as shown by behavioural audiometry and/or auditory brainstem response (306). Xylene mainly affected the mid-frequency range (8-24 kHz) of the cochlea (78). Gagnaire *et al* showed that outer hair cells were the primary targets within the organ of Corti and that only one of the isomers, *p*-xylene, was ototoxic (see Chapter 5). Thus, loss of outer hair cells was seen after exposure to 900 ppm *p*-xylene (6 hours/day, 6 days/week for 13 weeks), while no effects on the auditory system was found after exposure to up to 1 800 ppm of either *o*-xylene or *m*-xylene. At the lower level of 450 ppm *p*-xylene, hair cells were unaffected (140). Later, Manguin *et al* confirmed that only *p*-xylene was ototoxic in rats after exposure to 1 800 ppm (6 hours/day, 5 days/week for 3 weeks) (238).

7.3.2.2 Xylene combined with noise

No studies were identified.

7.3.2.3 Xylene combined with other agents

In studies by Rebert *et al*, combined exposure to iso-effective concentrations of mixed xylenes and chlorobenzene, and mixed xylenes and trichloroethylene caused additive effects on the diminished amplitudes of auditory brainstem responses in rats (Table 10) (328).

A recent study on rats using different mixed xylenes with a known content of the three isomers as well as of ethylbenzene showed loss of outer hair cells after exposure to 250 ppm of the mixture (≈ 50 ppm *p*-xylene and 50 ppm ethylbenzene) and increased auditory brainstem response thresholds at 1 000 ppm of the mixture (all exposures 6 hours/day, 5 days/week for 13 weeks) (141). The combined exposure caused enhanced ototoxicity compared to exposure to ethylbenzene alone (Section 7.4.2.2).

7.3.3 Observations in man

7.3.3.1 Xylene alone or combined with noise

No studies were identified.

7.3.3.2 Xylene combined with other agents

There are some studies in humans where xylene is one of several solvents. The effects of the mixed solvent exposures are reported in Section 7.8 (Table 20).

7.3.4 Conclusion on xylenes

No effects on the auditory system have been found in rats after exposure to *o*- or *m*-xylene only. Only *p*-xylene has been found ototoxic (140, 141, 238).

In rat inhalation studies, exposure to 450 ppm pure *p-xylene* for 13 weeks caused no auditory effects (NOAEL) whereas exposure to 900 ppm caused outer hair cell loss (LOAEL) (140).

Auditory effects were observed after exposure to 800 ppm *mixed xylenes* for 6 weeks (lowest dose tested) (306) and after exposure for 13 weeks to 250 ppm of a *mixture* (LOAEL, lowest dose tested) containing approximately 50 ppm *p*-xylene but also 50 ppm ethylbenzene (141). The combined exposure caused enhanced ototoxicity compared to exposure to ethylbenzene alone. Interaction with noise has not been studied.

No human studies of auditory effects of xylenes alone or in combination with noise were identified.

International 8-hour OELs for xylene vary from 25 to 100 ppm (Appendix 1). According to ACGIH, the suggested threshold limit value is primarily set to prevent irritation and acute as well as chronic effects on the central nervous system (4).

Table 9. Auditory effects in rats exposed to xylene (XYL) alone.

Exposure conditions		Auditory	Results and comments	Reference
XYL level (ppm)	Regimen	test		
Single XYL isomers				
0, 450, 900, 1 800 *o*-, *m*- or *p*-XYL	Inhalation: 6 h/d, 6 d/w, 13 w	ABR ME	*p*-XYL: OHC loss at ≥ 900 ppm (LOAEL) and increased auditory thresholds at 2, 4, 8 and 16 kHz at 1 800 ppm. NOAEL at 450 ppm. *o*-XYL and *m*-XYL: No effect on OHC loss or ABR.	(140)
0, 1 800 *o*-, *m*- or *p*-XYL	Inhalation: 6 h/d, 5 d/w, 3 w	ABR ME	*p*-XYL: Elevated auditory thresholds at all frequencies (2-32 kHz) and OHC loss: row 3 > row 2 > row 1. *o*-XYL and *m*-XYL: No effect on OHC loss or ABR.	(238)
900 mg/kg bw *o*-, *m*- or *p*-XYL (8.47 mmol/kg bw)	Gavage: 5 d/w, 2 w	ME	*p*-XYL: OHC loss in mid-frequency region of the cochlea: row 3 > row 2 > row 1. *o*-XYL and *m*-XYL: No effect	(139)
Mixed XYL				
0, 800, 1 000, 1 200 [a] 1 450 [a] 1 700 [a]	Inhalation: 14 h/d, 7 d/w, 6 w 8 h/d, 1 d or 3 d 4 h	CAR BA ABR	All repeated exposures (800-1 200 ppm, 6 w or 1 450 ppm, 3 days) caused elevated BA and/or ABR thresholds (LOAEL at 800 ppm). No effects after single exposures.	(306)
0, 1 000 [b]	Inhalation: 18 h/d, 7 d/w, 61 d	ABR	No effect on non-frequency specific click ABR.	(286)
0, 1 700, 2 000 [b]	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	No effect at 1 700 ppm. Diminished amplitudes at 2 000 ppm.	(328)
0, 1 800 [b]	Inhalation: 8 h/d, 5 d	RMA	Elevated RMA thresholds for the mid-frequency tones (8, 16 and 24 kHz).	(78)

[a] 10 % *o*-, 80 % *m*- and 10 % *p*-XYL.
[b] Composition not given.
ABR: auditory brainstem response, BA: behavioural audiometry, bw: body weight, CAR: conditioned avoidance response, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, RMA: reflex modification audiometry, XYL: xylene; *m*-: *meta*-, *o*-: *ortho*- and *p*-: *para*-.

Table 10. Auditory effects in rats of combined exposure to xylene (XYL) and other solvents, in order from lowest to highest exposure.

Exposure conditions		Auditory test	Result and comments	Reference
Level (ppm)	Regimen			
0, 250, 500, 1 000 or 2 000 mixed XYLs type 1 (≈ 0, 50, 100, 200 or 400 each of *p*-XYL and EBZ)	Inhalation: 6 h/d, 5 d/w, 13 w	ABR ME	OHC loss at 250 ppm (LOAEL). Elevated auditory thresholds at 2, 4, 8 and 16 kHz at 1 000 ppm.	(141)
0, 250, 500, 1 000 or 2 000 mixed XYLs type 2 (≈ 0, 25, 50, 100 or 200 each of *p*-XYL and EBZ)	Inhalation: 6 h/d, 5 d/w, 13 w	ABR ME	OHC loss in all rats at 1 000 ppm. Some rats in each group showed non-significant OHC losses also at 250 and 500 ppm, thus no NOAEL could be established. Elevated auditory thresholds at 2, 4, 8 and 16 kHz at 2 000 ppm.	(141)
Mixed XYL+*n*-HEX 1 000 + 1 000	Inhalation: 18 h/d, 7 d/w, 61 d	ABR	*n*-HEX potentiated the hearing loss caused by XYL.	(286)
Mixed XYL+CBZ iso-effective conc.: 1 700 + 0 1 275 + 600 850 + 1 200 425 + 1 800 0 + 2 400	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Additive effects on the diminished amplitudes of ABR.	(328)
Mixed XYL+TCE iso-effective conc.: 2 000 + 0 1 500 + 700 1 000 + 1 400 500 + 2 100 0 + 2 800	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Additive effects on the diminished amplitudes of ABR.	(328)

ABR: auditory brainstem response, CBZ: chlorobenzene, EBZ: ethylbenzene, HEX: hexane, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, TCE: trichloroethylene, XYL: xylene; *p*-: *para*-.

7.4 Ethylbenzene

7.4.1 General

Ethylbenzene is a colourless liquid aromatic solvent produced from benzene and ethylene. It is mainly used as a raw material in the production of styrene. It is also a component of technical xylene, which usually contains approximately 20 % ethylbenzene. The estimated yearly production in the western world is about 8-10 million tonnes (172)

Exposure to pure ethylbenzene is unusual in the work environment. As part of mixed xylenes, ethylbenzene is often one of many solvents in solvent mixtures, e.g. in paints and lacquers and in the rubber and chemical manufacturing industries (97, 172).

7.4.2 Effects in animals

7.4.2.1 Ethylbenzene alone

The ototoxic effect of ethylbenzene was investigated in a series of studies by Cappaert *et al* (51-54). Dose-dependent loss of outer hair cells (300-550 ppm) as well as increased hearing thresholds (400 and 550 ppm) were observed in rats exposed 8 hours/day for 5 days (51, 52, 54). Guinea pigs exposed to 2 500 ppm (6 hours/day for 5 days) did not display signs of ototoxicity. In another experiment, measurements of ethylbenzene blood levels after exposure to 500 ppm, 8 hours/day for 3 days showed that the guinea pigs had much lower levels than the rats (2.8 ± 0.1 µg/ml and 23.2 ± 0.8 µg/ml, respectively) (54).

Recently, Gagniere *et al* showed ototoxic effects in rats at all ethylbenzene exposure levels (200-800 ppm, 6 hours/day, 5 days/week for 13 weeks) (141).

In a comparison study of 21 different solvents, ethylbenzene (gavage for 2 weeks) was shown to be one of the most potent ototoxic solvent as measured by outer hair cell loss in rats (Table 11) (139).

7.4.2.2 Ethylbenzene combined with noise or other agents

Combined exposure (8 hours/day for 5 days) to 300 or 400 ppm ethylbenzene and 105 dB broadband noise induced synergistic effects on outer hair cell loss. Exposure to 95 dB noise caused no effect on hearing neither alone nor in combination with the solvent (52).

The ototoxic effect of two different xylene mixtures with controlled amounts of the xylene isomers as well as of ethylbenzene was investigated. Rats exposed to 250 ppm (6 hours/day, 5 days/week for 13 weeks) of the mixture containing 50 ppm ethylbenzene and 50 ppm *p*-xylene exhibited loss of outer hair cells (Table 12) (141).

Synergistic interaction was seen in rats exposed to 400 ppm toluene and 660 ppm ethylbenzene together with 93 dB noise (6 hours/day for 10 days) (133).

7.4.3 Observations in man

No studies on ethylbenzene alone or combined with noise were identified.

In several occupational studies, workers exposed to ethylbenzene were exposed also to other solvents. The effects of the mixed solvent exposures are reported in Section 7.8 (Table 20).

7.4.4 Conclusion on ethylbenzene

Ethylbenzene has proven ototoxic in rats (51-54, 139, 141) but not in guinea pigs (54). No NOAEL has been identified for rats. In rat inhalation studies, 200 ppm ethylbenzene (13 weeks, lowest level tested) caused outer hair cell loss (LOAEL) and 400 ppm caused hearing loss (51, 141).

In combination with noise (105 dB SPL), exposure for 5 days to 300 ppm ethylbenzene (lowest dose tested) induced synergistic effects on outer hair cell loss (LOAEL) (52). Exposure to 250 ppm of a commercial xylene mixture (13 weeks, lowest level tested) containing approximately 50 ppm ethylbenzene but also 50 ppm *p*-xylene induced outer hair cell loss (LOAEL) (141).

No human studies on the auditory effects of ethylbenzene alone or combined with noise were identified.

International 8-hour OELs for ethylbenzene vary from 5 to 100 ppm (Appendix 1). The ACGIH threshold limit value is set to minimise the potential risks of disagreeable irritations. Other main concerns are effects on the central nervous system, liver and kidneys. Ethylbenzene is classified as an animal carcinogen (5).

7.5 Chlorobenzene

Chlorobenzene is a colourless liquid with a mild aromatic odour. It is used primarily as a degreasing solvent, as a chemical intermediate in the synthesis of nitrochlorobenzenes, in the dry cleaning industry and in manufacture of resins, dyes, perfumes and pesticides (100).

The only animal study of chlorobenzene showed that 2 000 (LOAEL) and 2 400 ppm (8 hours/day for 5 days) was ototoxic in rats. Interaction with noise was not studied. Combined exposure to iso-effective concentrations of chlorobenzene and toluene or chlorobenzene and mixed xylenes caused additive effects on the diminished amplitudes of auditory brainstem responses in rats (Table 13) (328).

No human studies on the auditory effects of chlorobenzene were identified.

International 8-hour OELs for chlorobenzene vary from 1 to 75 ppm (Appendix 1). According to the EU Scientific Committee on Occupational Exposure Limits (SCOEL), the main concern is haematological, liver and kidney effects (100).

Table 11. Auditory effects in animals exposed to ethylbenzene (EBZ) alone, in order from lowest to highest exposure.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
EBZ level (ppm)	Regimen				
0, 200, 400, 600, 800	Inhalation: 6 h/d, 5 d/w, 13 w	Rats	ABR ME	Significant OHC loss at 200 ppm (LOAEL). Complete OHC loss at ≥ 400 ppm. Increased auditory thresholds at 2, 4, 8 and 16 kHz after 4 w to ≥ 400 ppm.	(141)
0, 300, 400	Inhalation: 8 h/d, 5 d	Rats	CAP DPOAE ME	Minor OHC loss at 300 ppm. OHC loss and increased CAP thresholds in mid-frequency regions at 400 ppm. No effect on CAP or DPOAE amplitude.	(52)
0, 300, 400, 550	Inhalation: 8 h/d, 5 d	Rats	CAP DPOAE ME	Minor OHC loss at 300 ppm. OHC loss and increased CAP thresholds in mid-frequency regions at ≥ 400 ppm. Lowered DPOAE amplitude growth at 550 ppm.	(51)
	Inhalation:				(54)
0, 550	8 h/d, 5 d	Rats	CAP	Increased hearing threshold and OHC loss in rats.	
0, 2 500	6 h/d, 5 d	Guinea pigs	ME	No effects in guinea pigs.	
0, 500	8 h/d, 3 d	Both species		Difference in susceptibility between species may be due to different EBZ blood concentrations.	
0, 800	Inhalation: 8 h/d, 5 d	Rats	RMA CAP ME	Increased RMA and CAP thresholds at all frequencies (1-24 kHz). OHC loss in mid-frequency regions.	(53)
900 mg/kg bw (8.47 mmol/kg bw)	Gavage: 5 d/w, 2 w	Rats	ME	Almost total OHC loss in the whole frequency region of the cochlea in all rows of OHCs. Small loss of inner hair cells.	(139)

ABR: auditory brainstem response, bw: body weight, CAP: compound action potentials, DPOAE: distortion product otoacoustic emissions, EBZ: ethylbenzene, LOAEL: lowest observed adverse effect level, ME: morphological examination, OHC: outer hair cell, RMA: reflex modification audiometry.

Table 12. Auditory effects in rats of combined exposure to ethylbenzene (EBZ) and other agents.

Exposure conditions			Auditory test	Results and comments	Reference
Level (ppm)	Noise (N)	Regimen			
0, 300, 400 EBZ	95 or 105 dB SPL broad-band 1.5-12.5 kHz	Inhalation: 8 h/d, 5 d N: 8 h/d, 5 d	CAP DPOAE ME	N: No effects at 95 dB. Effects on CAP and DPOAE and minor OHC loss at 105 dB. EBZ: OHC loss (minor at 300 ppm). EBZ+95 dB N: Same OHC loss as EBZ alone. EBZ+105 dB N: Interaction, i.e. greater OHC loss than sum of OHC losses by N and EBZ alone. Similar effect on DPOEA and CAP as by N. LOAEL at 300 ppm with 105 dB N.	(52)
660 EBZ + 400 TOL	95 or 93 dB OBN at 8 kHz	Inhalation: 6 h/d, 5 d or 10 d N: 6 h/d, 5 d (95 dB) 6 h/d, 10 d (93 dB)	CAP DPOAE ME	N: Small reversible effects on DPOAE, no effect on CAP and small OHC loss. TOL+EBZ: No effect on DPOAE and CAP, no OHC loss. TOL+EBZ+either N: Permanent effects on DPOAE, significant effect on CAP and larger OHC loss than by N. Interaction at the combined exposure of both solvents and N.	(133)
0, 250, 500, 1 000, 2 000 mixed XYLs type 1 (≈ 0, 50, 100, 200 or 400 each of *p*-XYL and EBZ)	No N	Inhalation: 6 h/d, 5 d/w, 13 w	ABR ME	OHC loss at 250 ppm (LOAEL). Elevated auditory thresholds at 2, 4, 8 and 16 kHz at 1 000 ppm.	(141)
0, 250, 500, 1 000, 2 000 mixed XYLs type 2 (≈ 0, 25, 50, 100 or 200 each of *p*-XYL and EBZ)	No N	Inhalation: 6 h/d, 5 d/w, 13 w	ABR ME	OHC loss in all rats at 1 000 ppm. Some rats in each group showed non-significant OHC losses also at 250 and 500 ppm. Increased auditory thresholds at 2, 4, 8 and 16 kHz at 2 000 ppm.	(141)

ABR: auditory brainstem response, CAP: compound action potentials, DPOAE: distortion product otoacoustic emissions, EBZ: ethylbenzene, LOAEL: lowest observed adverse effect level, ME: morphological examination, OBN: octave band noise, OHC: outer hair cell, N: noise, RMA: reflex modification audiometry, SPL: sound pressure level, TOL: toluene, XYL: xylene; *p*-: *para*-.

Table 13. Auditory effects in young rats exposed to chlorobenzene (CBZ) alone or combined with other solvents.

Exposure conditions		Auditory test	Results and comments	Reference
Level (ppm)	Regimen			
0, 2 000, 2 400	Inhalation: 8 h/d, 5 d	ABR at 16 Hz only	Diminished amplitudes of ABR at and above 2 000 ppm (LOAEL).	(328)
CBZ+TOL iso-effective conc.: 0 + 2 800 500 + 2 100 1 000 + 1 400 1 500 + 700 2 000 + 0	Inhalation: 8 h/d, 5 d	ABR at 16 Hz only	Additive effects on the diminished amplitudes of ABR.	(328)
CBZ+XYL iso-effective conc.: 0 + 1 700 600 + 1 275 1 200 + 850 1 800 + 425 2 400 + 0	Inhalation: 8 h/d, 5 d	ABR at 16 Hz only	Additive effects on the diminished amplitudes of ABR.	(328)

ABR: auditory brainstem response, CBZ: chlorobenzene, LOAEL: lowest observed adverse effect level, TOL: toluene, XYL: xylene.

7.6 Trichloroethylene

7.6.1 General

Trichloroethylene is a colourless liquid with a boiling point of 89 °C and an odour resembling that of chloroform (197).

The major use of trichloroethylene is as a degreaser in metal cleaning operations and in textile cleaning. It is also used as paint stripper, adhesive solvent, ingredient in paints and varnishes, and in the manufacture of organic chemicals. Occupational exposure is mainly by inhalation but skin uptake of liquid trichloroethylene may also pose a problem (197, 385).

7.6.2 Effects in animals

7.6.2.1 Trichloroethylene alone

The auditory effects of trichloroethylene are shown in Table 14. In an early study, Yamamura *et al* investigated the ototoxic effect of trichloroethylene in guinea pigs. Exposures to high concentrations (≥ 6 000 ppm) caused no effect in this species using electrophysiological measurements (389). However, findings from experiments on rats demonstrated auditory effects after exposure to trichloroethylene at and above 2 000 ppm. All the studies showed that the mid-frequency region of the rats' cochlea was affected after exposure to trichloroethylene (10, 74, 75, 78, 129, 179, 327-329). Crofton and Zhao calculated the benchmark concentrations (estimated as the 95 % lower bound of the concentration that produced a 15-dB increase in threshold) to be 1 418 ppm (4-week exposure data) and 1 707 ppm (13-week exposure data) in their extensive study. The experimental NOAEL was 1 600 ppm and the LOAEL was 2 500 ppm in this study (75).

Fechter *et al* exposed rats to 4 000 ppm trichloroethylene (6 hours/day for 5 days) and examined auditory changes through several tests and histology of the outer hair cells and spiral ganglion cells. Trichloroethylene caused increased hearing thresholds in the mid-frequency region of the cochlea as measured by reflex modification audiometry. Some test results and histopathological changes suggested that trichloroethylene affects the innervation of the cochlea more than it damages the outer hair cells (129).

Albee *et al* exposed rats to lower concentrations of trichloroethylene (250, 800 and 2 500 ppm, 6 hours/day, 5 days/week for 13 weeks) and confirmed a mid-frequency hearing loss by trichloroethylene as measured by auditory brainstem response at 2 500 ppm. The study also showed loss of outer hair cells in the basal turn of the cochlea at the same level (only dose tested). No auditory brainstem response effects were seen at lower exposure levels (250 and 800 ppm) (10).

Rebert *et al* showed that rats exposed to 2 000 ppm trichloroethylene (12 hours/day, 7 days/week for 3 weeks) had diminished amplitudes of auditory brainstem responses indicating a mid-frequency hearing loss whereas another strain exposed to 1 600 ppm for 12 weeks was unaffected (NOAEL) (327). Diminished amplitudes of auditory brainstem responses were also observed at higher concentrations of trichloroethylene for shorter duration (at and above 2 500 ppm, 8 hours/day for 5 days) (328, 329).

7.6.2.2 Trichloroethylene combined with noise or other agents

Muijser *et al* exposed rats to 3 000 ppm trichloroethylene (18 hours/day, 5 days/weeks for 3 weeks), alone or in combination with 95 dB SPL noise (Table 15). Trichloroethylene caused hearing loss at the low and middle frequencies but not at the high frequencies tested. Noise exposure caused hearing loss in the mid-frequency region of the cochlea only. The combined exposure caused greater threshold shifts than the sum of the individual effects at 4 kHz, whereas effects were additive at 8, 16 and 20 kHz (262).

Rebert *et al* exposed rats to iso-effective combinations of trichloroethylene and mixed xylenes, of trichloroethylene and toluene, and of trichloroethylene and styrene (Table 15). The combined exposures caused additive effects on the diminished amplitudes of auditory brainstem responses (328, 329).

7.6.3 Observations in man

7.6.3.1 Trichloroethylene alone and combined with other agents

Individual medical case histories implicate trichloroethylene as a toxicant with possible oto- and vestibulotoxic properties. A 54-year-old male dry cleaner with 10 years of occupational exposure to trichloroethylene had many health complaints and suffered from high frequency ($\geq$4 kHz) sensorineural hearing loss (371). No exposure details were given. The hearing impairment was considered to be caused by damage to the auditory nerve due to trichloroethylene exposure.

Among 40 trichloroethylene exposed workers examined, 26 cases of hearing loss were observed. The hearing loss was bilateral, sensorineural, and affected high frequencies ($\geq$4 kHz). Electronystagmographic investigations indicated lesions of the balance system (366). No details on occupations, noise or trichloroethylene or other solvent exposures were given, but the authors mentioned that the examined groups were often exposed to high concentrations of trichloroethylene (trichloroacetic acid in urine ranging from 40 to 200 mg/l).

There are some more recent studies in humans where trichloroethylene is one of several solvents. The effects of the mixed solvent exposures are reported in Section 7.8 (Table 20).

7.6.4 Conclusion on trichloroethylene

In rat inhalation studies, no auditory effects were observed after exposure to 1 600 ppm trichloroethylene alone (12 or 13 weeks) (NOAEL) (75, 327). Exposure to 2 000 ppm TCE alone for 3 weeks caused hearing loss (LOAEL) (327). The calculated benchmark concentrations causing a 15-dB increase in hearing threshold were 1 418 ppm (4-week exposure data) and 1 707 ppm (13-week exposure data) (75). Combined exposure to 3 000 ppm trichloroethylene (only dose tested) and 95 dB SPL noise caused a synergistic interaction (262).

Available human studies indicate that trichloroethylene may be ototoxic also in humans, but the exposure levels in these studies cannot be assessed (366, 371).

International 8-hour OELs for trichloroethylene vary from 10 to 100 ppm (Appendix 1). The EU SCOEL recommended an OEL based on avoidance of renal toxicity and thereby preventing renal cell cancer (103).

Table 14. Auditory effects in rats exposed to trichloroethylene (TCE) alone, in order from lowest to highest exposure.

Exposure conditions		Auditory test	Results and comments	Reference
TCE level (ppm)	Regimen			
0, 250, 800, 2 500	Inhalation: 6 h/d, 5 d/w 13 w	ABR ME	NOAEL at 800 ppm. Mid-frequency hearing loss (elevated ABR thresholds) and OHC loss in the upper basal turn of the cochlea at 2 500 ppm.	(10)
0, 1 000, 2 000, 4 000	Inhalation: 6 h/d, 5 d	RMA	NOAEL at 2 000 ppm. Elevated RMA thresholds at 8 and 16 kHz (tested between 0.5-40 kHz) at 4 000 ppm. Mid-frequency hearing loss.	(74)
0, 1 500, 3 000	Inhalation: 18 h/d, 5 d/w, 3 w	RMA	NOAEL at 1 500 ppm. Elevated RMA threshold at 20 kHz but not at 5 and 35 kHz at 3 000 ppm. Mid-frequency hearing loss.	(179)
0, 2 000, 3 200 0, 1 600, 3 200	Inhalation: 12 h/d, 7 d/w, 3 w 12 h/d, 7 d/w, 12 w	ABR	NOAEL at 1 600 ppm. Diminished amplitudes of ABR indicated mid-frequency hearing loss at 2 000 ppm (LOAEL) and above after 3 w.	(327)
0, 2 500, 3 000, 3 500	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Diminished amplitudes of ABR at all dose levels.	(329)
0, 2 800	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Diminished amplitudes of ABR.	(328)
0, 3 500	Inhalation: 8 h/d, 5 d	RMA	Elevated RMA thresholds for the mid-frequency tones (8 and 16 kHz).	(78)
0, 4 000, 6 000, 8 000 0, 1 600, 2 400, 3 200 0, 800, 1 600, 2 400, 3 200 0, 800, 1 600, 2 400, 3 200	Inhalation 6 h/d, 5 d/w: 1 d 1 w 4 w 13 w	RMA at 16 kHz only	NOAELs, LOAELs and BMCs [a] (ppm) for elevated RMA thresholds Duration NOAEL LOAEL BMC 1 d: 4 000 6 000 5 223 1w: 2 400 3 200 2 108 4 w: 2 400 3 200 1 418 13 w: 1 600 2 400 1 707	(75)
0, 4 000	Inhalation: 6 h/d, 5 d	RMA, CM CAP, ME	Elevated RMA thresholds in the mid-frequency region. Effects on CAP and damaged and lost spiral ganglion cells but no OHC loss.	(129)

[a] BMC: Benchmark concentration estimated as the 95 % lower bound of the concentration that produced a 15-dB increase in threshold.
ABR: auditory brainstem response, CAP: compound action potential, CM: cochlear microphonics, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, RMA: reflex modification audiometry, TCE: trichloroethylene.

Table 15. Auditory effects in rats of combined exposure to trichloroethylene (TCE) and other agents.

Exposure conditions			Auditory test	Results and comments	Reference
Levels (ppm)	Noise (N)	Regimen			
0, 3 000 TCE	95 dB SPL	Inhalation and N: 18 h/d, 5 d, 3 w	RMA	TCE: Elevated RMA thresholds in the low- and mid-frequency region (4-20 kHz). No effect at high frequencies (24 and 32 kHz). N: Mid-frequency hearing loss (8-20 kHz). TCE+N: Additive effect at 8, 16, 20 kHz, synergism at 4 kHz.	(262)
TCE+mixed XYL iso-effective conc.	No N	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Additive effects, for details see Table 10.	(328)
TCE+TOL iso-effective conc.	No N	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Additive effects, for details see Table 7.	(328)
TCE+STY iso-effective conc.	No N	Inhalation: 8 h/d, 5 d	ABR at 16 kHz only	Additive effects, for details see Table 4.	(329)

ABR: auditory brainstem response, N: noise, RMA: reflex modification audiometry, SPL: sound pressure level, STY: styrene, TCE: trichloroethylene, TOL: toluene, XYL: xylene.

7.7 *n*-Hexane

7.7.1 General

n-Hexane is a saturated aliphatic hydrocarbon (C_6H_{14}), liquid at room temperature with a boiling point of 68 °C. *n*-Hexane is widely used as a solvent in different industrial settings such as the rubber industry in the production of tires, as a solvent in glues for shoe production, as a cleaning solvent for textiles and leather, as a raw material in the production of other chemicals, and as an additive to petrol. It is often used together with other aliphatic solvents, toluene and acetone (201).

n-Hexane is neurotoxic to both the peripheral and the central nervous system. The formation of ketone metabolites is responsible for the dying back polyneuropathy of myelinated axons caused by *n*-hexane exposure in both animals and humans (201, 352).

7.7.2 Effects in animals

7.7.2.1 n-Hexane alone

Rats were exposed to *n*-hexane in seven studies (Table 16). High levels (4 000 ppm, 14 hours/day, 7 days/week for 9 weeks) caused prolonged latencies of the auditory brainstem response, i.e. a neurotoxic effect (305). Reversible neurotoxic effects of *n*-hexane at lower exposure levels (1 000 ppm, 24 hours/day, 5 or 6 days/week for 11 weeks) has been shown as prolongation of the latency of a late component of the auditory brainstem response (163, 322). In another study, a slight but persistent decrease of the amplitude of a late component of the auditory brainstem response at 1 000 ppm was observed (325). Exposure to 1 000 ppm (18 or 21 hours/day, 7 days/week for 28 or 61 days) did not cause any significant effects on non-frequency specific click auditory brainstem response. However, a marked decrease in peripheral nerve conduction velocity (neurotoxic effect) was observed (286, 287).

7.7.2.2 n-Hexane combined with other agents

The auditory effect of *n*-hexane in combination with xylene or toluene was tested (Table 16). Although no significant ototoxic effect was found after exposure to *n*-hexane alone (1 000 ppm for 28 or 61 days), a potentiation of the ototoxic effect caused by toluene (1 000 ppm for 28 days) or xylene (1 000 ppm for 61 days) was found (286, 287). In contrast, Pryor and Rebert did not observe any potentiation of the auditory effect after 9 weeks of combined exposure to *n*-hexane (4 000 ppm) and toluene (1 200 ppm) (305).

Interaction with noise has not been studied.

7.7.3 Observations in man

In a few studies on workers occupationally exposed to *n*-hexane, prolonged interpeak latencies of the auditory brainstem response and P300 waves were observed and interpreted as a sign of central nervous system neurotoxicity (32, 59, 60, 165).

There are some studies in humans in which *n*-hexane is one of several solvents. The effects of the mixed solvent exposures are reported in Section 7.8 (Table 20).

7.7.4 Conclusion on n-hexane

The neurotoxic effect of *n*-hexane was shown in several studies investigating its auditory effects in rats (163, 286, 287, 305, 322, 325). In most of these studies, inhalation exposure to 1 000 ppm *n*-hexane (up to 11 weeks) did not cause significant effects on the auditory brainstem response (non-frequency specific click) (NOAEL) (163, 286, 287, 322). In contrast, one study with the same exposure level (1 000 ppm, 11 weeks) caused prolonged latencies and decreased amplitude of the auditory brainstem response (LOAEL) (325).

Although 1 000 ppm *n*-hexane had only a slight ototoxic effect alone, it potentiated the effects caused by xylene (1 000 ppm) and toluene (1 000 ppm), respectively (286, 287). The ototoxicity of *n*-hexane in combination with noise has not been investigated.

In the human studies, observed auditory effects of *n*-hexane have been interpreted as a sign of its well-known central nervous system neurotoxicity (32, 59, 60, 165). Risk evaluation regarding this outcome is beyond the scope of this document.

International 8-hour OELs for *n*-hexane vary from 20 to 500 ppm (Appendix 1). The ACGIH threshold limit value is set to prevent central nervous system impairment, peripheral neuropathy and eye and mucous membrane irritation (4).

7.8 *n*-Heptane

n-Heptane is another aliphatic hydrocarbon (C_7H_{16}), which resembles *n*-hexane although it is not as widely used in industry. Like *n*-hexane, *n*-heptane is neurotoxic and depresses the central nervous system but it does not cause polyneuropathy (149).

In the only animal study investigating the ototoxicity of *n*-heptane alone, inhalation exposure to a high dose of 4 000 ppm *n*-heptane (6 hours/day, 7 days/week for 28 days) caused hearing loss (reduction in auditory brainstem response amplitudes) in rats (LOAEL). At 800 ppm, no such effects were observed (NOAEL) (Table 16) (344). Interaction with noise or other agents were not studied.

No human studies investigating the ototoxicity of *n*-heptane were identified.

International 8-hour OELs for *n*-heptane vary from 85 to 500 ppm (Appendix 1). The ACGIH threshold limit value for heptanes is based on the narcotic and irritative effects (4).

Table 16. Auditory effects in rats exposed to *n*-hexane (*n*-HEX) or *n*-heptane (*n*-HEP) alone or *n*-HEX combined with other solvents.

Exposure conditions		Auditory test	Results and comments	Reference
Level (ppm)	Regimen			
n-Hexane				
0, 500, 1 000, 1 500	Inhalation: 24 h/d, 5 d/w, 11 w	ABR	Reversible effect on the latency of late ABR components at and above 1 000 ppm.	(322)
0, 1 000	Inhalation: 21 h/d, 7 d/w, 28 d or 18 h/d, 7 d/w, 61 d	ABR	No significant effect on non-frequency specific click ABR (NOAEL).	(286, 287)
0, 1 000	Inhalation: 24 h/d, 5 d/w, 11 w	ABR	Decreased amplitude and increased latency of the 5th component of ABR (LOAEL).	(325)
0, 1 000	Inhalation: 24 h/d, 6 d/w, 11 w	ABR	Reversible effect on the latency of late ABR components.	(163)
0, 2 000	Inhalation: 14 h/d, 7 d/w, 14 w	ABR	Decreased amplitude of the 5th component but no substantial effect on ABR latencies.	(308)
0, 4 000	Inhalation: 14 h/d, 7 d/w, 9 w	CAR ABR	Prolonged ABR latencies.	(305)
Combined exposure				
1 000 *n*-HEX+ 1 000 TOL	Inhalation: 21 h/d, 7 d/w, 28 d	ABR	*n*-HEX potentiated the hearing loss caused by TOL.	(287)
1 000 *n*-HEX+ 1 000 XYL	Inhalation: 18 h/d, 7 d/w, 61 d	ABR	*n*-HEX potentiated the hearing loss caused by XYL.	(286)
4 000 *n*-HEX+ 1 200 TOL	Inhalation: 14 h/d, 7 d/w, 9 w	CAR ABR	No interaction between *n*-HEX and TOL.	(305)
n-Heptane				
0, 800, 4 000	Inhalation: 6 h/d, 7 d/w, 28 d	ABR	NOAEL at 800 ppm. At 4 000 ppm (LOAEL), reduction in ABR amplitudes equivalent to a hearing loss of approximately 10 dB at all tested frequencies.	(344)

ABR: auditory brainstem response, CAR: conditioned avoidance response, HEP: heptane, HEX: hexane, LOAEL: lowest observed adverse effect level, NOAEL: no observed adverse effect level, TOL: toluene, XYL: xylene.

7.7 Carbon disulphide

7.7.1 General

Pure carbon disulphide, CS_2, is a colourless liquid with a pleasant odour resembling that of chloroform. The carbon disulphide as used in most laboratory and industry processes is a colourless to faintly yellow liquid with a strong, disagreeable cabbage-like odour detectable at 0.016-0.42 ppm. It is highly flammable and slightly soluble in water (192).

Since the 1800s, carbon disulphide has been an important industrial chemical because of its ability to dissolve fats, rubbers, phosphorus, sulphur and other elements. Its fat-solving properties also make it indispensable in preparing fats, lacquers and camphor, in refining petroleum jelly and paraffin, and in extracting oil from bones, olives and rags (273).

The most important industrial use of carbon disulphide has been in the manufacture of regenerated cellulose rayon (by the viscose process) and cellophane. Other industrial uses for carbon disulphide include carbon tetrachloride production, the vulcanisation and manufacture of rubber and rubber accessories, the production of resins, xanthates, thiocyanates, plywood adhesives, flotation agents, solvent and spinning-solution applications, conversion and processing of hydrocarbons, petroleum-well cleaning, brightening of precious metals in electroplating, rust removal from metals, and removal and recovery of metals and other elements from waste water and other media (273).

7.7.2 Effects in animals

7.7.2.1 Carbon disulphide alone

Several animal experiments have addressed the effects of carbon disulphide on the auditory system (Table 17). In rats, the ototoxic effect after inhalation exposure to 800 ppm (11 or 15 weeks) or intraperitoneal administration (286-400 mg/kg body weight for 11 weeks) of carbon disulphide has been established using electrophysiological methods (157, 323, 324), while reflex modification audiometry was not affected after inhalation exposure to 500 ppm carbon disulphide (12 weeks) (66).

7.7.2.2 Carbon disulphide combined with noise or other agents

No studies were identified.

7.7.3 Observations in man

7.7.3.1 Carbon disulphide combined with noise

Several occupational studies have been conducted in viscose rayon factories regarding the auditory effects of carbon disulphide (Table 18).

All five studies involved combined exposure with noise. Comparable noise levels ranging from 80 to 92 dBA were reported in the plants. Due to limitations in the exposure histories, none of these studies tested for statistical interaction between noise and solvent. In four of these studies, the prevalence of hearing loss in carbon disulphide exposed workers (observed in pure-tone audiometric tests)

was compared with that of groups with comparable noise levels or unexposed controls. Consistently, not only was the percentage of hearing loss higher among the workers exposed to both agents (carbon disulphide and noise) but hearing losses were also more serious and had an earlier onset than if the only environmental factor had been noise exposure (58, 196, 251, 356). In the remaining study, workers were evaluated using auditory brainstem response and history of exposure in years. Exhaust ventilation apparatus had undergone major improvements 14 years ago. The results were consistent with the pattern of increased interpeak latency observed in experimental animals. Noise levels were not given (158).

7.7.4 Conclusion on carbon disulphide

In rats, inhalation exposure to 500 ppm for 12 weeks did not cause auditory effects (NOAEL), while significant effects were observed at 800 ppm (11 and 15 weeks, LOAEL) as measured by auditory brainstem response (66, 157, 323). Interaction with noise has not been studied.

In humans, age-adjusted ORs for audiometric hearing loss were significantly increased for exposures above 14 ppm in combination with noise in the range 80-91 dBA (58). Auditory effects (evoked potentials) were also observed in workers exposed for at least 20 years (ventilation improved 14 years ago). Current exposure ranged between 3 and 8 ppm. Past or present levels of noise were not reported in this study (158). The workers were weavers, and thus a rather high noise level could be expected.

International 8-hour OELs for carbon disulphide vary from 1 to 20 ppm (Appendix 1). According to the EU SCOEL, the critical effects in humans are neurotoxicity and cardiotoxicity (102).

Table 17. Auditory effects in rats exposed to carbon disulphide (CS_2) alone.

Exposure conditions		Auditory test	Results and comments	Reference
CS_2 level (ppm)	Regimen			
0, 200, 800	Inhalation: 6 h/d, 5 d/w, 15 w	ABR	NOAEL at 200 ppm. Reversible significantly delayed ABR interpeak latencies at 800 ppm (LOAEL).	(157)
0, 400, 800	Inhalation: 7 h/d, 7 d/w, 11 w	Peripheral nerve conduction time, ABR	Effects on peripheral conduction time, in the ABR latencies and in the visual system at 800 ppm (LOAEL). No significant effects at 400 ppm (NOAEL).	(323)
0, 500	Inhalation: 6 h/d, 5 d/w, 12 w	RMA	Auditory thresholds remained stable (NOAEL).	(66)
0, 172, 286, 400 mg/kg bw	Ip. injection: 5 d/w, 11 w	Sensory-evoked potentials, conduction velocity, ABR	NOAEL at 172 mg/kg bw. Prolonged latency of the 5th ABR peak at 286 and 400 mg/kg bw. No effects on conduction velocity in the ventral caudal nerve and latencies of somatosensory evoked potential components.	(324)

ABR: auditory brainstem response, CS_2: carbon disulphide, ip.: intraperitoneal, LOAEL: lowest observed adverse effect level, NOAEL: no observed adverse effect level, RMA: reflex modification audiometry.

Table 18. Occupational studies on auditory effects of combined exposure to carbon disulphide (CS_2) and noise, tested with pure-tone audiometry, unless otherwise stated.

CS_2 level (ppm) and exposure duration, mean or range	Noise (N)	No. of exposed and controls	Results and comments	Reference
Current exposure: 3-8 [a] Duration: > 20 yrs (current workers) 2-7 yrs (current workers) > 10 yrs (former workers)	Not given	34 CS_2 (> 20 yrs) 25 CS_2 (2-7 yrs) 16 CS_2 (> 10 yrs) 40 controls	Increased ABR latencies of waves/intervals V, III-V IPL and I-V IPL in the longest exposed group (> 20 yrs) compared to all other groups. The higher III-V IPL suggested that chronic exposure to CS_2 involves the auditory ascending tract in the brainstem. Latencies in the group with former workers were not different from those of controls.	(158)
Current exposure: 8.5 (3-12) Duration: ~ 20 ± 5 yrs	88-92 dBA (CS_2+N) 86-93 dBA (N)	120 CS_2+N 40 N	High prevalence (98 %, 78/80) of hearing loss with signs of central vestibular syndrome in subjects with diagnosed chronic CS_2 poisoning. Hearing loss of various degrees in 45 % (18/40) of workers free from clinical symptoms of chronic CS_2 poisoning; prevalence significantly higher than that of N only exposed workers.	(196)
Current exposure: 1.6-20.1 Duration: ≥ 20 yrs for 90/131	80-91 dBA (CS_2+N) 83-90 dBA (N) 75-82 dBA (controls)	131 CS_2+N 105 N 110 controls	Higher prevalence of hearing loss in CS_2 exposed workers (68 %) than in controls (24 %) and N exposed workers (32 %). Significantly increased age-adjusted OR for hearing loss for exposures > 14.6 ppm.	(58)
Current exposure: 10-12 Historic exposure: 33-300	84-88 dBA	259 CS_2+N 60 controls (with similar N)	Higher prevalence of sensorineural hearing loss in CS_2+N group (60 %) than in control group with similar N (33 %) as measured with auditory and otoneurological tests.	(356)
Current exposure: 28-30	86-89 dBA	258 CS_2+N	Higher prevalence of hearing loss among workers exposed to CS_2+N (60 % vs. 50 %), with an earlier onset than among those exposed to N (from a Brazilian database of audiometric thresholds of metal workers). Exposure duration associated with the occurrence of hearing loss.	(251)

[a] Ventilation improved 14 years ago.

ABR: auditory brainstem response, CS_2: carbon disulphide, IPL: interpeak latency, N: noise, OR: odds ratio.

7.8 Solvent mixtures

7.8.1 General

This part of the document addresses the most common exposure situation in the workplace, exposure to solvent mixtures of different compositions. In most human studies on solvent ototoxicity, exposure to such mixtures was investigated. Also studies on humans and animals exposed to solvents that are mixtures *per se* such as white spirit and jet fuels are included here.

Exposure estimation in human occupational studies is always difficult and even more complicated when mixed exposures are involved. A method often used to describe the influence of the different solvents on the total exposure load is the hazard index. The exposure hazard index, HI (also called hygienic or additive effect), is calculated according to this formula:

$$HI = C1/T1 + C2/T2 + C3/T3 \ldots$$

C1, C2, C3, etc. are the measured exposure levels of the different solvents and T1, T2, T3, etc. are the individual OELs of the corresponding solvent. If any of the quotas exceeds 1.0, that specific solvent exceeds its OEL. If the hazard index exceeds 1.0, the total exposure load is considered too high.

White spirit of different types, Stoddard solvent and kerosene are all refined petroleum solvents. They are all complex mixtures containing normal and branched paraffins, naphtenes and aromatic hydrocarbons. The contents of these different substances in the mixtures differ and depend on the raw petroleum product as well as the distillation process used (249). All the mentioned substances are colourless to yellowish, oily liquids with a strong, characteristic odour.

The different types of white spirit are characterised by the percentage contents of aliphatic versus aromatic hydrocarbons. White spirits are used as solvents and thinners in paints and lacquers as well as degreasers in industrial processes (249).

Jet fuels are also called aviation kerosene. Jet fuels have different names (such as JP-4, JP-5, JP-7 and JP-8) depending on the composition of the mixture from the distillates. Jet fuels differ due to distillation temperature and additives. The additives determine the specific uses of the fuel. Since jet fuels are often used by the military, the exact components are not known (14, 15).

7.8.2 Effects in animals

Animal studies of solvents that are mixtures *per se* such as white spirits and jet fuels are described in detail in Table 19. Animal studies involving mixtures of specific solvents have been reported earlier under the heading of the different solvents.

7.8.2.1 Mixtures per se alone

Lund *et al* exposed rats to 400 or 800 ppm dearomatised white spirit for 6 months. Central nervous system functions including auditory evoked responses (auditory

brainstem response) were studied. The exposure did not cause any effects on hearing thresholds (236).

In a study of Fechter *et al*, repeated but not single exposure to jet fuel (JP-8) caused auditory impairment in rats. Partial though not complete recovery was observed over a 4-week post-exposure period (for details, see 7.8.2.2) (132).

7.8.2.2 Mixtures per se combined with noise

Fechter *et al* studied the effects of jet fuel (JP-8) exposure with or without noise exposure in rats (Table 19). Exposure to JP-8 alone (1 000 mg/m^3, 4 hours/day for 5 days) caused some effects on outer hair cells as measured by distortion product otoacoustic emissions. The effect was more evident and permanent when JP-8 was followed by noise exposure (97 dB for 4 hours or 102 dB for 1 hour). After a single inhalation exposure as well as after repeated exposures over the course of 5 days, JP-8 exposure increased the susceptibility to noise exposure. In addition, JP-8 markedly depleted glutathione levels in liver tissue (132).

7.8.3 Observations in man

7.8.3.1 Solvents mixtures alone and combined with noise

Several occupational studies have addressed the effects of solvent mixtures on the auditory system, as well as the interaction between solvent mixtures and noise (Table 20). Single solvent studies are described under the respective headings in previous sections.

The central auditory system was investigated in two clinical studies using a variety of tests. Noise exposure levels were not reported but self-estimated data indicated low occupational levels (below 85 dBA). Varney *et al* showed that patients heavily exposed to solvents performed worse than age-matched controls in a dichotic listening test (379). Fuente *et al* used an extensive test battery to evaluate the central auditory system in a study of 10 workers long-term exposed to solvent mixtures in a furniture factory (current hazard index = 0.25). Apart from test of hearing by pure-tone audiometry, the test battery included filtered speech, random gap detection, dichotic listening and the hearing-in-noise test. Differences were detected in the filtered speech tests, random gap detection and dichotic listening but not in the hearing-in-noise test or in the pure-tone audiometry as compared to unexposed controls (137).

The relationship between self-assessed hearing disorders and occupational exposure to solvent mixtures was investigated in a cross-sectional study with 3 284 men. Exposure to solvents for 5 years or more resulted in an adjusted relative risk for hearing impairment of 1.4 in men without occupational exposure to noise. In the group reporting combined occupational exposure to solvents and noise, the effects from noise dominated. The occupational exposure to noise had an effect twice that of solvents. A sub-sample of 51 men was examined with pure-tone audiometry. Twenty of the 21 men who reported abnormal hearing also fulfilled an audiometric criterion for hearing impairment, giving an indication that the self-assessment of hearing loss was valid (178).

Table 19. Auditory effects in rats exposed to white spirit or jet fuel (JP-8) alone or combined with noise.

Exposure conditions			Auditory test	Results	Reference
Level	Noise (N)	Regimen			
0, 400, 800 ppm white spirit (dearomatised)	No N	Inhalation: 6 h/d, 5 d/w, 6 m	ABR	Increased amplitudes of the ABR (not a hearing effect) at 8 and 16 kHz. No effect on latencies or hearing thresholds.	(236)
0, 1 000 mg/m^3 JP-8	97, 102 or 105 dB OBN (8 kHz)	Inhalation: 4 h/d, 1 or 5 d, followed by N: 4 h (105 dB), 1 h/d, 5 d (102 dB) or 4 h/d, 5 d (97 dB)	DPOAE CAP ME	JP-8: No hearing loss after single exposure. Some hearing loss as shown by DPOAE after repeated exposures. JP-8+N: After single exposure, more decreased DPOAE amplitudes and more increased CAP thresholds than by N but OHC loss not different from N. After repeated exposure, worse hearing loss and OHC loss than by N alone.	(132)

ABR: auditory brainstem response, CAP: cochlear action potential, DPOAE: distortion product otoacoustic emissions, JP-8: jet fuel JP-8, ME: morphological examination, N: noise, OBN: octave band noise, OHC: outer hair cell.

Two early reports showed that workers exposed to combinations of solvents and noise might have a worse hearing loss than could be expected from noise exposure alone (23, 30). In a long-term follow-up of hearing tests performed at a paper mill, Bergström and Nyström found that 23 % of the workers in the chemical department, where the noise exposure was lower than in the rest of the paper mill plant, developed a severe hearing loss after 20 years. In other departments, without solvent exposure but with higher noise exposure (95-100 dB), only 8 % of the workers developed a severe hearing loss (30).

Clinical studies were conducted on auditory and vestibular functions of workers exposed to a mixture of unspecified alcohols, jet fuels and aromatic solvents. Noise exposure levels were not reported but because of the nature of the participants' occupations, noise was indicated to be a factor. The findings in pure-tone audiometry and speech discrimination testing were essentially normal for age and noise exposure history, not indicating measurable cochlear damage due to solvent exposure. However, significant abnormalities were found in tests that assessed more central portions of the auditory pathways such as distorted or interrupted speech (272, 294, 397). The central auditory system was also affected after long-term solvent exposure in a group of workers (214) as well as in a group of patients with suspected chronic toxic encephalopathy due to solvent exposure (278). Vestibular dysfunction was seen in 47.5 % and hearing loss in 42 % of 61 solvent exposed workers compared to 5 % (of each effect) in age-matched controls (357).

For a group of workers (n = 39) exposed to a mixture of solvents (mainly toluene and xylene) in a quiet environment, the OR for hearing loss was 5 times greater (95 % CI 1.4-17.5) compared to non-exposed controls (254).

Morata *et al* tested the hearing in solvent-exposed workers from a refinery (n = 438). Participants were exposed to a solvent mixture containing mainly toluene, xylene, cyclohexane and ethylbenzene. The exposures to noise and solvents were at or below the US Occupational Safety and Health Administration (OSHA) exposure limits. Workers were also exposed in their homes, evident from available water and air samples. The prevalence of hearing loss within the exposed groups ranged from 42 to 50 %, significantly exceeding the prevalence for unexposed groups (15-30 %). The adjusted ORs for hearing loss were 2.4 (95 % CI 1.0-5.7) for groups exposed to aromatics and paraffins, 3.0 (95 % CI 1.3-6.9) for the maintenance group and 1.8 (95 % CI 0.6-4.9) for the group from shipping, when compared to unexposed controls (255).

Workers (n = 517) in the paint and lacquer industry in Poland exposed to a mixture of organic solvents with or without concomitant noise exposure were investigated for hearing loss. Average work-life solvent exposures were calculated using the exposure index for the mixed solvents at each workplace multiplied with the duration of years at that workplace. The different products were then added and divided by the total number of exposed years thus creating a "work-life exposure index" (WEI). The group was subdivided according to exposure: 1) solvent mixture (mean WEI 0.6, range 0.3-1.6) with noise exposure above 85 dBA, 2) solvent mixture only (mean WEI 0.8, range 0.3-3.0, noise equal to or below 85

dBA), and 3) non-exposed controls (noise equal to or below 85 dBA). The relative risks for hearing loss for the solvent with noise group and the solvent-only group were significantly increased (2.8, 95 % CI 1.6-4.9 and 2.8, 95 % CI 1.8-4.3) when compared to the control group. In a subgroup of the solvent-only exposed group with noise equal to or below 80 dBA, the relative risk for hearing loss was 4.4 (95 % CI 2.3-8.1) as compared to the noise-matched control subgroup. The mean hearing thresholds at frequencies of 2-4 kHz were poorer for workers exposed to solvents and noise than for the solvent-only group (348, 350).

In a study of dockyard workers in Poland, the effect on hearing of exposure to a mixture of solvents together with noise (n = 517) was compared to a noise exposed group (n = 184) and non-exposed controls (n = 205). The OR for hearing loss in the noise alone group was 3.3 (95 % CI 2.1-5.4) and differed significantly from the group with combined exposure with an OR of 4.9 (95 % CI 3.1-7.7). The main solvent in the mixture was xylene and the current exposure hazard index for the mixture of solvents was 6.3 (0.8-23.2). The current noise levels were 93 dBA (range 85-102 dBA) in the combined exposure group and 90 dBA (range 85-100 dBA) in the noise alone group (349).

Śliwińska-Kowalska *et al* assessed the auditory effects in a large cohort of workers exposed to different solvents and solvents together with noise. In the group exposed to mixtures of solvents (n = 731), the WEI for mixture together with noise (90 dBA) was 4.8 (0.01-9.9), the prevalence of hearing loss was 63 % and the OR 2.4 (95 % CI 1.6-3.7). A group exposed mainly to a mixture of toluene and *n*-hexane (hazard index for mixture 1.6 (0.8-4.5)) and moderate noise levels (79 dBA) had a prevalence of hearing loss of 73 % and an OR of 5.3 (95 % CI 2.6-11). When the workers were divided into subgroups according to the noise exposure and adjusted for age, the OR for the solvent mixtures without noise (equal to and below 85 dBA) was 4.1 and with noise (above 85 dBA) 6.7, whereas in the group exposed to *n*-hexane plus toluene the OR was 8.0 without noise and 20.2 with noise (347).

Pure-tone audiograms were measured in 100 workers in a car painting factory. Half of the workers were exposed to an unspecified mixture of solvents (levels not known) together with moderate noise (81.5-85 dBA) and the other half to noise only, at a similar level. These workers were also compared to a group exposed to high levels of noise (92.5-107 dB). The group exposed to solvent mixtures displayed significantly higher hearing thresholds compared to the moderate noise only group. The hearing loss in the solvent group was of the same magnitude as in the group exposed to high levels of noise (95).

Using parts of a selected test battery (pure-tone audiometry including high frequencies 0.5-16 kHz and dichotic listening test), Fuente *et al* investigated 110 workers exposed to a solvent mixture with toluene and methyl ethyl ketone as the main components but including also other solvents. Samples taken over the past 20 years were available and the geometrical means of toluene in the air were 3.21 ppm (geometrical SD 3.17, range 0.3-26, n = 18) and 4.77 ppm (SD 4.18, range 0.001-131, n = 72) for the groups of workers classified as moderately and highly

exposed, respectively. Workers from the office (n = 20) served as controls. Noise levels the past 10 years ranged from 74 to 84 dBA. Pure-tone audiometry and high-frequency audiometry as well as the dichotic listening test showed significantly worse results in the highly exposed group compared to the control group. The multiple regression analysis showed that outcomes of pure-tone audiometry were best predicted by solvent exposure group and male gender. Outcomes of high-frequency audiometry and dichotic listening test were also predicted by solvent exposure group, but also by some factors such as race and ethnicity (Hispanic) (138).

Bernardi investigated 136 workers of which 90 were exposed to a combination of noise and a mixture of solvents, 24 exposed to noise alone, 10 exposed to *n*-hexane alone (reported under *n*-hexane) and 12 controls. The solvent mixture included among others *n*-hexane and petroleum and the exposure levels were subdivided into high, medium, and low. Auditory function was measured using pure-tone audiometry and long latency auditory evoked potentials. High and medium noise exposure caused a 4-fold increase in the OR for hearing loss (4.06, 95 % CI 1.28-13). A dose-response gradient was detected for the simultaneous exposure to noise and solvents and hearing loss, with a significant association for medium or high exposure to the solvents with noise (OR 9.5, 95 % CI 2-44.5). Also the long latency auditory evoked potential was affected by the solvent exposures (32).

Kaufman *et al* investigated the risk for hearing loss in military personnel (n = 138) exposed to jet fuels and/or noise. The exposure to jet fuels was estimated by measurements and historical records, with levels clearly below the US Air Force Exposure Standards of 700 mg/m^3 for JP-4 and 350 mg/m^3 for JP-8 (calculated cumulative annual JP-4 exposures ranged from 0 to 33 % of this maximum for JP-4 and from 0.5 to 11 % for JP-8). The exposure to noise was estimated through current and historical noise dosimetry measurements. Three years of jet fuel and noise exposure caused a 70 % increase in adjusted OR for hearing loss (1.7, 95 % CI 1.14-2.5). The OR increased to 2.41 (95 % CI 1.04-5.6) for 12 years of fuel and noise exposure (189).

In a study by Kim *et al*, the hearing of 328 workers in aviation industry was investigated. Workers were divided into 4 groups after a classification system, where noise exposure was scored from 0-3 (78-90 dB) and solvent exposure was scored from 0-2 (hazard index 0.046, 0.256, and 0.857). The subjects were classified as exposed to solvents when the cumulative index (exposure index × years) was above 10 (this was equal to e.g. more than 11 years of exposure to low-level solvents or more than 3 years of exposure to high-level solvents). The prevalence of hearing loss was 55 % in the group exposed concomitantly to noise and mixed solvents compared to 28 % in the group exposed to solvent mixtures only, 17 % in the noise-only and 6 % in unexposed controls. The relative risks adjusted for age were estimated to be 8.1 (95 % CI 2-32.5) for the noise and solvents group, 2.6 (95 % CI 0.6-10.3) for the solvents group and 4.3 (95 % CI 1.7-11) for the noise-only group (191).

Prasher *et al* investigated aircraft maintenance workers exposed to solvent mixtures and intermittent noise with an extensive audiological test battery. The group was compared to mill workers exposed to noise alone, to a small group exposed only to solvent mixtures and to unexposed controls. Besides pure-tone audiometry, the tests included acoustic reflex thresholds, transient and distortion product otoacoustic emissions, auditory brainstem responses as well as different vestibular tests. The noise alone group had a higher prevalence of hearing loss as well as decreased otoacoustic emissions. The group exposed to solvents and noise showed significant effects in the tests evaluating the central parts of the auditory system such as auditory brainstem responses and acoustic reflexes. In the group exposed to solvents and noise, 32 % showed abnormal results in the balance tests (301).

Hearing loss development during 5 years was investigated in petrochemical workers exposed long-term to low levels of solvents and different levels of noise. Twenty-six per cent of the workers in the department with solvent exposure and low noise exposure (< 83 dBA) had their hearing thresholds significantly worsened in the 5-year period, 27 % in the department with solvent exposure and moderate noise (≤ 88 dBA) and 36 % in the utility department without solvent exposure but higher noise levels (up to 91 dBA). Of all workers, 29 % had significant poorer thresholds in the year 2002 as compared to 1998. Such percentages can be considered excessive (86).

Prevalence of, and risk factors for, hearing loss in the Canadian armed forces were investigated using current and first time audiograms and a questionnaire. The prevalence of moderate to severe hearing loss progressed with age. In the oldest age group among flight engineers, signal operators and cooks, 15 % or more had a moderate to severe hearing loss of 40 dBHL or more at 4 or 6 kHz. The results from the questionnaire revealed that the poor use of hearing protection was the most frequent explanation for the prevalence of hearing loss but also the solvent exposure was of importance since more than 60 % of the total sample was exposed to solvents at work (3).

A retrospective cohort study examined the relationship between solvent exposure and hearing loss in a cohort of 1 319 (age 35 years or less) aluminium industry workers. Employment, industrial hygiene and audiometric surveillance records allowed for estimation of noise and solvent exposures over the study period. The most common solvent exposures identified through the industrial hygiene records were to xylene, toluene and methyl ethyl ketone. Recorded solvent exposure levels varied widely both within and between jobs. While the highest levels recorded were several times higher than the ACGIH threshold limit values for individual solvents, TWAs over the study period in general were well below. Significant risk factors for hearing loss (3-6 kHz average) included age, reported hunting and shooting, higher baseline hearing threshold and solvent exposure (OR 1.9, 95 % CI 1.2-2.9) (317).

7.8.4 Conclusion on solvent mixtures

There are only a few animal studies regarding ototoxicity of solvent mixtures. In a rat inhalation study, exposure to 800 ppm dearomatised white spirits for 6 months caused no effects on hearing thresholds (NOAEL) (236).

In rats, inhalation exposure for 5 days to 1 000 mg/m^3 JP-8 (only dose tested) caused some effects on outer hair cells (LOAEL). When JP-8 exposure was followed by noise, the effects were more evident and permanent and appeared after 1 day of exposure (132). Thus, JP-8 exposure increased the susceptibility to noise exposure.

In the human studies, it should be noted that the exposure to solvent mixtures varied regarding both levels and composition. Some studies with large study groups reported an association between low to moderate exposure to solvent mixtures and hearing disorders. However, exposure information is often incomplete, and current levels do not necessarily reflect exposure history. Even though solvent exposures were low in some of the studies described, it is possible that past exposure was much higher, and that peak non-trivial exposures (current or past) may have contributed considerably to the hearing losses.

Table 20. Occupational studies on auditory effects of exposure to solvent mixtures (MIX), tested with pure-tone audiometry, unless otherwise stated (in order as appearing in the text).

Exposure description		No. of exposed and controls	Results and comments	Reference
MIX specification and exposure duration	Noise (N)			
TCE, white spirits, XYL, "cleaning solvents" ≥ 100 peak exposures causing symptoms (e.g. dizziness). Levels not given. Duration: 9-15 yrs, not exposed for ≥ 1 yr before study.	Self-estimated data suggest < 85 dB	25 MIX (suspected POS) 25 controls (age- and education-matched)	MIX group had significantly worse scores in dichotic listening tests than controls. No differences in other neuropsychological tests (battery).	(379)
TOL, XYL and *n*-HEX Low levels, current HI 0.25 Duration (mean ± SD): 17.5 ± 10 yrs	Self-estimated data suggest < 85 dB	Furniture workers 10 MIX 10 controls	MIX exposed had normal PTA but significantly poorer results than controls in several tests from a battery of central auditory tests.	(137)
Exposure in various occupations Levels not given	Self-estimated	3 284 subjects	RR 1.4 (95 % CI 1.1-1.9) for self-assessed hearing loss in subjects exposed to solvents ≥ 5 yrs without N.	(178)
Various solvent Levels not given Duration: long-term	Not given, but long-term exposure	4 patients 30 shipyard painters	4 patients with more extended hearing loss than could be expected from N history. ≥ 50 % of shipyard workers had hearing loss. N exposure was long but interactions with MIX exposure not excluded.	(23)
Chemical department at paper mill factory Levels not given	80-90 dB (MIX) 95-100 dB (N)	Paper mill factory 47 MIX 164 N	23 % of the MIX exposed developed severe hearing loss after 20 yrs compared to 8 % in N group.	(30)
Aliphatic and aromatic solvents: No current exposure. Duration: 5-40 yrs Jet fuel: Levels not given. Duration: 15-41 yrs	Not given	23 MIX (confirmed/ suspected POS) 8 jet fuel	> 50 % of the exposed had abnormal speech discrimination, CRA and central vestibular dysfunction. More severe results in POS patients.	(397)

Table 20. Occupational studies on auditory effects of exposure to solvent mixtures (MIX), tested with pure-tone audiometry, unless otherwise stated (in order as appearing in the text).

Exposure description		No. of exposed and controls	Results and comments	Reference
MIX specification and exposure duration	Noise (N)			
White spirits, thinner, TOL and XYL Levels not given Duration: 4-45 yrs	Not given	60 MIX (suspected POS) 18 controls 9 MIX (confirmed POS) 9 controls	Lower scores on distorted speech and longer latencies of the CRA in MIX exposed.	(272, 278)
Exposures from work as colour mixers, painters, printers and petrol truck drivers Levels not given. Duration: 4-25 yrs	Not given	53 MIX (suspected POS)	25 patients had abnormal values in one or more vestibular test. 31 patients showed decreased discrimination scores at the interrupted speech. Normal PTA.	(294)
History of exposure to paints, varnish, glue, gasoline, STY, lead. Levels not given Duration: 1-20 yrs	Not given	33 MIX compared to normal clinical values	Mild to moderate hearing loss in some subjects. Abnormal results in central hearing tests as filtered speech and cognitive responses.	(214)
EBZ, XYL and TMB Current exposure, HI 0.8-1.3 (range 0.4-2.9) Cumulative doses used (current exp × yrs) Duration: long-term	60-75 dBA Leq (current)	61 MIX 40 controls, age-matched	Prevalence of hearing loss: 42 % in MIX compared to 5 % in controls. Vestibular dysfunction: 47.5 % in MIX compared to 5 % in controls.	(357)
TOL ≤ 70 ppm, XYL ≤ 40 ppm Current exposure, HI ≤ 1.53	< 85 dB	39 MIX (painters) 50 controls	OR 5 (95 % CI 1.4-17.5) for hearing loss in painters.	(254)
TOL ≤ 18 ppm, XYL < 5 ppm, EBZ < 1.8 ppm, cycloHEX < 14 ppm Levels below TLVs	< 85 dBA TWA	Oil refinery 438 workers	Hearing loss: OR 2.4 (95 % CI 1.0-5.7) for workers exposed to aromatics and paraffins. OR 3.0 (95 % CI 1.3-6.9) for N exposed from maintenance group. Hearing loss assessed by PTA and immitance audiometry reflexes suggests also central involvement.	(255)
Max work-life average: XYL 25 ppm, TOL 25 ppm, white spirit 90 ppm and EBZ 20 ppm Work-life exposure index 0.3-3.0 Mean duration: 12.5 yrs (range 0.5-39)	> 85 dBA (MIX+N) ≤ 85 dBA (MIX) ≤ 80 dBA (subgroup MIX)	Paint and lacquer 96 MIX+N 207 MIX 214 controls	OR 4.4 (95 % CI 2.3-8.1) for hearing loss for subgroup MIX (N ≤ 80 dBA) compared to N matched controls. Poorer mean hearing thresholds at 2-4 kHz for MIX+N group than for MIX group.	(348, 350)

Table 20. Occupational studies on auditory effects of exposure to solvent mixtures (MIX), tested with pure-tone audiometry, unless otherwise stated (in order as appearing in the text).

Exposure description		No. of exposed and controls	Results and comments	Reference
MIX specification and exposure duration	Noise (N)			
Main solvent XYL, minor TOL, EBZ, white spirit, *n*-butanol Current HI 6.3 (0.8-23.2, 82 % had HI > 1) Work-life HI 66 (0.1-346)	93 dB (MIX+N) 90 dB (N)	Dockyard workers 517 MIX+N 184 N 205 controls	Hearing loss: OR 4.9 (95 % CI 3.1-7.7) for MIX+N group, OR 3.3 (95 % CI 2.1-5.4) for N alone group	(349)
Main component XYL Work-life HI 4.8 (0.01-9.9) HEX+TOL Work-life HI 1.6 (0.8-4.5)	90 dBA (MIX) 79 dBA (HEX+TOL) 78 dBA (controls)	731 MIX 96 HEX+TOL 223 controls	Prevalence of hearing loss: 42 % in control group, 63 % in MIX group (OR 2.4, 95 % CI 1.6-3.7), 73 % in HEX+TOL (OR 5.3, 95 % CI 2.6-11).	(347)
Unspecified Levels not given Duration: 5-15 yrs	81.5-85 dBA (MIX+mod N) 81.5-85 dBA (moderate N) 92.5-107 dBA (high N)	Car painters 50 MIX+ moderate N 50 moderate N 60 high N	MIX+mod N group had significantly higher hearing thresholds than moderate N group. Hearing loss in MIX+mod N the same as in high N.	(95)
TOL, MEK, TCE, CBZ and others Geometric means: 3.2 ppm TOL, 5.8 ppm MEK (moderate) 4.8 ppm TOL, 12.5 ppm MEK (high) Duration: 20 yrs	74-84 dBA all groups (last 10 yrs)	Coated fabrics workers 18 MIX (moderate) 72 MIX (high) 20 controls (low)	MIX (high) group had significantly higher hearing thresholds and worse results in central hearing test compared to the control group. Solvent exposure group predicted hearing loss outcome.	(138)
n-HEX, petroleum and others *n*-HEX alone	≥ 85 dBA (MIX+N)	90 MIX+N 24 N 10 *n*-HEX 12 controls	Auditory function measured by PTA and auditory evoked P300. A dose-response gradient for simultaneous exposure to MIX and N and hearing loss, with a significant association for medium or high exposures to MIX and N, OR 9.5 (95 % CI 2-44.5).	(32)
Jet fuels Levels 34 % of US OSHA standards Cumulative exposures to N and jet fuels used. Duration: at least 3 yrs	≥ 85 dBA at least 3 yrs	Military personnel 138 jet fuel and/or N	Hearing loss after exposure to jet fuel+N: OR 1.7 (95 % CI 1.14-2.5) after 3 yrs of exposure, OR 2.4 (95 % CI 1.04-5.6) after 12 yrs of exposure.	(189)

Table 20. Occupational studies on auditory effects of exposure to solvent mixtures (MIX), tested with pure-tone audiometry, unless otherwise stated (in order as appearing in the text).

Exposure description		No. of exposed and controls	Results and comments	Reference
MIX specification and exposure duration	Noise (N)			
MEK, TOL, XYL, MIBK Low levels, HI < 1 Duration: long-term	≤ 80 dB (MIX) 81-92 dB (MIX+N) 85-101 dB (N) ≤ 80 dB (Controls)	Aviation industry 18 MIX 13 MIX+N 146 N 151 controls	Prevalence of hearing loss: 55 % in MIX+N group (OR 8.1, 95 % CI 2.0-32.5). 28 % in MIX group (OR 2.6, 95 % CI 0.6-10.3) 17 % in N group (OR 4.3, 95 % CI 1.7-11) and 6 % in controls.	(191)
TOL, XYL, jet fuel and others Levels not given	Intermittent > 95 dBA (MIX+N) Constant > 85 dBA (N)	Aviation industry 13 MIX 174 MIX+N 153 N 39 controls	Higher prevalence of hearing loss in N and in MIX+N compared to controls. ABR and acoustic reflexes differentiated between MIX+N and N. 32 % of MIX+N had abnormal balance test results.	(301)
XYL, TOL, benzene, Low levels, 0.05-20 ppm Duration: 5- ≥ 20 yrs	Low N < 83 dBA Moderate N ≤ 88 dBA Higher N ≤ 91 dBA	Petrochemical industry 172 + controls	Prevalence of hearing loss: 26 % in MIX+low N, 27 % in MIX+mod N, and 36 % in high N. 29 % of all workers had significantly worse hearing loss in 2002 compared to 1998.	(86)
Various solvents Levels not given Duration: ≈ 17 yrs to MIX+N	Severe exposure 80 % of time, but no levels given	Military personnel 1 057 (≥ 60 % MIX)	Prevalence of moderate to severe hearing loss progressed with age. In the oldest age group, 15 % had a hearing loss of ≥ 40 dB.	(3)
TOL, XYL, MEK HI 26 (1-134)	N groups: < 82 dBA (reference) 82-84 dBA 85-87 dBA ≥ 88 dBA	Aluminium industry 1 319 workers (age ≤ 35 years) 116 MIX	OR 1.9 (95 % CI 1.2-2.9) for high-frequency hearing loss for MIX. Other significant risk factors for such loss included age, reported hunting and shooting, and higher baseline hearing threshold level.	(317)

CBZ: chlorobenzene, CI: confidence interval, CRA: cortical response audiometry, EBZ: ethylbenzene, HEX: hexane, HI: hazard index, MEK: methyl ethyl ketone, MIBK: methyl isobutyl ketone, MIX: solvent mixtures, N: noise, OR: odds ratio, OSHA: Occupational Safety and Health Administration, POS: psycho-organic syndrome, PTA: pure-tone audiometry, RR: relative risk, SD: standard deviation, STY: styrene, TCE: trichloroethylene, TLV: threshold limit value, TMB: trimethylbenzene, TOL: toluene, TWA: time-weighted average, XYL: xylene.

8. Auditory effects of metals

8.1 Lead

8.1.1 General

Lead occurs naturally in the environment. It is a ubiquitous metal that has long been used by humans. Lead exposures can occur in mining and in polluted environments. Main industrial applications include the manufacture of car batteries, sheet metal, pipes and foil. Individuals employed in any of these occupations as well as professional shooters may bring lead dust on their bodies or clothing into their homes. Except in some developing countries, there is a declining use in paints, enamels and glazes. Hazard arises from inhalation of dust or fumes or certain motor vehicle emissions. Organic lead compounds can be absorbed through the skin (194).

8.1.2 Effects in animals

Several animal experiments have been conducted regarding the effects of lead exposure on the auditory system (Table 21).

In guinea pigs, lead exposure induced dysfunction of the vestibulo-cochlear nerve but it did not induce dysfunction of the organ of Corti and the stria vascularis (388). In contrast, cochlear effects by lead were reported in studies with monkeys (204, 331). Auditory brainstem response latencies were less affected by stimulus intensity, stimulus rate and noise masking level in unexposed monkeys as compared to humans (205).

Three of six monkeys exposed for life-time to lead exhibited elevated thresholds for pure tones. The monkeys had high current blood lead concentrations between 50 and 170 µg/dl with a history of moderate blood lead levels of 30 µg/dl. The relative contribution of current and long-term moderate blood lead concentrations to the observed results is unknown (331).

In another study of long-term exposed monkeys, blood lead levels of 35 µg/dl did not cause significant effects on evoked potentials (NOAEL). At a level of 55 µg/dl, these effects were significant (LOAEL) (221).

Blood lead levels of 35-40 µg/dl in monkeys exposed from birth up to 2 years of age had no significant effects on auditory function (NOAEL) (202). Also in monkeys, concentrations in dams at or above 85 µg/dl during pregnancy and 122 µg/dl during lactation were associated with cochlear dysfunction in 2/11 offspring. These 2 monkeys hade the highest blood levels at 0-6 months of age (46 and 70 µg/dl) (204). Interaction with noise has not been studied.

8.1.3 Observations in man

8.1.3.1 Lead alone or combined with noise.

Occupational studies have been conducted regarding the effects of lead exposure on the auditory system (Table 22). Noise levels were not always reported, particularly because most studies examined the effects of lead on the central auditory system, which is not considered to be affected by noise exposure.

However, due to the nature of the work performed, it is likely that the studied workers were also exposed to noise.

Studies conducted with lead-exposed workers consistently report an association between lead exposure and central auditory effects. Chronic lead exposure impaired conduction in the auditory nerve and the auditory pathway in the lower brainstem. Blood lead concentrations correlated significantly with abnormalities in the recorded evoked potentials in several investigations.

Farahat *et al* reported a significant correlation between current blood lead levels and hearing thresholds. The exposed workers (mean 37 µg/dl) had significantly elevated hearing thresholds compared to the controls (110). Also Forst *et al* reported a significant correlation between current blood lead levels and elevated hearing thresholds (defined as > 10 dB hearing loss) but only at 4 kHz (134). However, a hearing loss of 10 dB is not considered abnormal in workers 19-65 years of age.

Median blood lead levels of 30 µg/dl (range 10-64 µg/dl) was significantly associated with central auditory effects (evoked potentials and sensitised speech tests) but not peripheral ones (13, 177, 266).

Bleecker *et al* reported an association between current mean (28 µg/dl) and life-time weighted average (39 µg/dl) blood lead levels and auditory dysfunction (35). Several other studies have demonstrated auditory dysfunction at current mean blood lead levels of 42-57 µg/dl (70, 89, 90, 156, 386).

The only study that tested for statistical interaction between lead and noise exposure showed no significant interaction. Long-term exposure to 57 µg/dl of lead significantly affected pure-tone thresholds (386).

Studies on exposures outside the work environment are beyond the scope of the present document. However, in the case of lead, it is worth noting that several studies conducted with children have shown ototoxic effects (291-293, 340, 341), which were not seen in cases of extreme plumbism (40).

8.1.4 Conclusion on lead

In monkeys, a NOAEL of 35-40 µg/dl blood and a LOAEL of 55 µg/dl blood were identified (202, 221). Interaction with noise has not been studied in animals.

In humans, central auditory effects have been associated with current exposures and life-time weighted average blood lead concentrations of approximately 28-57 µg/dl (13, 35, 70, 89, 90, 110, 156, 177, 266, 386). Thus, auditory effects have been observed at blood lead levels found in the general population, e.g. in urban areas in Western Europe (37 µg/dl) (345). One study investigated but did not find any interaction between lead (57 µg/dl) and noise (386).

International 8-hour OELs for elemental and inorganic lead are 0.05-0.15 mg/m^3 inhalable aerosol (Appendix 1). The EU SCOEL has recommended a health-based OEL of 0.1 mg/m^3 and a biological exposure limit of 30 µg/dl blood to minimise the potential for adverse health effects including neurotoxicity, reproductive and developmental effects and carcinogenicity (99).

Table 21. Auditory effects in animals exposed to lead (Pb) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
Blood Pb level (µg/dl)	Regimen				
~30, stable until 10-11 yrs of age, after that ~50-170 <10 (controls)	2 mg Pb/kg/day, capsules, from birth through testing at 13 yrs of age	Monkeys	PTA	3 of 6 exposed monkeys had poorer thresholds than the age- and rearing-matched controls.	(331)
After 8-9 yrs: ~35 (350 mg) ~55 (600 mg) ~5 (controls)	0, 350 or 600 mg Pb acetate/kg diet, pre- and postnatally continuously until ~10 yrs of age	Monkeys	ABR	The 600-mg group exhibited the longest latencies (main effect on wave II) as recorded at 8 yrs of age. Also signs of latency decreases in the 350-mg group that did not reach significance.	(221)
Shortly after birth, and continuing for 1 or 2 yrs: 35-40 (exposed) <5 (controls)	Pb acetate in milk, adjusted to target dose (35-40 µg/dl), from birth to 1 or 2 yrs postnatally	Monkeys	Tympanometry DPOAE ABR	No significant differences for any of the tests as measured at least one yr after exposure. The Pb exposure had little effect on auditory function.	(202)
40 (0-6 month of age) 5 (2-4 yrs of age) <6 (controls, 0-4 yrs of age) In dams: ≥85 during pregnancy and lactation)	Pb acetate in drinking water daily during pregnancy and during 5 months of lactation	Monkeys	ABR DPOAE MLR	2 of 11 exposed offspring had abnormal DPOAE and ABR but no effect on MLR when assessed at 11 yrs of age. These monkeys had the highest blood Pb levels recorded (46 and 70 µg/dl at 0-6 months of age, and 6.4 and 8.0 µg/dl at 2-4 yrs of age).	(204)
80 (2×20 mg) 126 (4×20 mg) 142 (5×20 mg) 4.5 (controls)	Pb acetate (20 mg), injected 1 time/week for 1-5 weeks	Guinea pigs	Whole-nerve action potential	No change in cochlear microphonics after 5×20 mg of Pb acetate or in endocochlear potentials after 2×20 mg. Acute Pb exposure induced dysfunction of the vestibulo-cochlear nerve at all doses, but it did not induce electro-physiological dysfunction of the organ of Corti and the stria vascularis. 27 of 57 animals died.	(388)

ABR: auditory brainstem response, DPOAE: distortion product otoacoustic emissions, MLR: middle latency evoked responses, Pb: lead, PTA: pure-tone audiometry.

Table 22. Occupational studies on auditory effects of lead (Pb) exposure, in order from lowest to highest exposure level.

Current blood Pb level, (µg/dl) and exposure duration, mean ± SD, range	Noise (N)	No. of exposed and controls	Results and comments	Reference
1-18 (range)	Not given	183 Pb	A significant correlation between blood Pb levels and hearing thresholds (>10 dB) at 4 kHz only. However, hearing loss of 10 dB is not considered abnormal in workers aged 19-65 yrs.	(134)
10-20 (low) 21-60 (moderate) >61 (high)	79-86 dBA	26 Pb+N 17 N	No significant correlation between blood Pb levels or duration of exposure and audiometric results. Pb+N exposed had significantly poorer performances in sensitised speech tests than N exposed.	(177)
30 (median) 12-59 (range)	Not given	22 Pb 14 controls	Latencies of P300 and N100 significantly prolonged and significantly correlated with blood Pb levels and other indicators of Pb absorption.	(13)
12-64 (range) Duration: 1-18 yrs	Not given	22 Pb 22 controls, sex- and age-matched	Significantly prolonged event-related P300 and ABR latencies. ABR latencies significantly correlated with the indicators of Pb absorption. Same study group as above (13).	(266)
28 ± 8 (range 4-62) Life-time weighted average: 39 ± 12 (4-66) Life-time integrated blood Pb index: 719 µg-yr/dl Duration: 17 (0.2-26) yrs	Not given	359 Pb	Current Pb and life-time weighted average blood Pb levels were significantly associated with the ABR wave I latency while the life-time index was significantly associated with wave III latency after adjustment for age. Pb exposure interfered with ABR in a dose-dependent manner.	(35)
37 ± 4.4	42 dB (median), 40-50 dB (range)	45 Pb 45 controls	Significantly increased hearing thresholds at 1-8 kHz in Pb exposed compared to controls. A significant positive association between hearing thresholds and blood Pb levels, especially at 8 kHz. Age range 20-40 yrs to avoid effects of ageing.	(110)

Table 22. Occupational studies on auditory effects of lead (Pb) exposure, in order from lowest to highest exposure level.

Current blood Pb level, (µg/dl) and exposure duration, mean ± SD, range	Noise (N)	No. of exposed and controls	Results and comments	Reference
42 ± 16 31 ± 13 (previous 5 yrs) Duration: 17 (4-29) yrs	Not given	15 Pb 39 controls, age-matched	Significantly prolonged ABR interpeak latencies III-V in exposed compared to controls. No dose-effect relationship.	(156)
45± 20 (range 11-80) Duration: long-term	Not given	30 Pb	Mean audiometric thresholds revealed sensorineural hearing loss, which may be attributable to N exposure in combination with Pb intoxication. Delayed ABR wave latencies in those with high blood Pb level (mean 47 µg/dl).	(70)
47± 9 Duration: 9 yrs	Not given	22 Pb 22 controls, age- and sex-matched	Significant prolongation of wave I-V latencies. The interwave time longer in a Pb subgroup with the greatest mean level of Pb in blood.	(89)
55 ± 16 54 ± 16 (previous 3 yrs) Duration: 7 yrs	Not given	49 Pb 49 controls, age- and sex-matched	Significant prolongation of interpeak latency differences. Longer latencies in the subgroup with 3-yr average blood Pb level > 50 µg/dl.	(90)
56 (mean), 26-79 (range) Duration: 8 (2-17) yrs	Not given	36 Pb 15 controls	No significant differences in ABR latencies.	(267)
57 (mean)	86 dBA Leq	220 Pb 119 controls	Significant correlation between a high, long-term Pb exposure index (duration of employment and ambient Pb concentration) and decreased hearing ability. No significant correlation between short-term Pb exposure (blood Pb level) and hearing ability. Neither N exposure level alone nor the interaction between N exposure level and short- or long-term Pb exposure was significantly associated with hearing ability.	(386)
33-118 (children) [a] 19-56 (adults) [a]	Not given	14 Pb (children) 5 Pb (adults)	Children had normal hearing thresholds and DPOAE. Adults had diminished DPOAE consistent with their observed, probably N-related, hearing loss.	(40)

[a] non-occupational exposure: children and adults living in a highly Pb-contaminated environment.
ABR: auditory brainstem response, DPOAE: distortion product otoacoustic emissions, N: noise, Pb: lead, SD: standard deviation.

8.2 Mercury

8.2.1 General

Mercury is a naturally occurring element found in rocks and ores. Mercury is released into the atmosphere by evaporation from soils, from volcanic activity and from burning of fossil fuels such as coal, oil, petrol, asphalt, etc. (273).

Exposure to mercury and mercury containing products may occur through contaminated air, water and food, or through the skin. Mercury released into the environment is converted into methyl mercury by aquatic bacteria. Methyl mercury bioaccumulates in the tissues of fish and shellfish, and humans (and other animals) may be poisoned by consumption of seafood (273). However, ototoxic effects reported following such environmental exposure, as well as from poisoning, will not be included in this document.

Workers may be exposed to mercury and its compounds in mercury mines and refineries, chemical manufacturing, fluorescent light bulb manufacturing, dental/health fields and metal smelting. Workers in fossil fuel power plants and in cement manufacturing may be exposed to mercury compounds if they are exposed to gaseous process emissions. The nervous system is very sensitive to all forms of mercury. Exposure to high levels of any types of mercury can permanently damage the brain, kidneys and developing foetus. Effects on the nervous system may result in irritability, tremors, changes in vision or hearing and memory problems. High exposures to mercury vapour may cause chest pain, shortness of breath, and pulmonary oedema that can be fatal. Methyl mercury compounds and mercury metal vapours are especially harmful to the nervous system because more mercury reaches the brain. Mercury also accumulates in the body (192, 273).

8.2.2 Effects in animals and in vitro studies

8.2.2.1 Mercury alone

Mercury is known to cause neurotoxicity and sensorineural hearing deficits. A few animal experiments assessed the effects of mercury and mercury compounds on the auditory system (Table 23).

Cochlear effects were reported in studies with monkeys (332, 333). Monkeys exposed to methylmercuric chloride (CH_3HgCl) (50 µg Hg/kg body weight and day) from birth to 7 years of age exhibited elevated pure-tone thresholds, indicating a selective permanent high-frequency deficit, as measured at 14 years of age (333). In a later study by the same research team, monkeys were exposed to methylmercuric chloride (0, 10, 25 and 50 µg Hg/kg/day) throughout gestation and postnatally until 4 years of age. At 19 years of age, all exposed monkeys exhibited elevated pure-tone thresholds, generally across the full range of frequencies (332).

In rats, exposure to 0.4 or 1.6 mg Hg/kg body weight as mercuric chloride ($HgCl_2$), a soluble crystalline salt of mercury and one of its most toxic forms, daily for 12 weeks by gavage did not affect cortical auditory evoked potentials (112). Exposure of rats to 4 mg/m^3 mercury vapour (Hg^0) 2 hours/day on gestation days 6-15 did not significantly alter evoked responses in adult offspring (154).

In vitro experiments (not in table) provided a description of mercury toxicity on central auditory structures. Gopal used microelectrode array recordings to evaluate acute and chronic neurotoxic effects of mercuric chloride on auditory cortex networks. Neurons dissociated from auditory cortices of 14-day-old mouse embryos were grown on photo-etched microelectrode arrays. For acute electrophysiological experiments, the spontaneous spiking and bursting activity from auditory cortex networks were compared before and after application of various concentrations of mercuric chloride. Results of acute experiments indicated that concentrations below 75 mM of mercuric chloride had an excitatory effect of variable magnitude on the spontaneous activity of the auditory cortex networks. However, concentrations above 100 µM completely and irreversibly inhibited spike and burst activity. Chronic exposure to 10 mM mercuric chloride completely blocked the spontaneous activity. Morphological analysis indicated that 10 mM mercuric chloride caused neuronal cell death in 3 days (146).

Liang *et al* used the whole cell patch clamp technique on freshly isolated outer hair cells of the guinea pig cochlea to record outward and inward potassium currents after treatment with mercuric chloride. Treatment affected potassium currents in a dose-dependent manner. The effects of mercuric chloride at 1.0-100 mM were more pronounced on onset peak current than on steady-state end-current. Although the effect of mercuric chloride at 1.0 mM was partially washed out over several minutes, the effects at 10 and 100 mM were irreversible, also after wash-out. Hearing sensitivity may thus result from dysfunction of hair cells, as their potassium channels are targets for mercuric chloride ototoxicity (219).

8.2.2.2 Mercury combined with noise

No studies were identified.

8.2.3 Observations in man

8.2.3.1 Mercury alone or combined with noise

Occupational studies have been conducted regarding the effects of mercury exposure on the auditory system. Noise levels were not reported, particularly because most of the studies examined mercury's central auditory effects, which are not considered to be affected by noise exposure. However, due to the nature of the tasks performed, it is likely that the workers were also exposed to noise.

Discalzi *et al* used auditory brainstem responses to examine 22 workers exposed to lead, 8 exposed to mercury and 22 and 8 age- and sex-matched subjects, respectively, never exposed to neurotoxic substances. All participants had normal audiometric thresholds ($\leq$25 dBHL). Mean durations of exposure were 9.3 and 11.7 years for lead and mercury, respectively. The urinary mercury content at the end of the previous working day was 325 µg/g creatinine. Both mercury and lead exposed workers showed a significant prolongation of wave I-V time (89).

Counter *et al* measured mercury in blood and its auditory effects in children and adults in the remote Andean settlement of Nambija in Ecuador where mercury is used extensively in gold mining operations. The mean blood mercury level was 17.5 µg/l in 75 inhabitants (36 children and 39 adults) versus 3.0 µg/l in a second

group of 34 subjects (15 children and 19 adults) in a non-gold mining area. Audiological tests on 40 persons in the study area (21 children and 19 adults) showed hearing thresholds ranging from normal to mildly abnormal for children, and normal to severely abnormal for adults. Auditory brainstem evoked responses revealed a significant correlation between blood mercury levels and the I-III interpeak latencies on the right side. The results indicated that the study population had elevated blood mercury levels and may be at neurological risk from exposure to methyl mercury from the consumption of contaminated food and possibly from elemental mercury vapours inhaled during amalgam burning in the gold extraction process (71).

More recently, Moshe *et al* also used auditory brainstem responses to examine the effects of industrial exposure to inorganic mercury and chlorinated hydrocarbons on the auditory pathway. Forty workers were exposed to mercury, 37 workers to chlorinated hydrocarbons and a control group of 36 subjects were never exposed to neurotoxic substances. The mean duration of exposure to mercury and chlorinated hydrocarbons was 15.5 and 15.8 years, respectively. The air concentration of current mercury exposure was 0.008 mg/m^3. The current mean blood mercury level was inconsistently reported but was presumably 5 µg/l. A higher percentage of workers exposed to mercury and chlorinated hydrocarbons had abnormal prolongation of auditory brainstem response interpeak latencies I-III compared to control subjects (42 % and 34 % versus 18 %, respectively) (260).

Thus, studies conducted with mercury exposed workers were consistent in reporting an association between mercury exposure and central auditory effects. Chronic mercury exposure impaired conduction in the auditory nerve and the auditory pathway in the lower brainstem.

8.2.4 Conclusion on mercury

In monkeys, ingestion (orally during gestation and postnatally for 4 years) of 10 µg Hg/kg body weight/day as methylmercuric chloride was associated with permanent poorer hearing thresholds (LOAEL) (332). Interaction with noise was not studied.

In humans, current mercury concentration in air of 0.008 mg/m^3 and mean blood mercury levels of presumably 5 µg/l were significantly associated with effects as shown in central auditory tests (evoked potentials) (260). Higher concentrations were associated with similar outcomes (71, 89). Noise levels were not reported. International 8-hour OELs for elemental and inorganic mercury range from 0.02 to 0.1 mg/m^3 (Appendix 1). ACGIH has biological exposure limits for total inorganic mercury of 15 µg/l blood and 35 µg/g creatinine (7). In 2007, the EU SCOEL recommended biological limit values of 10 µg/l blood and 35 µg/g creatinine (101).

All countries listed in Appendix 1 have OELs of 0.01 mg/m^3 for organoalkyl mercury compounds. The ACGIH threshold limit values are primarily set to minimise effects on the central and peripheral nervous system and kidney damage (4).

Table 23. Auditory effects in animals exposed to mercury (Hg) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
Level	Regimen				
0, 4 mg/m^3 Hg (0)	Inhalation, 2 h/d on GD 6-15	Rats	ABR	No significantly altered evoked responses. All evoked potentials exhibited predictable changes as the stimulus was modified. This shows conclusively that the evoked responses were under stimulus control and that the study had sufficient statistical power to detect changes of these magnitudes.	(154)
0, 10, 25, 50 µg Hg/kg bw/day as CH_3HgCl	Oral, during gestation and postnatally until 4 yrs of age	Monkeys	PTA and behavioural testing	Elevated pure-tone thresholds in all exposed monkeys at 19 years of age compared to controls. Comparisons of performance at 11 and 19 yrs revealed greater deterioration in function in treated compared to control monkeys. This study suggests an acceleration of impairment of auditory function during ageing as a consequence of developmental methyl mercury exposure.	(332)
0, 50 µg Hg/kg bw/day as CH_3HgCl Blood levels: 0.6-0.9 ppm	Oral, from birth to 7 yrs of age	Monkeys	PTA	Selective permanent high-frequency deficit.	(333)
0.4, 1.6 mg Hg/kg bw as $HgCl_2$ or plain water or water with 5 % (v/v) EtOH	Gavage, daily for 12 weeks	Rats	Auditory cortical evoked potentials	No effects on auditory cortical auditory evoked potentials.	(112)
0, 2.5-25 mg/kg bw/day of $HgCl_2$ Total dose: 10-90 mg/kg bw	Injections, daily for up to 49 days	Guinea pigs	ME	Significant OHC loss, disarrangement or loss of hair cell bundles and affected internal structure of the mitochondria.	(12)

ABR: auditory brainstem response, bw: body weight, GD: gestation days, ME: morphological examination, OHC: outer hair cell, PTA: pure-tone audiometry.

8.3 Organotins (trimethyltins)

8.3.1 General

Organotin compounds can be inhaled in commercial/industrial work environments where such compounds are produced or used, as in the production of plastics in the chemical industry and as biocides in antifouling boat bottom paints. Ingestion is considered an unlikely route of entry in these environments. Skin absorption may occur in direct contact with organotins (18).

Alkyltins comprise a structurally related group of compounds that exhibit varied toxic effects. Trimethyltins and triethyltins have attracted considerable attention due to their close structural similarity and to the striking differences in their toxic actions (93). Trimethyltins have been extensively investigated for ototoxicity in animals. Trimethyltin is a neurotoxin, which damages areas of the limbic system, cerebral cortex and the brainstem. Instances of occupational exposure are rare but acute exposure to trimethyltin chloride may result in irritation of the eyes, skin, and mucous membranes, headache, blurred vision, facial flushing, excessive salivation, abdominal pain, nausea, vomiting, diarrhoea, vertigo and general malaise. Respiratory signs include coughing, shortness of breath and a burning sensation in the chest. Irregular heart rate, hypotension, cardiac arrhythmias and loss of consciousness may occur (18).

8.3.2 Effects in animals and in vitro studies

8.3.2.1 Trimethyltin alone

The effects of trimethyltin on the auditory system have been studied in several animal experiments (Table 24). Trimethyltin disrupts auditory function at doses far below those shown to elicit general neurotoxicity (67, 393).

Fechter *et al* investigated the development of ototoxicity in guinea pigs 6-48 hours following a single intraperitoneal injection of 2 mg/kg body weight of trimethyltin. At all time interval studied, trimethyltin reduced compound action potential sensitivity and cochlear microphonics. No disruption in endocochlear potential was observed between 6 and 24 hours. Morphological examination 12 hours following exposure showed permanent destruction of outer hair cells (128).

In a later study by the same research group, trimethyltin was shown to disrupt the compound action potential sensitivity particularly between 8 and 24 kHz in a dose-related manner at doses as low as 0.2 mg/kg/body weight (guinea pigs, single intraperitoneal injection). A dose of 0.5 mg/kg body weight produced a loss of sensitivity which approached 30 dB (118).

Young and Fechter investigated the ototoxicity in rats. A single intraperitoneal injection of trimethyltin chloride (2, 4 and 6 mg/kg) produced a frequency-specific dose-dependent auditory impairment, as well as decreased amplitude of the acoustically elicited startle response. At 2 mg/kg, no significant auditory effects were observed (393). In a later study by Crofton *et al*, rats were exposed to 3, 5 or 7 mg/kg of trimethyltin (single intraperitoneal injection). Auditory brainstem responses were determined at 5, 40 and 80 kHz and acoustic startle response at 5 and 40 kHz. The auditory brainstem response thresholds for 40-kHz tones were

elevated (30-50 dB) in the 5- and 7-mg/kg groups and for 80-kHz tones (17-31 dB) in all dose groups. The acoustic startle response thresholds for 40-kHz tones were elevated (24-39 dB) in all groups. Morphological examination revealed a dose-dependent increase in outer hair cell loss preferentially in regions associated with high-frequency loss (79).

The animal studies have consistently demonstrated cochlear effects of trimethyltin, which produces both hair cell and vascular damage in the cochlea. Trimethyltin disrupts function of the synapse between the inner hair cell and the Type 1 spiral ganglion cell. In all experiments with trimethyltin, the chemical was administrated by intraperitoneal injections in order to obtain information on ototoxic mechanisms rather than to evaluate potential risk to exposed populations. If extrapolations were made from dose levels in these experiments to the corresponding concentrations in air, the latter would be extremely high when compared to existing limits and human exposure to trimethyltin at these levels is unlikely.

In vitro experiments (not in table) provided further descriptions of trimethyltin toxicity on the cochlea. Clerici *et al* examined 77 outer hair cells from 45 pigmented male guinea pigs. The cells were isolated in primary culture and exposed for 90 minutes to trimethyltin or triethyltin concentrations of 0.030-1.0 mM. Significant shortening of the outer hair cell body occurred at all doses to both organotins, with a mean reduction in length of 15 and 20 % for 1.0 mM trimethyltin and triethyltin, respectively, compared to 3.4 % in control cells. The outer hair cells suffered a series of injuries, indicating that they represent one target of acute alkyltin ototoxicity (68).

Fechter and Liu examined whether trimethyltin exerted direct toxic effects on the postsynaptic spiral ganglion cells and the role of extracellular calcium in such an effect. Trimethyltin induced a marked and sustained elevation in extracellular calcium levels in the spiral ganglion cells. The study indicated that extracellular calcium levels increased due to both increasing extracellular uptake and release of extracellular calcium from intracellular stores. Thus, trimethyltin ototoxicity appears to include a direct postsynaptic toxic event (119). In 1996, Liu and Fechter tested pigmented guinea pig outer hair cells and spiral ganglion cells *in vitro* to determine the role of enhanced intracellular calcium levels in trimethyltin ototoxicity. The study showed that trimethyltin elevates intracellular calcium levels in both types of cells. The elevation of trimethyltin in spiral ganglion cells is much more rapid and greater than that in the outer hair cells (227).

8.3.2.2 Trimethyltin combined with noise

No studies were identified.

8.3.3 Observations in man

No studies were identified.

8.3.4 Conclusion on trimethyltin

In all animal studies, trimethyltin was administered by intraperitoneal injection. In guinea pigs, 0.2 mg/kg body weight resulted in auditory dysfunction (LOAEL) (118). In rats, 2 mg/kg body weight resulted in no effect on auditory function (NOAEL) (393), whereas 3 mg/kg resulted in auditory dysfunction and corresponding cochlear histopathology (LOAEL) (79). The reason for the difference between guinea pigs and rats is unknown, but it could possibly be explained by differences in uptake or metabolism between species. Interaction with noise was not studied.

The auditory effects of trimethyltin in humans have not been studied.

International 8-hour OELs for organotin compounds are 0.1 mg/m^3 in all countries listed (Appendix 1). The ACGIH threshold limit value is set to prevent effects on immune function and the central nervous system (4).

Table 24. Auditory effects in animals exposed to trimethyltin (TMT) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal	Auditory test	Results and comments	Reference
TMT level (mg/kg bw)	Regimen	model			
0, 0.2, 0.35, 0.5, 1	Single ip. injection	Guinea pigs	CAP CM SP	Latency delays in CAP at 0.2 mg/kg (LOAEL) but not in the CM isopotential curve. Profound reduction in the slope of the N1 input-output curve at 0.5 mg/kg. The results are consistent with the hypothesis that TMT disrupts function at the synapse between the inner hair cell and the Type 1 spiral ganglion cell.	(118)
0, 2	Single ip. injection	Guinea pigs	CAP CM EP ME 6-48 h post-treatment	Reduced CAP sensitivity and CM amplitude. No disruption of the EP 6-24 h post-exposure. OHC pathology in the basal turn 12 h after exposure. Type 1 spiral ganglion cells appeared swollen at 24 h with separation of myelin from the cell bodies. OHCs were identified as targets responsible for the loss of CM sensitivity after exposure as the EP was unaffected.	(128)
0, 2 (TMT) or 0, 12, 24 (TET)	Single ip. injection	Guinea pigs	CAP CM	CAP function severely disrupted while CM was unaffected by TMT and TET. TMT: Impaired CAP thresholds at all frequencies within 30 min of administration, which deteriorated further at 30-60 min. TET: Reduced sensitivity of the CAP to all frequencies. Both organotins initially disrupted the functional integrity of either inner hair cells or spiral ganglion cells within the cochlea.	(67)
0, 2 (~ same fraction of the LD_{50} that is ototoxic to rats) LD_{50} identified as 3-3.25	Repeated ip. injections: 3 or 7 d intervals and then weekly for 6 w	Guinea pigs	ABR ME after 6 w	Reversible high-frequency impairment and significant changes in the number of OHCs and in the condition of the stria vascularis. A marked increase in the diameter of the vessels of the stria vascularis along with signs of atrophy. Data confirm that TMT produced both hair cell damage and vascular pathology in the cochlea and the extent of the damage was greater in guinea pigs than in rats.	(117)
0, 2, 4, 6	Single ip. injection	Rats	ASR	No significant shifts at 2 mg/kg (NOAEL). Reversible frequency-specific dose-dependent auditory impairment and a decreased amplitude of the acoustically elicited startle response at 4 and 6 mg/kg.	(393)

Table 24. Auditory effects in animals exposed to trimethyltin (TMT) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal model	Auditory test	Results and comments	Reference
TMT level (mg/kg bw)	Regimen				
0, 3, 5, 7	Single ip. injection	Rats	ABR ASR ME	Functional endpoints demonstrated a high-frequency hearing loss. ABR thresholds were elevated in the 5- and 7-mg/kg groups for 40-kHz tones and in all dose groups for 80-kHz tones. Histology 13 w post-dosing indicated OHC loss preferentially in regions associated with high-frequency hearing in a dose-dependent manner from base to apex. LOAEL at 3 mg/kg.	(79)
0, 4	Single ip. injection	Rats	RMA ME	Mostly reversible audiometric threshold shifts. Losses of OHC in the basal turn of the cochlea as early as 48 h following exposure. More extensive pathology including the loss of type 1 spiral ganglion cells occurred at longer survival times.	(159)
0, 4, 5, 6	Single ip. injection	Rats	ASR, 2 h, 2 and 4 w post-dosing	Number of responses and response amplitude decreased 2 h post-dosing for all doses. These effects persisted 4 w. Increases in latency also seen following all dosages.	(334)
0, 5	Single ip. injection	Albino rats	Reflex inhibition audiometry	Frequency dependent loss of auditory sensitivity, most severe in the high-frequency range. The accumulation of TMT on cochlear melanin is not critical to the production of hearing impairment.	(93)
0, 5	Single ip. injection	Rats	RMA CAP CM 2 or 6-8 w post-dosing	CAP and CM confirmed a preferential high frequency effect and demonstrated a significant cochlear component to the ototoxic effects.	(120)
0, 100 µM	Applied directly to the round window	Guinea pigs	CAP CM SP	Disrupt function of the cochlea. Measurements of auditory function at supra-threshold levels clearly showed that TMT reduced the amplitude of response wave while having no measurable effect on the SP. These findings indicate that TMT blocks the recruitment of neuronal elements by loud sound.	(226)

ABR: auditory brainstem response, ASR: acoustic startle response, bw: body weight, CAP: compound action potential, CM: cochlear microphonics, EP: endocochlear potential, ip.: intraperitoneal, LD_{50}: lethal dose for 50 % of the exposed animals at single administration, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, RMA: reflex modification audiometry, SP: summating potential, TET: triethyltin, TMT: trimethyltin.

9. Auditory effects of asphyxiants

Asphyxiants are vapours or gases that can cause unconsciousness or death by suffocation (asphyxiation). They act by interfering with oxygen delivery or utilisation. Chemical asphyxiants reduce the blood's ability to carry oxygen (like carbon monoxide) or interfere with the body's utilisation of oxygen (like cyanide). The chemical asphyxiants that have been studied for their ototoxicity include carbon monoxide, hydrogen cyanide, acrylonitrile and 3,3'-iminodipropionitrile, each presented below.

9.1 Carbon monoxide

9.1.1 General

Carbon monoxide, CO, is an odourless, tasteless, colourless, toxic gas. It occurs as a product of incomplete combustion of fuels, coal, oil and wood. It is present in petrol-powered engine exhaust and tobacco smoke. Carbon monoxide is co-produced with hydrogen by steam reforming plants using methane or other hydrocarbons as feedstock. It is a raw material for making monomers and other chemical products. Besides exposure via engine exhaust and tobacco smoke, which make exposure very common, other potential sources of carbon monoxide exposure include the process of forging, melting, pouring and welding metals, in farm operations, fire fighting, sewage and water treatment jobs (281).

The carbon monoxide molecule consists of a carbon atom triply bonded to an oxygen atom. The toxicity of carbon monoxide results from its very tight binding to haemoglobin, the molecule carrying oxygen from the lungs to bodily tissues. Carbon monoxide readily combines with haemoglobin to form carboxyhaemoglobin, thereby inhibiting haemoglobin from transporting oxygen. Excessive accumulations of carboxyhaemoglobin cause hypoxic stress as a result of the reduced oxygen-carrying capacity of the blood. In essence, victims are slowly suffocated because their haemoglobin is consumed (281).

9.1.2 Effects in animals

9.1.2.1 Carbon monoxide alone and combined with noise and other agents

The auditory effects of carbon monoxide in combination with noise have been examined in numerous animal experiments (Table 25). The majority of studies were performed on rats. This species demonstrates a much higher resistance to acute carbon monoxide intoxication than humans. In rats, a lethal dose for a 30-minute exposure is 5 000 ppm, in humans, the lethal dose is 1 500 ppm (320). The experiments in rats show that carbon monoxide does not alter auditory function by itself (up to 1 500 ppm) (61). Alone or combined with toluene, carbon monoxide exposure may potentiate noise-induced hearing loss (233).

Carbon monoxide can potentiate noise-induced hearing loss at noise exposure conditions that have limited effects on auditory function alone as shown by e.g. Fechter *et al* (122) and Young *et al* (394). Studies by Chen *et al* (62) and Rao and

Fechter (320) indicate that under intermittent noise exposure with long quiet periods, carbon monoxide exposure may produce unexpectedly large, permanent threshold shifts. Surprisingly, the data did not validate the anticipated relationship between the percentage of time that noise is present (noise duty cycle) and increasing hearing loss. Instead, the mildest noise duty cycle (noise exposure interrupted with quiet breaks) produced maximal hearing loss when carbon monoxide was also present. Otherwise, when carbon monoxide was absent, hearing loss was reduced due to the quiet breaks.

Auditory function was compared in rats exposed 4 weeks earlier to carbon monoxide alone, noise alone, combined exposure to carbon monoxide and noise, or air in a chamber. The compound action potential threshold evoked by pure-tone stimuli was used as a measure of auditory sensitivity. The experimental NOAEL with respect to potentiation of noise-induced hearing loss was found to be 300 ppm carbon monoxide. Potentiation of noise-induced hearing loss by carbon monoxide increased linearly as the concentration increased between 500 and 1 500 ppm (experimental LOAEL 500 ppm) (61, 130). Benchmark dose software from the US Environmental Protection Agency (EPA) was employed to determine a benchmark concentration of carbon monoxide that produced either an increase in auditory threshold equivalent to 10 % of the effect of noise alone or produced a 5-dB potentiation of noise-induced hearing loss. The lower bounds for these benchmark concentrations were 194 and 320 ppm (LOAELs), respectively (130). In a later study, the experimental NOAEL (300 ppm) and LOAEL (500 ppm) for combined exposure to carbon monoxide and noise (impulsive noise, for 10 days, Leq 8h of 84 dB SPL) were confirmed (233). Without exposure to carbon monoxide, the noise effect was not significant, but in the combined exposure scenarios, responses were significantly poorer.

9.1.2.2 Carbon monoxide combined with anti-oxidants

Rao and Fechter explored the ability of phenyl-*N*-*tert*-butylnitrone (PBN), a spin trap agent that forms adducts with free radicals, to protect against the combined effects of noise and carbon monoxide on auditory function in rats. Intraperitoneal injection of PBN both pre- and post-exposure to carbon monoxide and noise protected against the permanent hearing loss. Protection did not occur when PBN was given only post-exposure (319). Thus, while these results help to establish a role for oxidative stress in the interaction between carbon monoxide and noise, PBN does not offer an effective therapeutic treatment strategy.

Table 25. Auditory effects in animals exposed to carbon monoxide (CO) alone or combined with other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
CO level (ppm)	Noise (N)	Regimen				
0, 300, 500, 700, 1 200, 1 500	100 dB at 13.6 kHz OBN	Inhalation: 9.5 h N: 8 h	Rats	CAP	CO+N: NOAEL at 300 ppm (experimental). Potentiation of NIHL increased linearly as CO levels increased between 500 ppm (LOAEL) and 1 500 ppm. The lower bounds for benchmark doses were 194 ppm for an increase in auditory threshold equivalent to 10 % of the effect of N and 320 ppm for a 5-dB potentiation of NIHL.	(130)
0, 300, 500, 700, 1 200, 1 500	100 dB Lin or 115 dB Lin, 4 kHz OBN	Inhalation: 9.5 h N: 8 h	Rats	CAP CM	CO: No effect. CO+N: NOAEL at 300 ppm. The elevation of the CAP threshold and the CM iso-amplitude curve by N was potentiated by CO at ≥ 500 ppm (LOAEL). The potentiation may be due to the reduction of the cell's ability to repair N induced damage by CO.	(61)
0, 300 or 500 CO + 0, 500 or 1 000 TOL	84 dB SPL with 75 % of the energy as impulses, 4-20 Hz	Inhalation and N: 6 h/d, 10 d	Rats	ABR DPOAE	N: Hearing loss at 8-14 kHz. TOL+N: Same hearing loss as N CO+N: NOAEL at 300 ppm. Dose-dependent increase in hearing loss with a synergistic interaction with 500 ppm CO (LOAEL). TOL+CO+N: Greater hearing loss than CO+N. Synergistic interaction that increased with exposure levels of both CO and TOL. Worst hearing loss after 1 000 ppm TOL+500 ppm CO+N.	(233)
0, 1 200	105 dBA broadband	Inhalation and N: 2 h	Rats	CAP CM ME	CO: No effects on auditory thresholds. N: Variable but quite limited permanent threshold shifts. Hair cell loss restricted to the basal turn of the cochlea. CO+N: Large threshold shifts at all frequencies tested, greatest at the highest frequency, an effect not consistent with the N power spectrum.	(122)

Table 25. Auditory effects in animals exposed to carbon monoxide (CO) alone or combined with other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
CO level (ppm)	Noise (N)	Regimen				
0, 1 200	110 dBA broadband	Inhalation: 3.5 h N: 2 h	Rats	RMA	CO: No effect. CO+N: Greater high-frequency threshold shifts than by N. The potentiation of NIHL by CO provides support for the hypothesised role of metabolic exhaustion or blood flow impairment in NIHL.	(394)
0, 1 200	95, 100 and 105 dB SPL	Inhalation: 5.5, 3.5 or 2.5 h N: 4, 2 or 1 h	Rats	CAP	CO+N groups: Greater threshold elevations than in N groups. Potentiation reduced as N levels increased (105 dB, 1 or 4 h).	(320)
0, 1 200	100 dB Lin at 13.6 kHz OBN	Inhalation: 3.5 h N: 2 h, intermittent, varying % of N during exposure	Rats	CAP CM ME	N: The intermittent exposure with a shorter N duty cycle induced a less permanent threshold shift than those with a longer N duty cycle (or less rest periods). CO+N: Threshold elevations much higher than in all N groups and independent of N duty cycle. While intermittent, short duty N cycle did not cause OHC loss by itself, the combined exposure caused remarkable OHC loss in the basal turn.	(62)
0, 1 200 Injection with PBN	100 dB Lin at 13.6 kHz OBN	Inhalation: 3.5 h N: 2 h	Rats	CAP CM	Pre- and post-exposure PBN injection promoted recovery of thresholds. Repeated PBN administered post-exposure offered some protection at low frequencies whereas single-dose administration did not. These results suggest that free radicals may be involved in the cochlear damage.	(319)
0, 1 200 Injection with two isomers of MK-801 [a]	100 dB Lin at 13.6 kHz OBN	Inhalation: 3.5 h N: 2 h	Rats	CAP ME	(+)-MK-801 protected against NIHL, while (-)-MK-801 did not. The potentiation of the permanent NIHL by CO exposure was not reduced by the administration of either.	(63)
0, 1 200 Injection with POBN	100 dB Lin 13.6 kHz OBN	Inhalation: 3.5 h N: 2 h	Rats	CAP CM EPR	CO+N: Significant permanent increase in CAP thresholds; POBN offered some protection. CM differences not significant. Significant higher levels of free radicals shown by EPR.	(321)

Table 25. Auditory effects in animals exposed to carbon monoxide (CO) alone or combined with other agents, in order from lowest to highest exposure.

Exposure conditions			Animal model	Auditory test	Results and comments	Reference
CO level (ppm)	Noise (N)	Regimen				
0, 35 ml/kg bw Pre-treatment with (+)-MK-801 [a] (0, 0.1 or 1 mg/kg or 1 mM)	No N	Ip. (CO) Ip. or applied directly to round window (MK-801) 15 min prior to CO	Guinea pigs	CAP	CO elevated CAP thresholds between 16-40 kHz, 30 min after treatment. Pre-treatment with MK-801 ip. prevented elevation of thresholds at 1 mg/kg, but not at 0.1 mg/kg MK-801. 1 mM MK-801 applied on the round window membrane provided protective effects against CO hypoxia from 15 up to 60 min. Cochlear impairment induced by CO hypoxia may result from excess extracellular concentrations of glutamate.	(225)
0, 35 ml/kg bw Pre-treatment with PBN (0, 100 mg/kg), allo-purinol (0, 100 mg/kg)	No N	Ip. (CO) Ip. (PBN, allopurinol) 1 h prior to CO	Guinea pigs	CAP CM	CO caused CAP threshold elevations between 16 and 40 kHz, no loss of CM amplitude. Both free radical inhibitors (PBN and allopurinol) blocked loss of auditory threshold sensitivity produced by CO. Data suggest that free radical generation may play a significant role in the CO induced auditory effect.	(123)
HbCO levels 56 %	No N	Ip. (CO)	Rats	CAP CM	High doses of injected CO yielded dose-dependent reversible loss of the CAP sensitivity.	(121)
0, 35 ml/kg bw Pre-treatment with KCN (0, 7 mg/kg)	No N	Ip. (CO) Ip. (KCN) prior to CO	Rats	CAP EP	Acute CO injection suppressed CAP but not EP. HbCO levels were elevated while cochlear function was impaired. Recovery of cochlear function preceded the return to normal oxyhaemoglobin levels.	(368)

[a] (+)-MK-801: NMDA receptor antagonist. (-)-MK-801: much less active isomer of MK-801 with a low affinity for the NMDA receptor.
ABR: auditory brainstem response, CAP: compound action potential, CM: cochlear microphonics, CO: carbon monoxide, DPOAE: distortion product otoacoustic emissions, EP: endocochlear potential, EPR: electron paramagnetic resonance spectroscopy, HbCO: carboxyhaemoglobin, ip.: intraperitoneal, KCN: potassium cyanide, Lin: linear, LOAEL: lowest observed adverse effect level, ME: morphological examination, N: noise, NIHL: noise-induced hearing loss, NOAEL: no observed adverse effect level, OBN: octave band noise, PBN: phenyl-*N*-*tert*-butylnitrone, POBN: α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone, RMA: reflex modification audiometry, SPL: sound pressure level, TOL: toluene.

9.1.3 Observations in man

9.1.3.1 Carbon monoxide alone or combined with noise

In humans, several reports have documented that hearing loss is one of the outcomes associated with acute carbon monoxide poisoning. Unlike the findings in animal studies, noise exposure was not a necessary factor for the auditory problems to occur. Only studies on the effects of occupational exposures to carbon monoxide are included in the present document.

In an early study, Lumio examined 700 patients suspected of having carbon monoxide poisoning. With the exception of a small part of the study population (6 % who worked in factories), most of the participants were from occupations in which workers were exposed to carbon monoxide from gas generators used in automobiles (56 % were drivers). The participants' mean exposure duration was 6 years, and 92 % of the participants' age ranged from 21 to 50 years (44 % being 31-40 years old). Occupational noise exposure and health indicators that could contribute to hearing loss were accounted for through a general medical examination and interview. Two hundred and sixty three participants (38 %) were diagnosed with chronic carbon monoxide poisoning. Among those, 78 % had hearing loss. Once participants with other risk factors for hearing loss were excluded from the sample, the percentage of cases of hearing loss decreased to 68 %. The hearing disorders were evident in the extended high-frequency region of the audiogram and 63 % of the workers with hearing loss reported tinnitus. Among the workers exposed to carbon monoxide that were not diagnosed as cases of chronic poisoning, 27 % had hearing loss, a percentage that was reduced to 14 % once subjects with other risk factors were excluded. In 11 % of the cases of mild hearing loss, there was some improvement in their hearing thresholds. Lumio (1948) concluded that carbon monoxide poisoning was associated with hearing loss, despite the lack of excessive noise exposure (232). The presence of other risk factors does not exclude the possibility that carbon monoxide did contribute to the observed hearing deficits.

Among 78 workers with a history of carbon monoxide exposure likely to have occurred in combination with noise (no exposure measurements), 66 % showed hearing loss while 76 % presented vestibular disorders (195).

A database containing workers' charts collected by the Quebec National Public Health Institute between 1983 and 1996 was examined. The database provided information on occupation, noise level (Leq8 hours), number of years of noise exposure for the current occupation, audiometric data and medical history. Carbon monoxide exposure status (yes or no) was determined for each occupation by a panel of 5 experienced assessors (2 industrial hygienists and 3 audiologists). Data from 6 812 audiometric assessments were retained for analysis and were divided among 4 groups: 1) carbon monoxide + noise $\geq$ 90 dBA, n = 1 872, 2) noise alone $\geq$ 90 dBA, n = 2 383, 3) carbon monoxide + noise < 90 dBA, n = 1 031, and 4) noise alone < 90 dBA, n = 1 526. The effect of carbon monoxide with noise exposure below 90 dBA was not significant at any frequency (0.5, 1, 2, 3, 4, 6 kHz). Workers who were exposed to carbon monoxide and to noise levels above 90 dBA

displayed significantly poorer hearing thresholds at high frequencies (3, 4, and 6 kHz) than workers without carbon monoxide exposure but with equivalent noise exposure. The magnitude of the shift in hearing thresholds was influenced by the number of noise exposure years. The results indicate that carbon monoxide exposure increases the magnitude of the hearing loss due to noise exposure. The ORs for a hearing deficit at least at one frequency were 1.4 (95 % CI 1.2-1.5) for the carbon monoxide plus noise ≥ 90-dBA group, 1.2 (95 % CI 1.0-1.3) for the noise ≥ 90-dBA group and 1.1 (1.0-1.3) for the carbon monoxide plus noise < 90-dBA group compared to the noise < 90-dBA group (presented as conference proceedings) (200, 217).

Studies conducted by the same team in Canada also examined if a combined, non-occupational exposure to noise and carbon monoxide could affect the hearing thresholds of workers with occupational noise exposure. Data from 6 395 audiograms obtained from noise-exposed workers were retained for analysis and divided into two groups: 1) 3 306 workers exposed to noise and carbon monoxide in their non-occupational activities and 2) 3 089 workers exposed only to noise in their non-occupational activities. Information was available on their occupational noise exposure levels but were estimated for their non-occupational exposures. Results indicated a significant interaction between audiometric results of a specific test frequency and non-occupational carbon monoxide exposure, years of occupational noise exposure and current occupational noise exposure level. Non-occupational noise exposure had a marginal effect on hearing thresholds when compared to non-occupational noise and carbon monoxide exposure (which had a larger effect on hearing thresholds). However, these effects were only observed in the group with at least 15 years of occupational noise exposure associated with concurrent non-occupational exposure to carbon monoxide and noise (presented as conference proceedings) (200, 217).

Further, the effects of occupational exposure to low concentrations of carbon monoxide and noise on hearing status of a small subsample of workers (n = 28) were explored. Participants were subdivided in 4 groups: carbon monoxide only (n = 2), carbon monoxide and noise (n = 2, 85-90 dBA), noise only (n = 3, 90-91 dBA) and unexposed controls (n = 21, < 80 dB). The environmental carbon monoxide levels ranged between 16 and 35 ppm and the biological (carboxyhaemoglobin) levels were 2-3 %. The audiometric data indicated that combined carbon monoxide and noise exposure had an effect on hearing at 8 kHz as measured via both pure-tone audiometry and distortion product otoacoustic emissions, but this was based on only two individuals (199).

Ahn *et al* conducted a nested case-control study in a cohort of male iron and steel workers exposed to low concentrations of carbon monoxide. The study group comprised 770 cases and 2 574 incidence density age-matched controls. Quantitative carbon monoxide or noise exposure data were available from a job-exposure matrix. The OR for hearing loss (4 kHz threshold ≥ 35 dB) was 2.5 (95 % CI 1.2-5.0) for exposure levels greater than 20 ppm of carbon monoxide,

after controlling for noise exposure level, body mass index, smoking, hypertension and diabetes (presented as an abstract) (9).

9.1.3.2 Interactions between smoking and noise exposure

Studies on the interaction between hearing loss and smoking offer some support for an ototoxic potential of carbon monoxide. According to recent studies, heavy smoking can affect hearing (41, 343, 383) and interact with noise, thus causing a more severe hearing loss (176, 247, 314, 372). In the study by Ahn *et al* (9), smoking was a variable significantly associated with hearing loss but with an OR for hearing loss barely above 1 (OR 1.01, 95 % CI 1.003-1.021) (data not shown, personal communication Thais Morata).

9.1.4 Conclusion on carbon monoxide

In rat inhalation studies, carbon monoxide alone did not affect the auditory system at concentrations up to 1 500 ppm (NOAEL) (61). However, it can potentiate the effects of noise even when noise levels alone would not cause a change in hearing. In combination with noise (95 or 100 dB at 13.6 kHz OBN), the experimental NOAEL was 300 ppm and the LOAEL 500 ppm (61, 130, 233). The calculated lower bounds for benchmark doses that affected the auditory thresholds were 194 and 320 ppm (LOAELs, for details, see Section 9.1.2.1) (130).

Acute human carbon monoxide poisoning has been associated with hearing loss, despite lack of excessive noise exposure. Most field studies lack noise exposure estimates. It is therefore not clear if noise exposure is a prerequisite for the auditory effects seen following long-term occupational exposure to carbon monoxide.

In a study analysing 6 812 audiograms, exposure to carbon monoxide and noise levels below 90 dBA had no effect on hearing thresholds, whereas workers who were exposed to carbon monoxide and to noise levels above 90 dBA displayed significantly poorer hearing thresholds at high frequencies (conference proceedings) (200, 217). In a small subset, the adjusted ORs for audiometric hearing loss were significant for exposures in the 16 to 35 ppm range in combination with noise exposure (two subjects) (199).

International OELs for carbon monoxide range from 25 to 50 ppm (Appendix 1). The ACGIH threshold limit value of 25 ppm is intended to maintain blood carboxyhaemoglobin levels below 3.5 %, to prevent neurobehavioural effects and to maintain cardiovascular work and exercise capacities (4).

9.2 Hydrogen cyanide

9.2.1 General

Cyanides are chemical compounds that contain a cyano functional group, CN^-. The cyanide ion has a single negative charge and consists of a carbon that is triply bonded to a nitrogen atom. Cyanide is often used as shorthand term for hydrogen cyanide.

Hydrogen cyanide, HCN, is a colourless and highly volatile liquid that boils slightly above room temperature at 26 °C, thereby generating hydrogen cyanide

gas. Hydrogen cyanide has a faint, bitter, almond-like odour. Hydrogen cyanide may be synthesised directly from ammonia and carbon monoxide or from ammonia, oxygen (or air) and natural gas. It is a by-product of the production of coke from coal and is recovered (along with hydrogen sulphide) from coke-oven exhaust gases. It may also be prepared by reacting a cyanide salt, e.g. calcium cyanide, with a strong acid, e.g. sulphuric acid, or by thermal decomposition of formamide (164).

Cyanide is used in tempering steel, dyeing, explosives, engraving, the production of acrylic resin plastic and other organic chemical products. Hydrogen cyanide is contained in the exhaust of vehicles, in tobacco smoke, and in the smoke of burning nitrogen-containing plastics.

A hydrogen cyanide air concentration of 300 ppm will kill a human within a few minutes. The toxicity is caused by the cyanide ion, which prevent cellular respiration (164).

9.2.2 Effects in animals

9.2.2.1 Hydrogen cyanide alone and combined with noise

One single investigation on the auditory effects of hydrogen cyanide in animals has been identified (Table 26) (126). Rats were exposed to 10, 30 or 50 ppm hydrogen cyanide alone for 3.5 hours or in combination with 2 hours of octave band noise exposure (100 dB, linear scale). Additional groups received noise exposure alone and no treatment other than placement in a quiet inhalation chamber with clean air. Hydrogen cyanide alone did not cause significant hearing loss or hair cell loss. Noise exposure alone impaired compound action potential (CAP) thresholds by about 10 dB (averaged across frequencies 12-40 kHz) and produced a 5 % loss of outer hair cells at the base of the cochlea, but no inner hair cell loss. The combined exposure to noise and hydrogen cyanide caused a cyanide dose-dependent CAP threshold impairment and outer hair cell loss that exceeded the noise exposure alone. At 30 ppm, the potentiation of noise-induced hearing loss achieved statistical significance. A risk assessment analysis was conducted for the auditory threshold data using benchmark dose software (from US EPA). A continuous model showed that the data could be described by a linear function. For a benchmark response corresponding to a 5-dB increase in the auditory threshold above the effect of noise alone, the lower bound of the 95 % CI for the benchmark dose was 9 ppm. The benchmark dose that impaired the auditory threshold 10 % above the effect of noise alone had a lower bound of 2 ppm. The lower bound of the hydrogen cyanide dose that produced a one standard deviation elevation in noise-induced hearing loss was 16 ppm (126).

9.2.3 Observations in man

No studies were identified.

9.2.4 Conclusion on hydrogen cyanide

In the only study available, hydrogen cyanide alone (up to 50 ppm) did not significantly affect the auditory system in rats (NOAEL). However, it can

potentiate the auditory effects of noise. At 30 ppm, the potentiation of noise-induced hearing loss achieved statistical significance (LOAEL). Exposure to 10 ppm and noise did not produce significant potentiation or pronounced outer hair cell loss (NOAEL). The lower bounds for benchmark doses that affected the auditory thresholds were 2, 9 and 16 ppm (LOAELs) (126).

Auditory effects of hydrogen cyanide in humans have not been studied.

International OELs for hydrogen cyanide vary from 0.9 to 10 ppm (Appendix 1). The ACGIH threshold limit value is set to prevent upper respiratory irritation, headache, nausea and symptoms of chronic exposure such as thyroid enlargement, and in addition to provide a sufficient margin of safety against acute poisoning (4).

9.3 Acrylonitrile

9.3.1 General

A nitrile is any organic compound that has a carbon atom and a nitrogen atom triply bonded together, i.e. a -C≡N functional group. The prefix cyano is used in chemical nomenclature to indicate the presence of a nitrile group in a molecule.

Acrylonitrile is a colourless, liquid, man-made chemical with a sharp, onion- or garlic-like odour. It dissolves in water and evaporates quickly. Acrylonitrile is used in the production of other chemicals such as plastics, synthetic rubber and acrylic fibres, and is one of the 50 most commonly produced industrial chemicals. Metabolism of ingested, inhaled or topically applied acrylonitrile releases cyanide that can produce acute respiratory arrest and central nervous system toxicity (16).

9.3.2 Effects in animals

9.3.2.1 Acrylonitrile alone and combined with noise and anti-oxidants

Four animal experiments on the effects of acrylonitrile on the auditory system have been conducted (Table 26) (127, 131, 297, 299). Acrylonitrile potentiates noise-induced hearing loss as a consequence of oxidative stress. The metabolism of acrylonitrile involves conjugation with glutathione, resulting in rapid and pronounced depletion of this antioxidant in many organs including brain, liver, and kidney. It also results in cyanide formation through a secondary oxidative pathway. The studies indicate that the outer hair cells are the main target of toxicity.

Acrylonitrile alone (50 mg/kg body weight, 1-2 subcutaneous injections) elevated auditory thresholds temporarily in rats. No effects were seen after 3 weeks. Acrylonitrile (50 mg/kg body weight, 1-2 subcutaneous injections) in combination with noise (108 dB octave band noise, 8 hours) increased auditory threshold impairment relative to rats receiving noise only when measured 3 weeks following exposure (131). Combined exposure for 5 days to acrylonitrile (50 mg/kg body weight, subcutaneous injections) and moderate noise (95 or 97 dB octave band noise, 4 hours) caused permanent hearing loss and outer hair cell loss in rats. Individually, neither acrylonitrile nor noise caused these effects (297).

Rats treated daily with phenyl-*N*-*tert*-butylnitrone (PBN, spin-trap agent that sequesters ROS) prior to and again following acrylonitrile (50 mg/kg body weight,

subcutaneous injection) and noise (105 dB octave band noise, 4 hours) treatment for 5 consecutive days showed approximately the same auditory impairment as did rats receiving noise only. Thus, PBN blocked the potentiation of noise-induced hearing loss (127). Pre-treatment of rats with L-*N*-acetylcysteine (antioxidant, pro-glutathione drug) decreased auditory loss and hair cell loss resulting from combined exposure to acrylonitrile (50 mg/kg, subcutaneous injection) and moderate noise (97 dB octave band noise, 4 hours, 5 days) (299).

All studies show that acrylonitrile exposure alone does not cause damage to the auditory system of the rat, however, it potentiates noise-induced hearing loss at noise levels that are realistic in terms of human exposure. However, the acrylonitrile exposure route used in the animal studies (subcutaneous injection) differs from that experienced by workers and the doses of acrylonitrile in the animal studies were greater. Because the widespread use of acrylonitrile in industry occurs in settings where noise exposure is also present, the identified synergistic mechanism may be of importance for occupational health.

9.3.3 Observations in man

No studies were identified.

9.3.4 Conclusion on acrylonitrile

In rat studies using subcutaneous injection, acrylonitrile (50 mg/kg body weight, only dose tested) did not induce permanent auditory effects (NOAEL). However acrylonitrile can potentiate noise-induced hearing loss. Combined exposure to acrylonitrile (50 mg/kg, only dose tested) and noise ($\geq$95 dB) caused permanent hearing loss and outer hair cell loss (LOAEL) (127, 131, 297, 299).

Auditory effects of acrylonitrile in humans have not been studied.

International 8-hour OELs for acrylonitrile vary from 1 to 30 ppm (Appendix 1). The ACGIH threshold limit value is set to minimise the potential for headache, nausea, respiratory difficulties, central nervous system effects and cancer (4).

Table 26. Auditory effects in rats exposed to hydrogen cyanide (HCN) or acrylonitrile (ACN), alone or combined with noise.

Exposure conditions			Auditory test	Results and comments	Reference
Level	Noise (N)	Regimen			
Hydrogen cyanide					
0, 10, 30, 50 ppm	No N or 100 dB Lin	Inhalation: 3.5 h N: 2 h	CAP ME	HCN: No effect (NOAEL at 50 ppm). HCN+N: NOAEL at 10 ppm. Potentiation of NIHL at 30 ppm (LOAEL). Benchmark dose (lower bound) at 2 ppm for impaired auditory threshold 10 % above the effect of N, at 9 ppm for a 5-dB increase in auditory threshold above the effect of N, and at 16 ppm for one standard deviation increase in NIHL.	(126)
Acrylonitrile					
0, 50 mg/kg bw	No N	Sc. injection	CAP	ACN: Reversible loss in auditory threshold sensitivity. No permanent threshold impairment (NOAEL).	(131)
0, 50 mg/kg bw or 2×50 mg/kg bw	No N or 108 dB OBN	Sc. injection N: 8 h		ACN+N: Increased threshold impairment relative to N when assessed 3 w following injection (LOAEL). Systemic blood CN levels not significantly elevated until 60-120 min following injection. Cochlear GSH levels showed significant depletion for ~4 h.	
0, 50 mg/kg bw, with or without pre-treatment with PBN	No N or 105 dB	Sc. injection: 5 d N: 4 h/d, 5 d	CAP	ACN: Transient loss of auditory threshold sensitivity (NOAEL). ACN+N: Significant elevation of NIHL (LOAEL). PBN (spin-trap agent) blocked elevation of NIHL by ACN.	(127)
0, 50 mg/kg bw	No N or 95 or 97 dB OBN	Sc. injection: 5 d N: 4 h/d, 5 d	CAP DPOAE ME	ACN or N: No permanent hearing or OHC loss, only a reversible temporary threshold shift in N exposed (NOAEL). ACN+N: Permanent threshold shifts, decreased DPOAE amplitudes and significant OHC loss (LOAEL).	(297)
0, 50 mg/kg bw, with or without pre-treatment with antioxidans (STS, 4-MP or L-NAC)	No N or 97 dB SPL	Sc. injection: 5 d N: 4 h/d, 5 d	CAP DPOAE	All antioxidants reduced blood CN levels significantly but only L-NAC protected GSH levels in both liver and cochlea significantly. L-NAC decreased the auditory loss and hair cell loss from ACN+N, suggesting that GSH is involved in the protection of the cochlea against ROS generated by moderate N levels. On the other hand, CN does not seem to be involved in this potentiation.	(299)

ACN: acrylonitrile, CAP: compound action potential, CN: cyanide, DPOAE: distortion product otoacoustic emissions, GSH: glutathione, HCN: hydrogen cyanide, Lin: linear, L-NAC: L-*N*-acetylcysteine, LOAEL: lowest observed adverse effect level, ME: morphological examination, 4-MP: 4-methylpyrazole, N: noise, NIHL: noise-induced hearing loss, NOAEL: no observed adverse effect level, OBN: octave band noise, OHC: outer hair cell, PBN: phenyl-*N*-*tert*-butylnitrone, ROS: reactive oxygen species, sc.: subcutaneous, SPL: sound pressure level, STS: sodium thiosulphate.

9.4 3,3'-Iminodipropionitrile (IDPN)

9.4.1 General

3,3'-Iminodipropionitrile, IDPN, is a colourless liquid, which has been extensively used as neurotoxic agent in animal experiments. Synonyms include 3,3'-iminobis-propionitrile, ββ'-iminodipropionitrile, and *N, N*-bis(2-cyanoethyl)amine. No reports on occupational exposures to IDPN were identified (69).

9.4.2 Effects in animals

Four animal experiments have been conducted on the effects of IDPN on the auditory system (Table 27) (73, 77, 80, 144). These studies show that IDPN (administrated by intraperitoneal injection) alone causes extensive hearing loss and loss of neural structures in the cochlea of the rat.

No significant effects were registered after intraperitoneal injection of 100 mg/kg body weight/day for 3 days, but decreased acoustic startle response amplitude were registered in rats following 150 mg/kg body weight/day (LOAEL). Elevated auditory thresholds were reported for 5- and 40-kHz tones for the 200-mg/kg group, representing approximate increases of 25 dB and 50 dB, respectively. The onset of this auditory dysfunction in the 200-mg/kg group, as demonstrated by a loss of reflex inhibition, was 2 days for the 40-kHz tone and 4 days for the 5-kHz tone (73).

Exposure of rats to 167 and 200 mg/kg body weight of IDPN for 3 consecutive days caused elevated auditory thresholds (as measured by reflex modification audiometry and/or auditory brainstem response) over a broad range of frequencies while no such effects were observed at 133 and 150 mg/kg body weight. Histology made on rats dosed with 200 mg/kg body weight showed loss of hair cells and spiral ganglion cells, and damage to the cochlear nerve in a basal-to-apical fashion. At 100 mg/kg body weight, IDPN had virtually no effect on structure (80).

The interaction with noise has not been studied.

9.4.3 Observations in man

No studies were identified.

9.4.4 Conclusion on IDPN

In rat studies, intraperitoneal injections of 150 mg/kg body weight/day for 3 consecutive days caused auditory changes (LOAEL). No auditory effects were observed at 100 mg/kg (NOAEL) (73). The interaction with noise has not been studied. Auditory effects of IDPN in humans have not been studied.

No OELs for IDPN were identified.

Table 27. Auditory effects in rats exposed to 3,3'-iminodipropionitrile (IDPN) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal	Auditory		Reference
Level (mg/kg bw/day)	Regimen	model	test		
0, 50, 100, 150, 200	Ip. injection for 3 consecutive days	Rats	RMA, 1, 3, and 9 w post-dosing	NOAEL at 100 mg/kg. Decreased acoustic startle response amplitude in 150 (LOAEL) and 200-mg/kg groups. Elevated auditory thresholds for 5- and 40-kHz tones for the 200-mg/kg group, representing approximate increases of 25 dB and 50 dB, respectively. The onset of this auditory dysfunction in the 200-mg/kg group, as demonstrated by a loss of reflex inhibition, was 2 days for the 40-kHz tone and 4 days for the 5-kHz tone.	(73)
0, 75, 150, 225, 300	Ip. injection at PND 5-7	Rats	ASR at PND 23, 61 and 62	Elevated auditory thresholds for a high-frequency (40 kHz) but not for a low-frequency tone (5 kHz) for the 225 and 300-mg/kg/day groups.	(77)
0, 300	Ip. injection for 3 consecutive days: GD 15-17, or PND 1-3, 5-7, 15-17, 20-22, 25-27, 30-32, 40-42 or 70-72	Rats and their offspring	RMA	All animals tested as adults for auditory thresholds to 5- and 40-kHz tones. Adult-like susceptibility to IDPN was not reached until approximately PND 30-32. Early exposures (PND 5-22) induced a high frequency selective hearing loss. Prenatal or early neonatal (PND 1-3) exposure resulted in >70 % mortality. The data suggest either a unique site of action for IDPN in the cochlea or the possibility of two different mechanisms, one operating at early postnatal ages and one at later ages.	(144)
Functional data: 0, 100, 150, 200 or 0, 133, 167, 200 (kanamycin sulphate positive control for high-frequency hearing loss) *Histology:* 0, 100, 200, 400	Ip. injection for 3 consecutive days	Rats	ABR 9-10 w and 11-12 w post-exposure. RMA 9-10 w post-exposure. ME 12-14 w post-exposure	*Functional data:* NOAEL at 150 mg/kg. Doses ≥167 mg/kg (LOAEL) resulted in a broad-spectrum hearing loss ranging from 0.5 kHz (30 dB deficit) to 80 kHz (40 dB deficit) as compared to a hearing deficit in kanamycin-exposed animals that was only apparent at frequencies greater than 5 kHz. *Histology:* NOAEL at 100 mg/kg. Hair cell loss in all turns of the organ of Corti, with greater damage in the basal turns than in the more apical turns at 200 mg/kg (LOAEL). Complete destruction of the organ of Corti at 400 mg/kg. There was also a dosage-related loss of spiral ganglion cells in all turns of the cochlea.	(80)

ABR: auditory brainstem response, ASR: acoustic startle reflex, bw: body weight, GD: gestation days, IDPN: 3,3'-iminodipropionitrile, ip.: intraperitoneal, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, PND: postnatal days, RMA: reflex modification audiometry.

10. Auditory effects of other substances

10.1 Pesticides

10.1.1 General

The term “pesticide” has been used to represent any chemical substance used to prevent, destroy, repel or mitigate pests of weeds. The major classes of pesticides include herbicides, insecticides, fungicides and fumigants. Pesticides vary in their uptake, mode of action, metabolism, toxicity and elimination from the body. People can be exposed to pesticides in agriculture but also through the contamination of food, air, drinking water and dust. Epidemiological studies have discovered associations between pesticide exposure and long-term effects on health in three main areas, cancer, reproductive disorders and neurotoxic effects (22).

In this document, the term pesticide refers to the active ingredient of a pesticide product or formulation. A pesticide product applied by farmers usually contains one or more active pesticidal ingredients and ingredients which can enhance the effect of the active ingredient. The pesticides that have been studied for their auditory effects include organophosphates (paraoxon, parathion), pyrethroids, pyridyliums (paraquat) and hexachlorobenzene. Paraquat was banned in Sweden in 1983. The substance has also been banned in several other countries including Denmark, Finland and Norway. The EU allowed paraquat in 2004. Sweden supported by Denmark, Finland and Austria brought the EU to court. In 2007, the court annulled the directive authorising paraquat as an active plant protection substance. In the US, paraquat is available for use only by commercially licensed users (56, 284, 359).

10.1.2 Effects in animals

Few studies on the auditory effects of pesticides in laboratory animals were identified (Table 28) (28, 33, 148, 276, 330). Two of five studies were conducted with paraquat and reported irreversible cochlear effects (33, 276). One study on the organophosphorus compound paraoxon reported only reversible effects (28). The only study on hexachlorobenzene noted irreversible threshold shifts but no hair cell loss (148). Interaction with noise was not studied.

10.1.3 Observations in man

10.1.3.1 Pesticides alone or combined with noise

Few occupational studies have been conducted regarding the effects of pesticide exposures on the auditory system (Table 29) (26, 27, 72, 98, 150, 315, 369). Noise levels have not always been reported in these studies. However, due to the nature of the work performed, it is likely that the studied workers were also exposed to hazardous noise.

10.1.4 Conclusion on pesticides

Risk evaluation is not attempted, because of the lack of exposure information in the few experiments conducted.

Table 28. Auditory effects in animals or *in vitro* following exposure to pesticides.

Exposure conditions		Animal	Auditory	Results and comments	
Level	Regimen	model	test		
Organophosphorus compounds					
27 mg/kg bw paraoxon	Single ip. injection	Mini pigs	ABR	Reversible prolongation of interpeak latencies I-V. No characteristic signs of paraoxon intoxication. Acute intoxication was associated with temporary limited hearing impairment and increased interpeak latency.	(28)
0, 0.1 mg/kg bw parathion	In diet, daily for 148 days	Squirrel monkeys	PTA (conditioned)	Significant increase in the standard deviation of hearing thresholds after 40 days. The magnitude of the standard deviation continued to grow for 54 additional days and thereafter declined. Mean hearing thresholds between the control and exposed groups did not vary significantly. Daily oral doses of parathion caused decrement in tone reporting behaviour.	(330)
Paraquat (pyridylium compound)					
0, 0.01-10 mM	*In vitro* incubation of tissues for 24 h	Mice, cochlear organo-typic cultures	ME	Number of OHC and inner hair cells systematically decreased with increasing concentration of paraquat. To inactivate the superoxide radical generated by paraquat, M40403, a non-peptidyl mimetic of superoxide dismutase was added to some cultures. M40403 significantly increased hair cell survival.	(276)
0, 3, 5, 10 mM	Round window administration by injection	Chinchillas	IC EVP ME	EVPs increased in a dose-dependent manner, peaked 1-7 days post-exposure, and showed a small amount of recovery before reaching significant permanent threshold shift by day 22. OHC and inner hair cell losses were consistent with threshold shifts.	(33)
Hexachlorobenzene					
0, 0.16, 4, 16 mg/kg/bw/day	Orally, for 4 w, in olive oil	Rats	ABR ME	Reversible threshold changes (2-16 kHz) at 0.16 mg/kg. Permanent threshold shifts at all frequencies tested (1-32 kHz) at 16 mg/kg. No cochlear hair cell loss or alteration of stereocilia.	(148)

ABR: auditory brainstem response, bw: body weight, IC EVP: inferior colliculus evoked potential thresholds, ip.: intraperitoneal, ME: morphological examination, OHC: outer hair cell, PTA: pure-tone audiometry.

Table 29. Occupational studies on auditory effects of exposure to pesticides.

Exposure descriptors	Noise (N)	No. of exposed and controls	Results and comments	Reference
Organophosphorus chemicals (7.5 % malathion and 15 % metamidophos)	Not given	Case report	Patient with acute intoxication from spraying pesticides had profound bilateral hearing loss associated with residual peripheral neuropathy in the extremities.	(150)
Organophosphorus chemicals (malathion, monocrotophos, dimethoate)	Not given	34 exposed workers 34 controls	No significant difference in sensorineural hearing loss or auditory brainstem responses between the groups. No information on N exposure or other risk factors was given.	(98)
History of exposure to organophosphate and pyrethroid insecticides	Previous or current N exposure history, no levels given	92 exposed farm workers: 41 no excessive N 51 excessive N 54 controls: 36 no excessive N 18 excessive N	64 % of workers exposed to insecticides had hearing loss and 67 % of those exposed to insecticides+N. Mean hearing thresholds poorest among workers exposed to both agents. The median exposure time necessary to detect hearing loss was 3.4 yrs for workers exposed to both agents and 7.3 yrs for workers exposed to insecticides only. Central auditory effects were associated with insecticide exposure. The RR for central auditory disorders was 7.6 (95 % CI 2.9-19.8) for the group exposed to insecticides only when compared to the controls, 6.5 (95 % CI 2.2-20.0) for the group exposed to insecticides+N when compared to the non-exposed group, and 9.8 (95 % CI 1.4-64.5) when compared to the control group exposed to N. Lack of exposure records prevented evaluation of any interaction between N and the insecticides.	(369)
History of spraying crops with insecticides (including organophosphates and pyrethroids)	Not given	185 farmers	Hearing loss associated with a history of spraying crops with insecticides. Other factors associated with hearing loss were age, gender, high school education, firearms use and grain dryer operation.	(27)

Table 29. Occupational studies on auditory effects of exposure to pesticides.

Exposure descriptors	Noise (N)	No. of exposed and controls	Results and comments	Reference
History of spraying crops with insecticides (including organophosphates and pyrethroids compounds)	Not given	65 farmers, re-interviewed subjects from the study by Beckett *et al* (27)	Contrary to previous findings (27), no significant association between chemical exposure (combining mixing and applying pesticides) and hearing loss. The authors indicated that the study was limited by a smaller sample size than the original study, non-random selection of participant subjects and exposure estimation based on recall.	(26)
History of exposure to pesticides	History of N exposure, levels not given	150 migrant workers	More than half of the subjects had hearing loss in the higher audiometric frequencies. More than 35 % of respondents complained of subjective difficulty hearing or understanding speech.	(315)
History of exposure to pesticides and other toxicants (self-reports)	History of N exposure, levels not given	14 229 male licensed private pesticide applicators	35 % of the study group reported hearing loss. Logistic regression was performed with adjustment for location, age, noise, solvents and metals. The OR for hearing loss for the highest quartile of exposure was 1.19 (95 % CI 1.04-1.35) for insecticides and 1.17 (95 % CI 1.03-1.31) for organophosphate insecticides. ORs were elevated for high pesticide exposure events (1.38, 95 % CI 1.25-1.54), pesticide-related doctor visits (1.38, 95 % CI 1.17-1.62) or hospitalisation (1.81, 95 % CI 1.25-2.62) and diagnosed pesticide poisoning (1.75, 95 % CI 1.36-2.26).	(72)

CI: confidence interval, N: noise, OR: odds ratio, RR: relative risk.

10.2 Polychlorinated biphenyls (PCBs)

10.2.1 General

Polychlorinated biphenyls (PCBs) are synthetic organic chemicals, which are either oily liquids or solids and are colourless to light yellow. They have high boiling points and are practically non-flammable except at high temperatures. Some PCBs are volatile and may exist as vapour in air. PCB production started in the late 1920s (174) and PCBs were used extensively in the manufacture of transformers, capacitors and other heat transfer devices through the late 1970s. In 1979, their manufacture and importation was banned in the US based on mounting evidence that they were toxic to humans and wildlife. In Sweden, the use of PCBs was restricted in 1972 to only allow PCBs in closed systems. New equipment containing PCBs was not allowed for use from 1978, whereas old installations containing PCBs were allowed until 1995 (8, 29). No new PCB-containing products have been allowed in Norway since 1980, in Finland since 1985, in Denmark since 1986 and in Iceland since 1988 (8).

Occupational exposure to PCBs can take place during the renovating and demolishing of buildings, repair and maintenance of PCB transformers, accidents, fires, or spills involving PCB transformers and older computers and instruments, and disposal of PCB materials. In addition to older electrical instruments and fluorescent lights that contain PCB-filled capacitors, also caulking materials, elastic sealants and heat insulation have been known to contain PCBs. Exposure in the contaminated workplace occurs mostly by breathing air containing PCBs and by touching substances that contain PCBs (223).

PCBs are classified as probable human carcinogens and are listed in the top 10 % of the US EPA's most toxic chemicals (17, 167). Reproductive and developmental effects may also be related to consumption of PCB contaminated fish and possibly occupational exposure to PCBs. Skin conditions, such as acne and rashes, may occur in people exposed to high levels of PCBs.

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) is presently preparing a criteria document on PCBs (223).

10.2.2 Effects of PCBs

Aroclor 1254 is the PCB mixture most investigated for its auditory effects but also other PCB mixtures as well as the individual congener PCB 126 (3,3',4,4',5-pentachlorobiphenyl) have been studied for their ototoxicity in the offspring of rats (Table 30). All studied PCBs were found to be ototoxic with a suggested cochlear and/or auditory nerve site of lesion. Aroclor has also been shown to affect development of the hearing organ through induction of hypothyroidism (145, 300).

PCB 126 administrated by feed (35 days prior to breeding to postnatal day 21) caused no significant auditory effects at 0.25 µg/kg body weight/day (NOAEL), whereas elevated hearing thresholds were observed at 1 µg/kg body weight (LOAEL) (76).

Aroclor 1254 administered by oral gavage (from gestation day 6 to postnatal day 21) caused permanent auditory deficits at 1 mg/kg body weight/day (LOAEL, lowest dose tested) (153).

Oral gavage of a mixture of PCBs containing 35 % Aroclor 1242, 35 % Aroclor 1248, 15 % Aroclor 1254 and 15 % Aroclor 1260 (28 days prior to breeding to postnatal day 21) caused no auditory effects at 1 mg/kg body weight/day (NOAEL) but auditory deficits at 3 mg/kg (LOAEL) (300).

Interaction with noise has not been studied in animals nor have auditory effects of PCBs in humans.

10.2.3 Conclusion on PCBs

PCB mixtures as well as the individual congener PCB 126 were ototoxic with a suggested cochlear and/or auditory nerve site of lesion in the offspring of rats. Interaction with noise was not studied. No human studies were identified.

International OELs for PCBs range from 0.001 to 0.5 mg/m^3 (Appendix 1).

Table 30. Auditory effects in offspring of rats exposed to polychlorinated biphenyls (PCBs) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal model	Auditory test on offspring	Results and comments	Reference
Maternal dose level	Regimen				
PCB 126					
0, 0.25, 1.0 µg/kg bw/day in corn oil	In diet, 5 d/w for 35 days prior to breeding to PND 21	Nulliparous rats	ASR RMA	NOAEL at 0.25 µg/kg. Elevated auditory thresholds for 0.5- and 1-kHz tones at 1.0 µg/kg (LOAEL). Thresholds not significantly affected at higher frequencies.	(76)
Aroclor 1254					
0, 1, 4, 8 mg/kg bw/day in corn oil	Gavage from GD 6 to PND 21	Rats	ASR RMA	NOAEL at 1 mg/kg. Permanent auditory deficits (20-30 dB threshold shift) at the lowest frequency tested (1 kHz) at 4 mg/kg (LOAEL) and 8 mg/kg. Auditory thresholds not significantly affected at higher frequencies (4, 16, 32 or 40 kHz).	(145)
0, 1, 4, 8 mg/kg bw/day in corn oil	Gavage from GD 6 to PND 21	Rats	ABR at ~1 yr of age	Decreased ABR amplitudes at 1 and 4 kHz but not at 16 or 32 kHz in all dose groups (LOAEL at 1 mg/kg). A dose-related decrease in the baseline-to-peak P1A amplitude for 1-kHz (80-dB) stimulus. Decreased peak-to-peak amplitudes. Such decreases in peak P1A amplitudes are consistent with a dysfunction of the cochlea and/or auditory nerve. Data confirm that Aroclor 1254 produce a permanent low- to mid-frequency auditory dys-function and suggest a cochlear and/or auditory nerve site of action.	(153)
0, 6 mg/kg bw/day in corn oil	Gavage from GD 6 to PND 21	Rats	ABR DPOAE at ~18 months of age	Reduced DPOAE amplitudes and increased auditory thresholds. Deficits most pronounced at the lowest frequencies tested (2.1-3.2 kHz) but also observed at higher frequencies (3.7-8.6 kHz). In contrast, ABR latencies and amplitudes were not altered. Evidence supports a cochlear site of damage in PCB-induced hearing loss.	(203)

Table 30. Auditory effects in offspring of rats exposed to polychlorinated biphenyls (PCBs) alone, in order from lowest to highest exposure level.

Exposure conditions		Animal model	Auditory test on offspring	Results and comments	Reference
Maternal dose level	Regimen				
0, 6 mg/kg bw/day in corn oil	Gavage from GD 6 to PND 21	Primiparous rats	RMA	On the day of birth, half of the treated and half of the control litters were cross-fostered: Ctrl/Ctrl (controls), A1254/A1254 (perinatal exposure), A1254/Ctrl (prenatal exposure only), Ctrl/A1254 (postnatal exposure only). Permanent hearing deficits (20-dB increase) at the low frequency (1 kHz) in the A1254/A1254 and Ctrl/A1254 groups.	(81)
0, 8 mg/kg bw/day in corn oil	Gavage from GD 6 to PND 21	Rats	RMA ME 6 w after RMA	Elevated auditory thresholds (26 dB) for 1-kHz tones. Thresholds not significantly affected at higher frequencies (4, 16 and 40 kHz). Mild to moderate loss of OHC in the upper-middle and apical turns of the cochlea. Inner hair cells and ganglion cells were not affected.	(82)
PCB mixture (35 % Aroclor 1242, 35 % Aroclor 1248, 15 % Aroclor 1254, 15 % Aroclor 1260)					
0, 1, 3, 6 mg/kg bw/day in corn oil	In diet, 28 days prior to breeding to PND 21	Rats	ABR DPOAE	NOAEL at 1 mg/kg. Decreased DPOAE amplitudes and elevated DPOAE and ABR thresholds across a range of frequencies at 3 mg/kg (LOAEL) and 6 mg/kg.	(300)

ABR: auditory brainstem response, ASR: acoustic startle response, bw: body weight, DPOAE: distortion product otoacoustic emissions, GD: gestation day, LOAEL: lowest observed adverse effect level, ME: morphological examination, NOAEL: no observed adverse effect level, OHC: outer hair cell, PCB: polychlorinated biphenyl, PCB 126: 3,3',4,4',5-pentachlorobiphenyl, PND: postnatal day, RMA: reflex modification audiometry.

11. Dose-effect and dose-response relationships

The chemicals examined in the present document have been associated with auditory effects in animals. They are substances with diverse chemical structures. This implies multiple targets for injury within the auditory system and multiple possible underlying mechanisms. This complexity represents an obstacle in identifying the features necessary for a chemical to be ototoxic (113, 116).

Another complexity is that different species respond differently to the same chemical, basically due to metabolic differences. On the other hand, this has offered some clues as to the toxic action (84, 117, 213). Since noise is often present in the occupational arena, there is a need to incorporate noise exposure in the investigations of ototoxicity of industrial chemicals. This adds to the complexity of the problem. Little is known about combined chemical exposures, and even less is known about mechanisms for interaction between a chemical and a physical agent, in this case noise, which makes prediction of the outcome challenging.

At this writing, the existing human studies were designed to generate or test hypotheses instead of examining dose-response relationships. Limitations of the reviewed studies (study designs, insufficient characterisation of the exposure levels of chemicals and noise, lack of details on if and how other risk factors were accounted for, etc.) preclude the use of their results in estimating dose-response relationships and in identifying NOAELs or LOAELs for the chemicals covered in the present document.

Overall, studies conducted with experimental animals provide the most robust evidence regarding mechanisms and dose-effect relationships between agents and effects on the auditory function or physiology.

Styrene, toluene, lead and carbon monoxide are the substances that have been more extensively studied to date, due to the relevance to occupational health and evidence of their general toxicity or neurotoxicity.

NOAELs and LOAELs for auditory effects in animals for the chemicals covered in this document are summarised in Table 31. If a chemical exposure has not been shown to potentiate noise-induced hearing loss, the level given is considered as the NOAEL for the substance in the combined exposure scenario.

Table 32 presents the most relevant human studies indicating auditory effects by chemical exposure.

Styrene

The ototoxicity of styrene has been confirmed in numerous studies and 300 ppm was found to be the LOAEL in inhalation experiments with rats forced to be active (210). A more recent study using gavage exposure of non-active rats showed effects at 200 mg/kg body weight (corresponding to approximately 250 ppm) (64). Synergistic interaction between styrene and noise was manifested only at concentrations above the LOAEL for styrene alone, i.e. at and above 400 ppm (208, 210, 269).

Several occupational studies were also conducted and the preponderance of the evidence indicates that workers exposed to styrene are at increased risk of hearing loss. This was demonstrated not only by increased prevalences of cases of hearing loss, but also by significant differences in hearing thresholds and in other tests evaluating central auditory function compared to control populations. Such tests allowed for a distinction between the effects of the solvent and effects of noise. A limitation of the occupational studies is the incomplete historical characterisation of the exposure to styrene. This calls for caution when interpreting dose-effect relationships from human studies. The lowest current average exposures among styrene-only and styrene and noise exposed workers exhibiting hearing loss (compared to noise-only or no noise-no styrene workers) were 2.8-3.5 ppm. However, neither current nor life-time (average 14-18 ppm) styrene air concentrations correlated with hearing loss, whereas current urinary MA levels were positively correlated with hearing thresholds (187, 256). Other studies have, on the other hand, demonstrated hearing loss in workers currently exposed to average styrene air levels of 5-22 ppm and noise below 85 dB (240, 258, 259). Śliwińska-Kowalska *et al* found a significant positive correlation between the styrene exposure (work-life average 14 ppm) and hearing loss (346, 347). However, more recently, Triebig *et al* observed hearing loss in workers exposed to styrene only after exposure during at least 10 years to levels around 30-50 ppm styrene, with higher levels in the past (374).

Toluene

In studies with rats, 900 ppm was identified as the NOAEL for toluene (308). In studies of combined exposure to toluene and noise, a NOAEL of 500 ppm was identified (39, 234). Synergistic interaction between toluene and noise was manifested at the same concentration as the LOAEL for toluene alone, i.e. at and above 1 000 ppm (39, 186).

Several occupational studies were conducted and the preponderance of the evidence indicates that workers exposed to toluene are at increased risk for auditory disorders. One human study did not find significant effects that could be attributed to life-time weighted average toluene exposure at around 50 ppm (342). Other observations suggested that the lowest current exposures associated with significant hearing loss were 10-50 ppm (31, 257, 384). Historic toluene and/or noise exposure levels were not well characterised and some groups of workers were co-exposed to other solvents. It is likely that participants were exposed to higher concentrations in the past, as well as to peaks of high exposure in the present that could explain the observed effects. In one study, chronic exposure (12-14 years) to an average of 97 ppm caused hearing loss but noise levels were not given (2).

Carbon disulphide

Most investigations on the neurotoxicant carbon disulphide have focused on its central auditory effects but cochlear effects have also been reported. In rats, 500 ppm was identified as the NOAEL, while significant effects were observed at 800 ppm (LOAEL) as measured by auditory brainstem response (66, 157, 323).

Interaction with noise was not studied. In humans, the age-adjusted risk for audiometric hearing loss was significantly increased for exposures above 14 ppm in combination with noise in the range 80-91 dBA (58). Auditory effects (evoked potentials) were also observed in workers exposed to carbon disulphide (current exposure levels 3-8 ppm and higher exposure in the past) for at least 20 years. Noise levels were not reported (158). The workers were weavers, and thus a rather high noise level could be expected.

Other solvents

Xylene, ethylbenzene, chlorobenzene, trichloroethylene, *n*-hexane and *n*-heptane have been less studied for ototoxicity than styrene and toluene, but the existing animal data indicate that they cause auditory effects. A synergistic interaction has been shown between noise and exposure to ethylbenzene and trichloroethylene, respectively (the only substances investigated). No or limited human data were identified concerning auditory effects of these solvents.

Solvent mixtures

Solvent mixtures are important even if risk assessment is impossible due to the different nature of mixtures used at workplaces. However, some studies indicate that exposure to solvent mixtures containing ototoxic solvents (e.g. styrene, toluene, xylenes, ethylbenzene, trichloroethylene, *n*-hexane and jet fuels) and with a hazard index exceeding 1 may cause auditory effects.

Lead

Most investigations on lead have focused on its central auditory effects but also cochlear effects have been reported. In monkeys, blood concentrations of 35-40 µg/dl (NOAEL) did not cause significant changes in evoked potentials, whereas 55 µg/dl did (LOAEL) (202, 221). In humans, mean/median and life-time weighted average blood lead concentrations of 28-57 µg/dl were significantly associated with auditory effects (13, 35, 70, 89, 90, 110, 156, 177, 266, 386). Noise exposure was not reported in most studies, since they focused on central auditory effects. However, the only study that controlled for noise did not detect an interaction between the two agents. Peripheral auditory effects were associated with a mean blood lead level of 57 µg/dl (386).

Mercury

Mercury is known to cause neurotoxicity and sensorineural hearing deficits. In monkeys, ingestion (orally during gestation and postnatally for 4 years) of methylmercuric chloride at 10 Hg µg/kg body weight/day was associated with permanently poorer hearing thresholds (LOAEL) (332). Interaction with noise was not studied. In humans, current mercury concentration in air of 0.008 mg/m^3 and mean blood mercury levels of presumably 5 µg/l were significantly associated with effects as shown in central auditory tests (evoked potentials) (260). Higher concentrations were associated with similar outcomes (71, 89). Noise levels were not reported.

Trimethyltins
Intraperitoneally injected trimethyltin caused auditory effects in both guinea pigs and rats. Interaction with noise was not studied. No human studies were identified.

Carbon monoxide
In rats exposed to noise and carbon monoxide, the calculated LOAELs (lower bounds for the benchmark concentrations) were 320 ppm for a 5-dB potentiation of noise-induced hearing loss and 194 ppm for an increase in auditory threshold equivalent to 10 % of the effect of noise alone (100-dB octave band noise for 8 hours) (130). Without concomitant noise exposure, no auditory effects of carbon monoxide were reported in rats (130, 233). A more serious implication is that in scenarios in which *noise exposure alone did not have a significant effect,* noise in combination with carbon monoxide exposure produced unexpectedly large, permanent threshold shifts (61, 62, 130, 320). The data did not validate the anticipated positive relationship between the percentage of time that noise is present (noise duty cycle) and hearing loss. Instead, the mildest noise duty cycle (short duration exposures interspersed by quiet periods) produced maximal hearing loss when carbon monoxide was also present. The potentiation was more likely to occur with moderate noise exposure levels (octave band noise: 100 dB for 2 hours or 105 dB for 1 hour) than more severe noise exposures. Rao and Fechter used the US OSHA's 5-dB time-intensity exchange rate to manipulate their noise exposures. The greatest potentiation by carbon monoxide occurred at levels equivalent to the US permissible exposure level of 90 dB for 8 hours (320). Their observations raise the issue of the appropriateness of the time-intensity paradigm in combined exposure circumstances.

Most field studies lack noise exposure estimates. It is therefore not clear if noise exposure is a prerequisite for the auditory effects seen following long-term occupational exposure to carbon monoxide. In a study analysing 6 812 audiograms, exposure to carbon monoxide and noise levels below 90 dBA had no effect on hearing thresholds, whereas workers who were exposed to carbon monoxide and to noise levels above 90 dBA displayed significantly poorer hearing thresholds at high frequencies (conference proceedings) (200, 217). In humans, the only dose-response data available is based on two individuals for which audiometric data indicated a significant effect for exposures in the 16-35 ppm range when combined with noise (85-90 dBA) (199).

Other asphyxiants
Hydrogen cyanide (inhalation) and *acrylonitrile* (subcutaneous injection) alone were not ototoxic in rats but potentiated the effects of noise. *IDPN* (intraperitoneal injection) alone caused auditory changes in rats. Interaction with noise was not studied. No human studies on auditory effects by these agents were identified.

Pesticides
Results from animal studies as well as human data indicate that pesticides (e.g. organophosphorus compounds, paraquat and hexachlorobenzene) may affect

auditory function. Interaction with noise was not studied. Due to the sparse amount of data on this diverse group of chemicals, no hazard assessment can be performed.

PCB

PCB mixtures (including Aroclor 1254) as well as the individual congener PCB 126 were ototoxic with a suggested cochlear and/or auditory nerve site of lesion in the offspring of rats. Interaction with noise was not studied. No human studies were identified.

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Styrene (STY)							
STY alone							
-	200 mg/kg bw ~ 250 ppm	-	Gavage: 5 d/w, 3 w	Rats	ABR ME	OHC loss at 200 mg/kg bw (lowest dose tested). Hearing loss at 300 mg/kg bw.	(64)
-	300	-	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats, active	ABR ME	OHC loss at 300 ppm (lowest dose tested). Hearing loss at 500 ppm.	(210)
-	500	-	Inhalation: 6 h/d, 5 d/w, 4 w	Young rats, sedentary	ABR ME	OHC loss at 500 ppm (lowest dose tested). Hearing loss at 850 ppm.	(210)
300	600	-	Inhalation: 12 h/d, 5 d/w, 4 w	Rats	ABR ME	Hearing loss and OHC loss.	(268)
STY combined with noise (N)							
-	400	85 dB Leq8h, OBN at 8 Hz, (86.2 dB SPL)	Inhalation and N: 6 h/d, 5 d/w, 4 w	Young rats, active	ABR ME	OHC loss (synergism, only dose tested). Hearing loss not different from N.	(210)
300	600	100-105 dB SPL	Inhalation and N: 12 h/d, 5 d/w, 4 w	Rats	ABR ME	Hearing loss and OHC loss at 600 ppm (synergism). Hearing loss not different from N at 300 ppm.	(269)
Toluene (TOL)							
TOL alone							
-	1 000	-	Inhalation: 6 h/d, 5 d/w, 4 w	Rats	ABR ME	OHC loss (lowest dose tested). No hearing loss.	(46, 229)
-	1 000	-	Inhalation: 16 h/d, 5 or 7 d/w, 2 w	Young rats	ABR	Hearing loss (only dose tested).	(182, 185, 186)
700	1 000	-	Inhalation: 14 h/d, 7 d/w, 16 w	Young rats	ABR BA CAR	Hearing loss.	(311)

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
900	1400	-	Inhalation: 14 h/d, 7 d/w, 14 w	Weanling rats	ABR, BA CAR	Hearing loss.	(308)
TOL combined with noise (N)							
500	-	90 dB SPL, steady WBN 4-20 kHz (~87 dB Leq8h)	Inhalation: 6 h/d, 5 d/w, 90 d N: 4 h/d, 5 d/w, 90 d	Rats	ABR DPOEA	Hearing loss not different from N (highest dose tested).	(234)
500	1 000	96 dB SPL (~90 dB Leq8h)	Inhalation: 6 h/d, 10 d N: 2 h/d, 10 d (after TOL exposure)	Young rats	ABR	Hearing loss at 1 000 ppm (synergism). Hearing loss not different from N at 500 ppm.	(39)
-	1 000	100 dB Leq8h	Inhalation: 16 h/d, 5 d/w, 2 w N: 10 h/d, 7 d/w, 4 w (after TOL exposure)	Young rats	ABR	Hearing loss (synergism, only dose tested).	(186)
Xylene (XYL)							
p-XYL alone, mixed XYL (10 % o-, 80 % m- and 10 % p-XYL, EBZ content not given) and mixture (o-, m- and p-XYL and EBZ)							
450 *p*-XYL	900 *p*-XYL	-	Inhalation: 6 h/d, 6 d/w, 13 w	Rats	ABR ME	OHC loss (*o*- and *m*-xylene not ototoxic).	(140)
-	1 800 *p*-XYL	-	Inhalation: 6 h/d, 5-6 d/w, 3 or 13 w	Rats	ABR ME	Hearing loss and OHC loss (*o*- and *m*-xylene not ototoxic, only dose tested).	(140, 238)
-	800 mixed XYL	-	Inhalation: 14 h/d, 7 d/w, 6 w	Rats	ABR BA CAR	Hearing loss (lowest dose tested).	(306)
-	250 Mixture (~50 *p*-XYL+50 EBZ)	-	Inhalation: 6 h/d, 5 d/w, 13 w	Rats	ABR ME	OHC loss (synergism, lowest dose tested). Hearing loss at 1 000 ppm mixture (synergism).	(141)
XYL combined with noise (N) No data							

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Ethylbenzene (EBZ)							
EBZ alone and in mixture (EBZ and o-, m- and p-XYL)							
-	200	-	Inhalation: 6 h/d, 5 d/w, 13 w	Rats	ABR ME	OHC loss (lowest dose tested). Hearing loss at 400 ppm.	(141)
300	400	-	Inhalation: 6 h/d, 5 d	Rats	CAP ME	Hearing loss and OHC loss.	(51)
-	250 Mixture (~50 EBZ+50 *p*-XYL)	-	Inhalation: 6 h/d, 5 d/w, 13 w	Rats	ABR ME	OHC loss (synergism, lowest dose tested). Hearing loss at 1 000 ppm mixture (synergism).	(141)
EBZ combined with noise (N)							
-	300	95 or 105 dB SPL broadband 1.5-12.5 kHz	Inhalation: 6 h/d, 5 d N: 8 h/d, 5 d	Rats	CAP DPOEA ME	OHC loss (when combined with 105 dB), (synergism, lowest dose tested). Hearing loss not different from N at 400 ppm.	(52)
Chlorobenzene (CBZ)							
CBZ alone							
-	2 000	-	Inhalation: 6 h/d, 5 d	Rats	ABR at 16 kHz only	Hearing loss (lowest dose tested). The only study identified.	(328)
CBZ combined with noise (N) No data							
Trichloroethylene (TCE)							
TCE alone							
-	2 000	-	Inhalation: 12 h/d, 7 d/w, 3 w	Rats	ABR	Hearing loss (mid-frequency, lowest dose tested).	(327)
1 600	2 400 1 707 (BMC)	-	Inhalation: 6 h/d, 5 d/w, 13 w	Rats	RMA at 16 kHz only	Hearing loss (mid-frequency). BMC: 15-dB increase in hearing threshold.	(75)
2 400	3 200 1 418 (BMC)	-	Inhalation: 6 h/d, 5 d/w, 4 w	Rats	RMA at 16 kHz only	Hearing loss (mid-frequency). BMC: 15-dB increase in hearing threshold.	(75)

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
1 600	3 200	-	Inhalation: 12 h/d, 7 d/w, 12 w	Rats	ABR	Hearing loss (mid-frequency).	(327)
TCE combined with noise (N)							
-	3 000	95 dB SPL	Inhalation: 18 h/d, 5 d/w, 3 w	Rats	RMA	Hearing loss (mid-frequency, only dose tested). Generally additive effect. Synergism at 4 kHz.	(262)
n-Hexane (n-HEX)							
n-HEX alone							
1 000	-	-	Inhalation: 18-21 h/d, 7 d/w, 28 or 61 d	Rats	ABR	No auditory effects (only dose tested).	(286, 287)
1 000	-	-	Inhalation: 24 h/d, 6 d/w, 11 w	Rats	ABR	Reversible auditory effects (only dose tested).	(163)
-	1 000	-	Inhalation: 24 h/d, 5 d/w, 11 w	Rats	ABR	Auditory effects (prolonged latencies and decreased ABR amplitudes, only dose tested).	(325)
n-HEX combined with noise (N)							
No data							
n-Heptane (n-HEP)							
n-HEP alone							
800	4 000	-	Inhalation: 6 h/d, 7 d/w, 28 d	Rats	ABR	Auditory effects (decreased ABR amplitudes). The only study identified.	(344)
n-HEP combined with noise (N)							
No data							

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Carbon disulphide (CS_2)							
CS_2 alone							
200	800	-	Inhalation: 6 h/d, 5 d/w, 15 w	Rats	ABR	Auditory effects (reversible delayed ABR interpeak latencies).	(157)
400	800	-	Inhalation: 7 h/d, 7 d/w, 11 w	Rats	ABR	Auditory effects (effects on ABR latencies).	(323)
500	-	-	Inhalation: 6 h/d, 5 d/w, 12 w	Rats	RMA	No hearing loss (only dose tested).	(66)
CS_2 combined with noise (N)							
No data							
Solvent mixture (per se) (MIX)							
White spirit (dearomatised) alone							
800 ppm	-	-	Inhalation: 6 h/d, 5d/w, 6 m	Rats	ABR	No hearing loss.	(236)
Jet fuel (JP-8) alone							
-	1 000 mg/m^3	-	Inhalation: 4 h/d, 1 or 5 d	Rats	CAP DPOEA ME	Hearing loss (decrease in DPOAE amplitude) after repeated exposure. No OHC loss. Only dose tested.	(132)
Jet fuel (JP-8) combined with noise (N)							
-	1 000 mg/m^3	97, 102 or 105 dB OBN 8 kHz	Inhalation: 4 h/d, 1 or 5 d N: 4 h (105 dB), 1 h/d, 5 d (102 dB) or 4 h/d, 5 d (97 dB)	Rats	CAP DPOEA ME	Hearing loss (decrease in DPOAE amplitude) and OHC loss greater than by N. OHC loss only after repeated exposure. Only dose tested.	(132)

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Lead (Pb) (blood level)							
Pb alone							
35 µg/dl	55 µg/dl	-	In diet: prenatal to ~ 10 yrs of age	Monkeys	ABR	Auditory effects (prolongations of ABR latencies).	(221)
35-40 µg/dl	-	-	In milk: birth to 1 or 2 yrs of age	Monkeys	ABR, DPOAE, Tympano-metry	No auditory effects.	(202)
Pb combined with noise (N)							
No data							
Mercury (Hg)							
Hg alone							
-	10 µg Hg/kg bw/d as CH_3HgCl	-	Orally: gestation to 4 yrs of age	Monkeys	PTA	Hearing loss in all animals at 19 yrs of age. The deterioration in hearing between 11 and 19 yrs was more pronounced in exposed than in control monkeys. Lowest dose tested.	(332)
Hg combined with noise (N)							
No data							
Trimethyltins (TMT)							
TMT alone							
-	0.2 mg/kg bw	-	Single ip. injection	Guinea pigs	CAP, CM, SP	Auditory effects (latency delays in CAP, lowest dose tested).	(118)
2 mg/kg bw	4 mg/kg bw	-	Single ip. injection	Rats	ASR	Hearing loss.	(393)
-	3 mg/kg bw	-	Single ip. injection	Rats	ABR, ASR, ME	Hearing loss (ABR thresholds) and OHC loss. Lowest dose tested.	(79)
TMT combined with noise (N)							
No data							

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Carbon monoxide (CO)							
CO alone							
1 500	-	-	3.5-9.5 h	Rats	CAP CM	No auditory effects.	(61)
CO combined with noise (N)							
300	500	110 or 115 dB Lin, 4 kHz OBN	Inhalation: 9.5 h N: 8 h	Rats	CAP CM	Potentiation of NIHL (synergism).	(61)
300	500 194, 320 (BMCs)	100 dB Lin, 13.6 kHz OBN	Inhalation: 9.5 h N: 8 h	Rats	CAP	Potentiation of NIHL increased linearly as CO increased between 500 and 1 500 ppm (synergism). BMCs (lower bounds): Increase in auditory threshold equivalent to 10 % of the effect by N at 194 ppm and 5-dB potentiation of NIHL at 320 ppm.	(130)
300	500	84 dB SPL Leq8h, impulsive	Inhalation: 8 h/d, 10 d N: 6 h/d, 10 d	Rats	ABR DPOAE	Potentiation of NIHL (synergism).	(233)
Hydrogen cyanide (HCN)							
HCN alone							
50	-	-	Inhalation: 3.5 h	Rats	CAP ME	No hearing loss or OHC loss. The only study identified.	(126)
HCN combined with noise (N)							
10	30 2-16 (BMCs)	100 dB Lin, OBN	Inhalation: 3.5 h N: 2 h	Rats	CAP ME	Potentiation of NIHL and OHC loss. BMCs (lower bounds): Impaired auditory threshold 10 % above the effect by N at 2 ppm, 5-dB potentiation of NIHL at 9 ppm and 1 SD elevation of NIHL at 16 ppm. The only study identified.	(126)

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Acrylonitrile (ACN)							
ACN alone							
50 mg/kg bw	-	-	Sc. injection: 1, 2 or 5 d	Rats	CAP	No hearing loss (transient loss of auditory threshold sensitivity immediately after exposure). Only dose tested.	(127, 131)
50 mg/kg bw	-	-	Sc. injection: 5 d	Rats	CAP DPOEA ME	No hearing loss or OHC loss. Only dose tested.	(297)
ACN combined with noise (N)							
-	50 mg/kg bw	108 dB OBN	Sc. injection: 1 or 2 d N: 8 h/d, 1 d	Rats	CAP	Potentiation of NIHL (permanent loss of auditory threshold sensitivity). Only dose tested.	(131)
-	50 mg/kg bw	105 dB Lin OBN	Sc. injection: 1 or 5 d N: 4 h/d, 1 or 5 d	Rats	CAP	Potentiation of NIHL (permanent loss of auditory threshold sensitivity). Only dose tested.	(127)
-	50 mg/kg bw	95 or 97 dB OBN	Sc. injection: 5 d N: 4 h/d, 5 d	Rats	CAP DPOEA ME	Hearing loss (permanent threshold shifts, decrease in DPOEA amplitudes) and OHC loss. No effect of ACN or N alone. Only dose tested.	(297, 299)
3,3´-Iminodipropionitrile (IDPN)							
IDPN alone							
100 mg/kg bw	150 mg/kg bw	-	Ip. injection: 3 consecutive days	Rats	RMA	Hearing loss (decreased amplitude of acoustic startle reflex).	(73)
150 mg/kg bw	167 mg/kg bw	-	Ip. injection: 3 consecutive days	Rats	ABR RMA	Hearing loss (elevated auditory thresholds for a broad spectrum of frequencies).	(80)
IDPN combined with noise (N)							
No data							

Table 31. Critical studies on auditory effects in animals with corresponding NOAELs and LOAELs (in ppm, if not otherwise stated) for the substances reviewed in the present document. For abbreviations, see end of table.

NOAEL	LOAEL	Noise (N)	Exposure regimen	Species	Auditory test	Auditory effect	Reference
Pesticides							
Insufficient data							
Polychlorinated biphenyls (PCBs)							
PCBs alone							
0.25 µg/kg bw PCB 126	1 µg/kg bw PCB 126	-	Gavage: 5 d/w, 35 d prior to breeding to PND 21	Nulli-parous rats, offspring	ASR RMA	Hearing loss (elevated auditory thresholds for low-frequency tones but not for higher frequencies).	(76)
-	1 mg/kg bw Aroclor 1254	-	Gavage: GD 6 to PND 21	Rats, offspring	ABR	Hearing loss (decreased ABR amplitude for low-frequency tones but not for higher frequencies). Lowest dose tested.	(153)
1 mg/kg bw PCB mixture	3 mg/kg bw PCB mixture	-	In diet: 28 days prior to breeding to PND 21	Rats, offspring	ABR DPOEA	Hearing loss (elevated ABR and DPOEA thresholds across a range of frequencies. Decreased DPOEA amplitudes).	(300)
PCBs combined with noise (N)							
No data							

Substance abbreviations: ACN: acrylonitrile, CBZ: chlorobenzene, CO: carbon monoxide, CS_2: carbon disulphide, EBZ: ethylbenzene, HCN: hydrogen cyanide, HEP: heptane, HEX: hexane, Hg: mercury, IDPN: 3,3´-iminodipropionitrile, MIX: solvent mixture, Pb: lead, PCB: polychlorinated biphenyl, STY: styrene, TCE: trichloro-ethylene, TOL: toluene, TMT: trimethyltin, XYL: xylene.

Other abbreviations: ABR: auditory brainstem response, ASR: acoustic startle response, BA: behavioural audiometry, BMC: benchmark concentration, bw: body weight, CAP: compound action potential, CAR: conditioned avoidance response, CM: cochlear microphonics, DPOAE: distortion product otoacoustic emissions, GD: gestation day, ip.: intraperitoneal, Leq8h: equivalent level of noise during 8 hours, Lin: linear, LOAEL: lowest observed adverse effect level, MAEP: middle latency auditory evoked potentials, ME: morphological examination, N: noise, NIHL: noise-induced hearing loss, NOAEL: no observed adverse effect level, OBN: octave band noise, OHC: outer hair cell, PND: postnatal day, PTA: pure-tone audiometry, RMA: reflex modification audiometry, sc.: subcutaneous, SD: standard deviation, SP: summating potential, SPL: sound pressure level, WBN: wide band noise.

Table 32. Critical studies on auditory effects in humans for the substances reviewed in the present document (exposure levels in ppm, if not otherwise stated). Hearing loss means changes measured with pure-tone audiometry. Auditory dysfunction means changes measured in the central auditory system with evoked potential testing or other central tests. For abbreviations, see end of table.

Current exposure level, mean ± SD (range)	Exposure duration	Noise (N)	Size of study group	Auditory effect	Reference
Styrene (STY)					
3.5 (0.05-22) (STY) 2.8 (0.007-12) (STY+N) Average work-life exposure: 18 (STY) 14 (STY+N)	17 (1-39) yrs (STY) 15 (2-37) yrs (STY+N)	≤84 dBA (STY) 89 dBA (STY+N) 86 dBA (N)	65 STY 89 STY+N 78 N 81 controls	Hearing loss and auditory dysfunction (speech) in STY and STY+N compared to N exposed and controls. Biological marker for STY (urinary MA) associated with hearing loss.	(187, 256)
~5 (0.7-14) (estimated from urinary MA+PGA)	7±6.2 yrs	73 dBA	32 STY 60 controls	Hearing loss in STY compared to the age-matched controls.	(240)
8 (0.1-93) < 50 for 87/93 workers	9.4±8.9 yrs	≤85 dB	44 STY 49 STY in mixture incl. TOL 33 controls	Hearing loss in high-frequency range in STY sub-group (n=54) exposed to >16 ppm for ≥5 yrs. The effect correlated to STY in air and to biological marker of STY (urinary MA).	(258)
Average work-life exposure: 14 ± 9.3 (STY) 8 ± 6 (STY+N)	-	80 dBA (STY) 89 dBA (STY+N) 89 dBA (N)	194 STY 56 STY+N 66 N 157 controls	Hearing loss in STY and in STY+N compared to N and controls. Average work-life exposure to STY correlated to hearing loss.	(346, 347)
22 (3.7-46)	5.4 yrs	69-76 dBA (STY) 82-86 dBA (N)	19 STY 18 N 11 controls	Hearing loss in high-frequency range in STY exposed compared to N and controls.	(259)
~2-50 (range of means, estimated from urinary MA+PGA)	6 (1-26) yrs	75-83 dBA	248 STY (low, medium, high)	Hearing loss in STY sub-group exposed to high levels (30-50 ppm, n=17) at least 10 yrs with levels >50 ppm in the past.	(374)

Table 32. Critical studies on auditory effects in humans for the substances reviewed in the present document (exposure levels in ppm, if not otherwise stated). Hearing loss means changes measured with pure-tone audiometry. Auditory dysfunction means changes measured in the central auditory system with evoked potential testing or other central tests. For abbreviations, see end of table.

Current exposure level, mean ± SD (range)	Exposure duration	Noise (N)	Size of study group	Auditory effect	Reference
Toluene (TOL)					
26 ± 20 Life-time weighted exposure: 45 ± 17	-	81-82 dBA	192 TOL No controls	No hearing loss. TOL levels or duration not associated with hearing loss. NOAEL estimated to 50 ppm by authors.	(342)
(9-37)	12 (2-24) yrs (TOL+N) 6 (3-15) yrs (N)	88-98 dBA TOL+N and N	50 TOL+N 50 N 40 controls Workers without signs of hearing loss only.	> 60 % of TOL+N and N groups had no response in TEOAE vs. 27 % of controls. 49 % of TOL+N group had no contralateral inhibition in TEOAE vs. 17 % of N and 7 % of controls. The OR for absence of contralateral inhibition was 12 (95 % CI 3.1-43.5).	(31)
34 (2-89) (estimated from TOL in blood)	21.4 (4-30) yrs	Not given	49 TOL 59 controls	Auditory dysfunction of ABR.	(384)
(0.04-244) in mixture+N ≤ 50 for 109/124 workers incl. ethyl acetate and ethanol	7.7 (1-25) yrs	71-93 dBA	124 TOL (in mix) + N No controls	Hearing loss in 49 %. Biological marker (urinary hippuric acid) for TOL correlated with hearing loss.	(257)
97	12-14 yrs	Not given	40 TOL 40 controls	Auditory dysfunction of ABR shown in TOL exposed (only workers with normal PTA included).	(2)
Carbon disulphide (CS_2)					
3-8 (ventilation improved 14 yrs ago)	2-7 yrs up to > 20 yrs	Not given	25 CS_2 (2-7 yrs) 34 CS_2 (> 20 yrs) 40 controls	Auditory dysfunction of ABR shown in CS_2 exposed > 20 yrs.	(158)
1.6-20.1	≥ 20 yrs for 90/131	80-91 dBA (CS_2+N) 83-90 dBA (N)	131 CS_2+N 105 N 110 controls	Higher prevalence (68 %) of hearing loss in exposed than in N group and controls. Greatly increased risk for exposures > 14.6 ppm.	(58)

Table 32. Critical studies on auditory effects in humans for the substances reviewed in the present document (exposure levels in ppm, if not otherwise stated). Hearing loss means changes measured with pure-tone audiometry. Auditory dysfunction means changes measured in the central auditory system with evoked potential testing or other central tests. For abbreviations, see end of table.

Current exposure level, mean ± SD (range)	Exposure duration	Noise (N)	Size of study group	Auditory effect	Reference
Xylene (XYL), ethylbenzene (EBZ), chlorobenzene (CBZ), trichloroethylene (TCE), n-hexane (n-HEX), n-heptane (n-HEP)					
No data					
Solvent mixtures					
Not sufficient data					
Lead (Pb) (blood level)					
(10-61) µg/dl	-	79-86 dBA	26 Pb+N 17 N	No hearing loss but auditory dysfunction (sensitised speech) in Pb+N group.	(177)
(12-64) µg/dl	1-18 yrs	Not given	22 Pb 22 controls	Auditory dysfunction (P300 and ABR). ABR correlated with Pb blood levels. Same study group as in Araki *et al* (13).	(266)
Median: 30 (12-59) µg/dl	1-18 yrs	Not given	22 Pb 14 controls	Auditory dysfunction (P300) in Pb exposed. Blood Pb level correlated with auditory dysfunction.	(13)
37 ± 4.4 µg/dl	-	< 50 dB	45 Pb 45 controls	Hearing loss in Pb exposed. Blood Pb level correlated with hearing loss.	(110)
28 ± 8 (4-62) µg/dl Life-time weighted average: 39 ± 12 (4-66) µg/dl Life-time integrated blood Pb index: 719 µg-yr/dl	17 (0.2-26) yrs	Not given	359 Pb No controls	Pb exposure interfered with ABR in a dose-dependent manner. Current and life-time weighted average blood Pb level associated with the ABR wave I latency while the life-time index was associated with the wave III latency.	(35)
42 ± 16 (13-67) µg/dl Previous 5 yrs of exposure: 31 ± 13 (18-70) µg/dl	17 (4-29) yrs	Not given	15 Pb 39 controls	Auditory dysfunction (ABR) in Pb exposed.	(156)

Table 32. Critical studies on auditory effects in humans for the substances reviewed in the present document (exposure levels in ppm, if not otherwise stated). Hearing loss means changes measured with pure-tone audiometry. Auditory dysfunction means changes measured in the central auditory system with evoked potential testing or other central tests. For abbreviations, see end of table.

Current exposure level, mean ± SD (range)	Exposure duration	Noise (N)	Size of study group	Auditory effect	Reference
45 ± 20 (11-80) µg/dl	-	Not given	30 Pb No controls	Auditory dysfunction (ABR) in Pb sub-group with mean Pb level 47 µg/dl. Sensorineural hearing loss (increased audiometric thresholds), which may be attributable to N combined with Pb intoxication.	(70)
47 ± 9.2 µg/dl	9 yrs	Not given	22 Pb 22 controls	Auditory dysfunction (ABR) in Pb group. Strongest effect when mean Pb level was > 50 µg/dl.	(89)
55 ± 16 µg/dl	7 yrs	Not given	49 Pb 49 controls	Auditory dysfunction (ABR) in Pb group. Strongest effect when mean Pb level was > 50 µg/dl for 3 yrs.	(90)
57 µg/dl	Long-term	86 dBA Leq	220 Pb 119 controls	Hearing loss correlated to high and long-term Pb exposure index (duration of employment and ambient Pb concentration). No correlation to N alone or to the interaction between N and short- or long-term Pb exposure.	(386)
Mercury (Hg)					
0.008 mg/m^3, presumably 5 µg/l blood	16 yrs (mean)	Not given	40 Hg 36 controls)	Auditory dysfunction (ABR) more frequent in exposed than in controls (42 % vs. 18 %).	(260)
325 ± 464 µg/g creatinine in urine	12 yrs (mean)	Not given	8 Hg 8 controls	Auditory dysfunction (ABR).	(89)
17.5 µg/l blood (exposed) 3.0 µg/l blood (controls)	-	Not given	36 Hg (children) 39 Hg (adults) 34 controls	Auditory dysfunction (ABR). Correlation between Hg blood levels and ABR.	(71)
Trimethyltin (TMT)					
No data					

Table 32. Critical studies on auditory effects in humans for the substances reviewed in the present document (exposure levels in ppm, if not otherwise stated). Hearing loss means changes measured with pure-tone audiometry. Auditory dysfunction means changes measured in the central auditory system with evoked potential testing or other central tests. For abbreviations, see end of table.

Current exposure level, mean ± SD (range)	Exposure duration	Noise (N)	Size of study group	Auditory effect	Reference
Carbon monoxide (CO)					
(16-35)	-	85-90 dBA CO+N 90-91 dBA N	2 CO 2 CO+N 3 N 21 controls	Hearing loss in CO+N at 8 kHz.	(199)
Hydrogen cyanide (HCN), acrylonitrile (ACN), 3,3´-iminodipropionitrile (IDPN)					
No data					
Pesticides					
Not sufficient data					
Polychlorinated biphenyls (PCBs)					
No data					

Substance abbreviations: ACN: acrylonitrile, CBZ: chlorobenzene, CO: carbon monoxide, CS_2: carbon disulphide, EBZ: ethylbenzene, HCN: hydrogen cyanide, HEP: heptane, HEX: hexane, Hg: mercury, IDPN: 3,3´-iminodipropionitrile, Pb: lead, PCB: polychlorinated biphenyl, STY: styrene, TCE: trichloroethylene, TOL: toluene, TMT: trimethyltin, XYL: xylene.

Other abbreviations: ABR: auditory brainstem response, CI: confidence interval, MA: mandelic acid, N: noise, NOAEL: no observed adverse effect level, N: noise, OR: odds ratio, PGA: phenylglyoxylic acid, PTA: pure-tone audiometry, SD: standard deviation. TEOAE: transient evoked otoacoustic emissions.

12. Evaluations and recommendations by national and international bodies

In its 1996 National Occupational Research Agenda, the US NIOSH identified both hearing loss and multiple exposures as research priorities for the occupational safety and health community. In two publications, NIOSH also argued for broadening the scope of risk assessment of hearing risks and preventive initiatives (280, 282). NIOSH and the American College of Occupational and Environmental Medicine both recommend that hearing loss prevention programmes take chemical exposures into account when monitoring hazards, assessing hearing and controlling exposures (11, 280, 282). These recommendations do not include specific information on exposure levels of concern.

ACGIH states that periodic audiograms are advised and should be carefully reviewed in settings where there may be exposures to noise and to carbon monoxide, lead, manganese, styrene, toluene or xylene. Other substances under investigations for ototoxic effects include arsenic, carbon disulphide, mercury and trichloroethylene (7).

In 1998, the US Army started requiring consideration of ototoxic chemical exposures for inclusion in hearing conservation programmes, "particularly when in combination with marginal noise" (375).

The most detailed and specific recommendation to date is one offered in 2003 by the US Army, in which it is stated that since the exposure threshold for ototoxic effects is not known, audiometric monitoring is necessary to find out if the substance is affecting the hearing of exposed workers. Yearly audiograms are recommended for workers whose airborne exposures (without regard to the use of respiratory protection) are at 50 % of the most stringent criteria for OELs (either of the US OSHA permissible exposure limit or the ACGIH threshold limit value) for toluene, xylene, *n*-hexane, organic tin, carbon disulphide, mercury, organic lead, hydrogen cyanide, diesel fuel, kerosene fuel, jet fuel, JP-8 fuel, organophosphate pesticides or chemical warfare nerve agents, regardless of the noise level (376).

Best practice guidelines recommending hearing tests for those exposed to ototoxic agents were also published in Australia and New Zealand, without information on exposure levels (20). Legislation regarding compensation for hearing loss associated with chemical exposure at work has changed in Australia (19) and Brazil (246) making it possible for workers to apply for compensation for hearing loss because of exposure to ototoxic chemicals in the workplace.

In February 2003, the European Parliament published the Directive 2003/10/EC on minimum health and safety requirements regarding the exposure of workers to the risks arising from noise. In the Directive, it is stated that when carrying out risk assessments, employers should "…give particular attention to: any effects on workers' health and safety resulting from interactions between noise and work-related ototoxic substances…" (108). In April 2004, because of its demonstrated ototoxicity, toluene was labelled as R48/20: Danger of serious damage to health

by prolonged exposure through inhalation. It was stated that toluene-induced chronic impairment of auditory function had been demonstrated in a number of animal studies, substantiated by morphological evidence of cell loss in the rat cochlea and that existing data suggest that humans are sensitive to this effect at exposure levels which may be encountered in the working environment (106, 107).

Besides the present document, comprehensive evaluations of ototoxic substances (387) and of the hazards of combined workplace exposure to noise and ototoxic chemical substances (105) have recently been published by other bodies.

The Canadian Occupational Health and Safety Research Institute (IRSST) (387) did not include data on the interaction between noise and chemicals. Still, the conclusions are in agreement with those of the present document in classifying lead and its inorganic compounds, toluene, styrene and trichloroethylene as "ototoxic substances".

The report published by the European Agency for Safety and Health (105) included noise interactions and focused on the qualitative properties of chemicals inducing ototoxic effects. The list of chemicals included is slightly different from that in the present document as is the rating strategy used. Still, conclusions are in agreement. The report from the European Agency also highlights policies from specific member states and the possible impact of the 2007 new regulations Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH), which will not be repeated here.

13. Evaluation of human health risks

13.1 Assessment of risks of hearing impairment

Robust evidence from a large number of animal studies has demonstrated that toluene, styrene, solvent mixtures, lead and carbon monoxide (the latter only in combination with noise) are ototoxic. For these substances, the number of existing studies is relatively large, and comprehensive approaches have been taken in investigating their ototoxicity (testing of different exposure parameters and combinations of agents, attempting benchmark dose calculations, testing of hypothesis for the inhibition of the observed effects).

Other chemicals that have been studied in less detail with respect to ototoxicity include xylenes, ethylbenzene, chlorobenzene, trichloroethylene, *n*-hexane, *n*-heptane, carbon disulphide, mercury, organotins, hydrogen cyanide, acrylonitrile, IDPN, pesticides and PCBs. Hitherto, the existing evidence indicates that also these substances have ototoxic properties (in some cases, only with concurrent noise).

Cochlear histopathology has been investigated in studies on several solvents, pesticides, PCBs, organotins and mercury, in conjunction with functional tests, often of evoked potentials. The studies have consistently demonstrated a greater effect with increasing exposure, independently of noise. This is also true for lead

exposures even if no cochlear histopathology reports were identified. However, an association between increasing blood lead levels and greater auditory dysfunction has been reported.

For chemicals such as *n*-hexane, *n*-heptane, carbon disulphide, lead and mercury, the auditory effect is connected to the neurotoxic effect of these substances. Thus, they exhibit more central neurotoxic effects than pure ototoxic effects.

Human data supports the evidence from animal studies that toluene, styrene, solvent mixtures and lead are ototoxic. Although less investigated, also trichloroethylene, carbon disulphide, mercury, carbon monoxide and some pesticides have been associated with auditory effects in humans. Observed auditory effects of *n*-hexane have been interpreted as a sign of its well-known central nervous system toxicity. No human studies on the ototoxicity of xylenes, ethylbenzene, chlorobenzene, *n*-heptane, organotins, hydrogen cyanide, acrylonitrile, IDPN and PCBs were identified, even though xylenes, ethylbenzene and chlorobenzene are common components in solvent mixtures that have been shown to be ototoxic in humans.

Early reports on solvents suggested that the exposure levels needed to cause an auditory effect in experimental animals were rather high in relation to OELs. In contrast, several occupational reports (on styrene, toluene, solvent mixtures and lead) indicated that much lower levels in industrial settings were associated with hearing deficits. The reasons for the difference between the lowest levels that cause an effect in humans and in animals, respectively, are not understood. However, recent research in animals has demonstrated that addition of other stressors (such as impact or continuous noise, other chemicals or drugs, or keeping the animals active during chemical exposure) reduces the lowest solvent exposure level needed to elicit an auditory effect (210, 233, 234).

In contrast to experimental animals, humans are characterised by great individual variability. The variability can be explained by genetic differences that create individual susceptibility for hearing loss, but also from individual differences in medical and exposure histories. This variability makes it challenging to characterise risk and to separate the effects of each agent in a combined exposure scenario, and to measure with precision the interaction between agents such as noise and chemicals. When investigating causal associations of a certain factor, it is of utmost importance to determine known medical factors such as past diseases, intake of certain ototoxic drugs, noise or head trauma accidents as well as non-medical risk factors such as leisure time or past occupational noise and/or chemical exposures and life-style factors associated with the outcome to be investigated. In several of the reviewed investigations, this was accomplished by the examination of existing health or exposure records or by a questionnaire, while others did not report on even the most recognised confounders, such as noise exposure. Still, particularly in occupational studies, complete historical exposure information for hazards is rarely available. Given all these limitations, a large body of knowledge shows that hearing losses are more common in work settings where certain chemical exposures occur. Chemical-induced hearing losses are often moderate

to severe, as is also the case with noise-induced hearing loss. The audiometric high-frequency "notch" common in noise-induced hearing loss is often present following long-term chemical exposures, although some reports indicate that a wider range of audiometric frequencies are affected when compared to the range of frequencies affected by noise.

It should also be noted that the prevalence of chemical-induced hearing loss (identified through pure-tone audiometry) varies across studies. This is often also the case in studies with noise-induced hearing loss, a finding that has been explained by the wide range of possible exposure scenarios and the modifying or confounding factors mentioned above. Further, different definitions of hearing loss have been used. Still, more relevant limitations are insufficient or unreliable exposure history data and lack of comparability between study and control groups regarding solvent and noise exposures.

Mausner and Bahn stated that five conditions should be met before a causal relationship between exposure and disease is established (242). These conditions were: 1) exposure to the factor must antedate the onset of disease, 2) the association between exposure and outcome must be biologically plausible, 3) the outcome must be specific, 4) must be strong and 5) must be consistent with other findings.

The first criterion could not be evaluated in the reviewed human studies because of the study design.

The second criterion of biological plausibility was considered by most publications reviewed in the present document. Thus, robust animal research has indicated that the reviewed chemicals have ototoxic properties. Many of these chemicals also have recognised neurotoxic properties and the mechanisms of action of neurotoxicants lend support to the possibility of central auditory effects of chemicals such as solvents, metals, asphyxiants and pesticides (23, 311, 326, 352, 396).

In several of the reviewed studies, central auditory tests were performed to complement the findings from pure-tone audiometry in workers exposed to solvents or metals (136, 137, 187, 254, 257, 301, 379, 395, 396). Although noise is particularly damaging to the cochlea, industrial chemicals can affect both the cochlear structures and the central auditory system. This means that not only the detection but also the discrimination of sounds may be impaired (i.e. not only will sounds be perceived as less loud, but also as distorted). Such audiological test results indicate that the third criterion, which addresses specificity of response, was met in the case of organic solvents and lead. With solvents, both peripheral and central effects have been confirmed in several studies. Lead has been clearly associated with central auditory effects.

The fourth criterion for evaluating the likelihood of a causal association is the strength of an association as expressed in the ratios of disease in exposed versus non-exposed, which should be analysed for each study.

Finally, as Mausner and Bahn stated, support for the causal nature of an association exists if the findings persist in other study populations (242). As discussed

earlier, with some exceptions (toluene, styrene and solvents mixtures), there are only a few studies on the ototoxicity of industrial chemicals, most of which have been conducted with experimental animals. Still, they indicate that chemicals found in the workplace can have deleterious effects on the auditory system.

In summary, the findings of the association between industrial chemical exposure and hearing impairments are biologically plausible, the association between exposure and outcome is strong, but because of the large confidence intervals, the possibility of no effect exists. The association is confined to specific effects on the auditory system.

In 2008, Hoet and Lison proposed a "noise notation" inspired by the widely used "skin notation" (skin notation criteria were introduced almost 50 years ago as a qualitative indicator of a hazard related to dermal absorption at work). They suggested that "a noise notation" could be added to the OELs of chemical agents for which there is significant concern about a possible ototoxic effect, e.g. when experimental data suggest that ototoxicity is the critical health effect or that ototoxic effects occur at a level close to the OEL (160).

As combined exposure (e.g. chemical and noise) is currently not taken care of in the regular OEL setting procedure, a noise notation can be used to indicate an increased risk of hearing loss after exposure to the chemical with concurrent noise exposure.

13.2 Groups at extra risk

There is no firm evidence to identify groups of humans at extra risk for developing hearing impairment. However, factors that have been shown to influence the occurrence and degree of hearing loss other than noise and chemicals include age, foetal and neonatal development, gender, race, socio-economic and life-style factors, physical work load and use of medications (94, 372).

Age

Age is an important factor to consider when examining hearing disorders. Animal experiments suggest that young animals are more susceptible to the effects of noise (198, 290) than older ones. Similarly, young rats (14 weeks of age) were more vulnerable to the effects of styrene than aged rats (49). Toluene and noise were found to accelerate the age-related hearing loss in mice with a genetic predisposition for age-induced hearing loss, but not in mice from a strain without this predisposition (218). These studies suggest that younger populations may be more susceptible to hearing loss, but that has not yet been clearly demonstrated in humans. On the other hand, toughening of ears through low intensity noise exposures has been demonstrated in animals and might make young ears more resistant to noise (316, 355).

Foetal and neonatal development

Ototoxic effects of chemical exposure of rats during pregnancy and early lactation (a period in which the auditory system develops rapidly in rats) were investigated

for toluene, lead, mercury, IDPN and PCBs and were demonstrated in the offspring (76, 144, 145, 153, 154, 162, 204, 332). Similar findings have not been reported in humans.

Gender and race

Gender and race seem to be associated with susceptibility to noise-induced hearing loss. Studies conducted with groups with similar jobs and exposures have indicated that Caucasian males have poorer auditory thresholds and higher prevalence of noise-induced hearing loss, while African American females have the lowest prevalence of hearing loss (91, 365). The issue of gender is not fully understood since both environmental and occupational noise exposure histories can be heavily influenced by gender. The issue of race and susceptibility to noise could be explained by the protective role played by the presence of melanine in the inner ear (24, 25). Regarding solvents, albino and pigmented rats have been used in ototoxicity experiments and both species are susceptible to auditory effects but no formal investigation compared species for this specific feature. Eastman, Young and Fechter examined the role of melanine following animal exposures to trimethyltin and did not observe significant effects (93).

Socio-economic and life-style factors

Low social class in childhood and adulthood was also found to be associated with poorer hearing thresholds (94) and is likely to interact with occupational risks, leisure noise or non-occupational chemical exposures, and medical history factors such as middle ear disease, lack of appropriate treatment or use/abuse of medication.

Studies on the interaction between hearing loss and smoking indicate that heavy smoking can affect hearing (41, 343, 383) and interact with noise, thus causing a more severe hearing loss in humans (176, 247, 353, 372). Other epidemiological investigations of solvents have controlled for smoking and no significant associations were reported (254, 256, 257, 346, 348). Similarly, epidemiological studies (176, 254, 256, 257, 346, 348) have not confirmed that alcohol consumption potentiates the effect of solvent exposure on hearing as demonstrated in animals (44). Information about alcohol consumption can be considered sensitive and is thereby difficult to obtain in human studies.

Physical workload

Physical exercise has been shown to increase the susceptibility to noise (87, 224). It has also been demonstrated that styrene concentrations required to induce auditory damage were much lower for active rats in comparison to sedentary rats (210). Studies indicate that the total absorbed styrene dose can be increased sixfold with physical work and increased respiratory rate (96). It has been suggested that auditory effects of solvents may be observed at lower concentrations in humans because humans are generally exposed to solvents in combination with a multitude of other factors (several combined exposures, physical demands, etc.), whereas animal experiments typically involve isolated chemical exposures (210).

Medication
Finally, the ototoxicity of therapeutic drugs has been recognised for a long time (Table 2) but their interaction with work-related risk factors has rarely been examined. A synergistic interaction between acetyl salicylic acid and toluene was shown by Johnson. Acetyl salicylic acid did not cause hearing loss but potentiated the ototoxic effect caused by toluene (182). These results might be of interest since pain killers of this type in lower doses are likely to be used by workers including those exposed to toluene.

13.3 Scientific basis for occupational standards

Ototoxicity in animals has been reported for all substances included in this review. Except for hydrogen cyanide, ototoxicity has only been observed at high exposure levels (far above most common OELs). For some substances, data for combined chemical and noise exposure are also available.

Auditory effects have also been indicated in humans for all substances covered in this document for which there are data. However, most human studies lack information on historic as well as current peak chemical and noise exposure. Styrene and toluene are the substances most studied to date, because of the ubiquity in use and the magnitude of the exposed populations.

As combined exposure (e.g. chemical and noise) is currently not taken care of in the regular OEL setting procedure, a noise notation can be used to indicate an increased risk of hearing loss after exposure to the chemical at a level close to the OEL (or biological exposure limit) with concurrent noise exposure.

The strength of evidence for ototoxicity differs between the agents covered but falls basically into three categories discussed in the following:

1) Human data indicate auditory effects under or near existing OELs. There are also robust animal data supporting an effect on hearing from exposure.
2) Human data are lacking whereas animal data indicate an auditory effect under or near existing OELs.
3) Human data are poor or lacking. Animal data indicate an auditory effect well above existing OELs.

Category 1. Human data indicate auditory effects under or near existing OELs. There are also robust animal data supporting an effect on hearing from exposure.

Styrene In occupational studies, the lowest current exposure levels associated with hearing loss were 3.5-22 ppm. One study demonstrated that an average work-life styrene exposure of 14 ppm was associated with an increased risk for hearing loss. Noise levels in these studies were below 85 dB. A synergistic interaction with noise was demonstrated in animals only above the lowest effect level for styrene alone. In humans, the interaction with noise is not clear.

Toluene In some occupational studies, ototoxic effects have been associated with current exposure levels of approximately 10-50 ppm. Historic toluene and/or noise levels were not well characterised. In one study, chronic exposure to an average of 97 ppm caused hearing loss but noise levels were not given. No auditory effects were observed in a study in which both current and historic levels of toluene were up to 50 ppm and noise levels were low. A synergistic interaction with noise was demonstrated in animals only at high levels. In humans, the interaction with noise is not clear.

Carbon disulphide The less investigated solvent carbon disulphide increased the risk for hearing loss significantly at current occupational exposure levels of 3-8 ppm (noise levels not reported but expected to be rather high) and above 14 ppm (noise levels at 80-91 dB), respectively. No animal studies investigating interaction with noise were identified.

Lead In occupational studies, current and life-time weighted average blood levels of 28-57 µg/dl (i.e. levels also found in the general population) have been associated with auditory effects. Effects in monkeys have been reported at approximately the same levels. Noise levels were not reported in most studies, since they focused on central auditory effects. The only study that controlled for noise did not detect an interaction between lead and noise exposure in humans.

Mercury The human data are weak but a few studies indicate auditory effects around the current biological exposure limits. Interaction with noise was not studied, neither in animals nor humans.

Carbon monoxide The animal data are strong. Without concomitant noise exposure, no auditory effects were reported in rats at high exposure levels. However, combined exposure to carbon monoxide and noise produced unexpectedly, large and permanent thresholds shift even in scenarios in which noise exposure alone had no effect. Decreasing noise exposure did not always decrease the severity of the auditory effect. Human data are sparse but suggest that carbon monoxide can affect hearing even in the absence of excessive noise exposure. The only dose-response data identified (based on two individuals) indicated an effect at 16-35 ppm in combination with noise.

Category 2. Human data are lacking whereas animal data indicate an auditory effect under or near existing OELs.

Xylenes *p*-Xylene, but not *m*- or- *o*-xylene, has shown to be ototoxic in rats at high levels. However, combined exposure to a mixture containing only 50 ppm each of *p*-xylene and ethylbenzene (as well as the other xylene isomers) caused enhanced auditory effects compared to ethylbenzene alone in rats. The interaction with noise was not studied.

Ethylbenzene Auditory effects of ethylbenzene have been demonstrated in rats at 200 ppm and at 50 ppm in combination with *p*-xylene (see above, the lowest doses tested). A synergistic interaction with noise was shown at 300 ppm, the only exposure level tested.

Hydrogen cyanide In the only study available, hydrogen cyanide alone was not ototoxic at levels up to 50 ppm in rats but potentiated noise-induced hearing loss at 30 ppm. The lower bounds for benchmark doses that affected the auditory thresholds were 2-16 ppm.

Category 3. Human data are poor or lacking. Animal data indicate an auditory effect well above existing OELs.

Trichloroethylene Available studies indicate that trichloroethylene may be ototoxic in humans, but exposure levels were not reported and there was also a lack of data on noise and possible co-exposures to other chemicals. In one animal study, high trichloroethylene exposure was shown to interact synergistically with noise.

n-Hexane Auditory effects in workers have been interpreted as a sign of the substance's well-known central nervous system toxicity.

Trimethyltin In animal studies, trimethyltin (intraperitoneal injection) disrupted auditory function (effects on the peripheral auditory system) at doses far below those shown to elicit general neurotoxicity. Interaction with noise was not studied. No human studies were identified.

Chlorobenzene and *n-heptane* Only one animal study was identified for each substance and no human studies were identified.

Solvent mixtures Effects on the auditory system have been indicated in several occupational studies.

Acrylonitrile In animal studies, acrylonitrile (subcutaneous injection) alone was not ototoxic but potentiated noise-induced hearing loss at a high dose (only dose tested). No human data were identified.

IDPN Auditory effects in humans have not been investigated.

Pesticides Effects on the auditory system have been indicated in several occupational studies.

PCB mixtures as well as the individual congener *PCB 126* have not been investigated for auditory effects in humans.

14. Research needs

The state-of-the art and research needs in the area of ototoxic chemicals have been examined in two workshops (in 2002 and 2006) dedicated to the issue (253, 351). Consensus on research needs was reached by the participant experts on several topics presented below.

OELs are often based on an adverse health effect detected at the lowest exposure level. The auditory system is not an endpoint tested routinely but consideration should be given to including it in a toxicity evaluation battery. It could also be used as an early indicator of neurotoxicity.

Because of the enormous number of existing industrial chemicals and the thousands of new ones that are placed in the market every year, it is of crucial importance to understand the mechanisms by which chemicals affect the auditory system. Such an understanding could lead to a prediction of which chemicals to target by preventive efforts. This is a very complex task, a challenge that was scrutinised by Fechter (115, 116).

Some of the specific issues to be considered in mechanistic research include:

- Different species respond differently to the studied chemicals. Examining these differences could offer clues to the mechanism of ototoxicity.
- Toxic interactions among agents present the need to manipulate exposure parameters: doses, duty cycles, presentation order (sequential or simultaneous exposures).
- Physical or endogenous factors should also be taken into consideration: health status of the study participants (blood pressure, respiration, etc.), genetics, and age.

Identifying priority chemicals is of utmost importance, not only for research purposes but also for establishing recommendations or identifying best practices for hearing loss prevention. The potential for human exposure and the magnitude of exposed populations is an important factor in giving priority to a specific chemical. A second criterion for the inclusion of a chemical in the research priority list is evidence of the chemical's ototoxicity, general toxicity as well as neurotoxicity (since most of the chemicals found to affect the auditory system are potentially neurotoxic). Information on whether a chemical produces ROS could also help in the decision to examine that agent's ototoxicity (253, 351).

A few studies of the reviewed chemicals have reported effects in the vestibular system and balance function (252, 271, 277, 301, 373, 395, 397, 398). Their findings underscore the need for further research on this outcome.

The issue of how the ototoxicity of chemicals should be assessed in humans through audiological tests needs further investigation. When pure-tone audiometry is the only test performed, information by means of a questionnaire on speech discrimination difficulties or other auditory problems that are inconsistent with pure-tone thresholds can help in detecting some of the effects by chemicals on the auditory system. Comparing prevalences of hearing disorders between groups with different exposure conditions and calculating risk ratios may also

allow for the detection of effects caused by chemicals. A more robust approach involves auditory testing to assess more central portions of the auditory system, as a complement to the findings from pure-tone audiometry and a help in distinguishing between noise and chemically induced effects.

A golden standard auditory test battery is not yet available. Although ideal, a comprehensive audiological test battery in occupational studies may not be feasible because of time and cost constraints. Screening workers to select those who should undergo further testing can also prove to be a fruitful approach. When selecting hearing tests, administration time, ease of analysis, sensitivity and specificity, and the site of the auditory system should be evaluated.

Dose-response information for ototoxicity is needed for most of the chemicals in this document. Such information could be used in the examination of inclusion criteria for workers exposed to chemicals (alone or in combination with noise) in hearing loss prevention programmes. Such expansion of the current recommendation to incorporate also chemical exposures would consequently require examination of the recommended action to be taken.

In summary, research needs on ototoxic chemicals include:

- Assessing potential interactions to allow for decision making on strategic directions and priorities of mixed exposures research.
- Evaluating mechanisms of interaction to provide a rational basis for extrapolation of toxicological information across different mixtures, dose levels, exposure parameters and routes.
- Conducting multidisciplinary epidemiological investigations, which include careful mixed-exposure assessment, preferably collected by personal air monitoring and biological monitoring.
- Evaluating non-occupational risk factors and individual variability in response to occupational environmental and organisational factors.
- Improving the toxicity testing of new chemicals to properly evaluate their ototoxicity.
- Identifying levels of simultaneous noise and specific chemicals' exposures that can be considered safe to the human auditory system.

15. Summary

Johnson A-C, Morata TC. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 142. Occupational exposure to chemicals and hearing impairment.* Arbete och Hälsa 2010;44(4):1-177.

Research conducted over the last two decades has brought attention to the ototoxicity of chemicals in the workplace and their interaction with noise. Chemicals that have been specifically studied for their ototoxicity include solvents, metals, asphyxiants, PCBs and pesticides.

Noise exposure is particularly damaging to the cochlea, a part of the peripheral auditory system, whereas chemicals tend to affect both the cochlear structures and the central auditory system. Reduced blood flow and free radical formation are important ototoxic mechanisms shared by noise and chemical exposures. Solvents and asphyxiants may also disrupt intrinsic anti-oxidant defences and make the ear more vulnerable to the effects of e.g. noise exposure.

The chemicals reviewed in the present document have all been associated with auditory effects in animals. Some of the solvents and the asphyxiants interact synergistically with noise or potentiate noise effects on the auditory system. Combinations of chemical exposure with noise and other stressors such as physical activity during exposure may lower the concentration of the chemical exposure necessary for induction of an auditory effect.

Auditory effects have also been indicated in humans for all agents covered in this document for which there are data. Noise is often present in the occupational arena, which makes prediction of the outcome challenging.

As combined exposure (e.g. chemical and noise) is currently not taken care of in the regular occupational exposure limit (OEL) setting procedure, a *noise notation* can be used to indicate an increased risk of hearing loss after exposure to the chemical at a level close to the OEL with concurrent noise exposure. The strength of evidence for ototoxicity differs between the agents but falls basically into three categories, i.e. agents for which:

1) human data indicate auditory effects under or near existing OELs and robust animal data support an effect on hearing from exposure (styrene, toluene, carbon disulphide, lead, mercury and carbon monoxide),
2) human data are lacking whereas animal data indicate auditory effects under or near existing OELs (*p*-xylene, ethylbenzene and hydrogen cyanide),
3) human data are poor or lacking and animal data indicate an auditory effect well above the existing OELs (chlorobenzene, trichloroethylene, *n*-hexane, *n*-heptane, some solvent mixtures, trimethyltin, acrylonitrile, 3,3'-iminodipropionitrile, pesticides and PCBs).

Keywords: asphyxiant, auditory, hearing, metal, noise, occupational exposure limit, ototoxic, PCB, pesticide, review, risk assessment, solvent.

16. Summary in Swedish

Johnson A-C, Morata TC. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 142. Occupational exposure to chemicals and hearing impairment.* Arbete och Hälsa 2010;44(4):1-177.

Att kemikalier som är vanliga i arbetslivet kan vara skadliga för hörseln och också kan samverka med buller har uppmärksammats i forskningsrapporter de senaste 20 åren. De kemiska ämnen som speciellt har uppmärksammats som hörselskadande är lösningsmedel, metaller, cellandningshämmare, PCB och bekämpningsmedel.

Buller ger främst skador på det perifera hörselsystemet genom att påverka hårcellerna i innerörat medan kemiska ämnen kan orsaka skador både på det perifera och centrala hörselsystemet. Både exponering för buller och ototoxiska kemikalier kan orsaka skador i innerörat via gemensamma mekanismer såsom minskat blodflöde och bildandet av fria radikaler. Lösningsmedel och cellandningshämmare kan också störa eller hindra kroppens eget antioxidantförsvar mot fria radikaler och därmed göra innerörat mer mottagligt för skador av t.ex. buller.

Alla kemiska ämnen som presenteras i denna översikt har i djurförsök visats ha en effekt på hörseln. Några av lösningsmedlen och cellandningshämmarna kan interagera med buller och potentiera bullrets skadliga effekter på hörselsystemet. Exponering för kemikalier i kombination med buller, andra kemikalier eller faktorer såsom fysisk aktivitet har visats kunna ge hörselskador vid lägre nivåer än exponering enbart för ett kemiskt ämne.

Humandata saknas för flera av ämnena i denna översikt. För de ämnen som studerats har dock tecken på hörselpåverkan också setts hos människa. Buller förekommer ofta på samma arbetsplatser som kemiska ämnen, vilket gör effekten svår att förutsäga.

Kombinationsexponeringar (t.ex. för kemikalier och buller) hanteras för närvarande inte i gränsvärdessättningen. En *bullermärkning* skulle därför kunna användas för att uppmärksamma en ökad risk för hörselskador efter exponering för en kemikalie vid nivåer nära gränsvärdet vid samtidig bullerexponering.

Styrkan i data som visar ototoxicitet varierar för de olika kemiska ämnena men faller huvudsakligen i tre kategorier, dvs. ämnen för vilka:

1) humandata antyder hörselpåverkan vid nivåer under eller i närheten av nuvarande gränsvärden och starka djurdata stöder att hörselskador uppkommer efter exponering (styren, toluen, koldisulfid, bly, kvicksilver och kolmonoxid),
2) humandata saknas men djurdata antyder effekter på hörseln vid nivåer under eller i närheten av nuvarande gränsvärden (*p*-xylen, etylbensen och cyanväte),
3) humandata är svaga eller saknas och djurdata antyder hörselpåverkan klart över nuvarande gränsvärden (klorbensen, trikloretylen, *n*-hexan, *n*-heptan, vissa lösningsmedelsblandningar, trimetyltenn, akrylnitril, 3,3'-iminodipropionitril, pesticider och PCB).

Nyckelord: bekämpningsmedel, buller, cellandningshämmare, hygieniskt gränsvärde, hörsel, lösningsmedel, metaller, ototoxisk, PCB, riskbedömning, översikt.

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18. Data bases used in the search for literature

Experimental and epidemiological studies included in this review were identified through a literature search covering the period between 1950 and November 2007. The search was not limited to studies published in English. Full-text copies of each of these articles were obtained and read in detail by at least one of the authors. In addition, the bibliography of each article was scanned to identify potential references that were missed by our searches. All studies were collected without consideration of research design. Databases searched included:

PubMed (US NLM)
Toxline (US NLM)
NIOSHTIC-2 (US NIOSH)
Arbline (Stockholm University, Sweden)
RISKLINE (Swedish Chemicals Agency)
LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde) (Biblioteca Regional de Medicina, Brazil, Pan-American Health Organization and the Latin American and Caribbean Health Sciences Center, Brazil)
The dissertation and theses bank (Banco de Teses da CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil)

A final search in PubMed was performed in February 2010.

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Appendix 1. Occupational exposure limits in different countries for the substances reviewed

Table A. Occupational exposure limits in ppm (mg/m^3) in different countries for the solvents reviewed in this document.

Substance	Denmark (1)	Finland (2)	Norway (3)	Sweden (4)	The Nether-lands (5)	Germany (6)	UK (7)	EU (9-11)	US OSHA (12)	US NIOSH (12)	ACGIH (13)
Styrene											
8-TWA	-	20 (86)	25 (105)	20 (90)	-	20 (86)	100 (430)	-	100 (426)	50 (215)	20 (86)
STEL	25 (105) C	100 (430)	37 (157)	50 (200)	-	40 (172)	250 (1 080)	-	200 (852) C	100 (425)	40 (172)
Toluene											
8-TWA	25 (94)	25 (81)	25 (94)	50 (200)	40 (150)	50 (190)	50 (191)	50 (192)	200 (754)	100 (375)	20 (75)
STEL	50 (188)	100 (380)	37 (141)	100 (400)	100 (384)	200 (760)	100 (384)	100 (384)	300 (1 131) C	150 (560)	-
Xylene (*m*-, *o*- and *p*-isomers)											
8-TWA	25 (109)	50 (220)	25 (108)	50 (200)	47 (210)	100 (440)	50 (220)	50 (221)	100 (435)	100 (435)	100 (435)
STEL	50 (218)	100 (440)	37 (162)	100 (450)	100 (442)	200 (880)	100 (441)	100 (442)	-	150 (655)	150 (655)
Ethylbenzene											
8-TWA	50 (217)	50 (220)	5 (20)	50 (200)	50 (215)	-	100 (441)	100 (442)	100 (435)	100 (435)	100 (435)
STEL	100 (434)	200 (880)	10 (40)	100 (450)	100 (430)	-	125 (552)	200 (884)	-	125 (545)	125 (545)
Chlorobenzene											
8-TWA	5 (23)	5 (23)	5 (23)	-	5 (23)	10 (47)	1 (4.7)	5 (23)	75 (350)	-	10 (46)
STEL	10 (46)	15 (70)	10 (46)	-	15 (70)	20 (94)	3 (14)	15 (70)	-	-	-
Trichloroethylene											
8-TWA	10 (55)	10 (50)	10 (50)	10 (50)	-	-	100 (550)	-	100 (537)	25 (134)	10 (55)
STEL	20 (110)	-	20 (100)	25 (140)	-	-	150 (820)	-	200 (1 074) C	-	25 (140)
n-Hexane											
8-TWA	20 (72)	20 (72)	20 (72)	25 (90)	20 (172)	50 (180)	20 (72)	20 (72)	500 (1 800)	50 (180)	50 (180)
STEL	40 (144)	-	30 (108)	50 (180)	40 (144)	400 (1 440)	-	-	-	-	-
n-Heptane											
8-TWA	200 (820)	300 (1 200)	200 (800)	200 (800)	300 (1 200)	500 (2 100)	500 (2 085)	500 (2 085)	500 (2 000)	85 (350)	400 (1 600)
STEL	400 (1 640)	500 (2 100)	250 (1 000)	300 (1 200)	400 (1 600)	500 (2 100)	-	-	-	440 (1 800) C	500 (2 100)
Carbon disulphide											
8-TWA	5 (15)	5 (16)	5 (15)	5 (16)	-	5 (16)	10 (32)	5 (15)	20 (62)	1 (3)	1 (3)
STEL	10 (30)	-	10 (30)	8 (25)	-	10 (32)	-	-	30 (93) C	10 (30)	-

C: ceiling value (15 min), STEL: short-term exposure limit (15-min TWA), TWA: time-weighted average (8 hours or for NIOSH up to 10 hours).

Table B. Occupational exposure limits in mg/m^3 (if not otherwise stated) in different countries for the metals reviewed in this document.

Substance	Denmark (1)	Finland (2)	Norway (3)	Sweden (4)	The Nether-lands (5)	Germany (6)	UK (7)	EU (8,11)	US OSHA (12)	US NIOSH (12)	ACGIH (13)
Lead (Pb)											
Elemental and inorganic forms[a] (as Pb)											
8-h TWA	0.05	0.1	0.05	0.05	-	-	0.15	0.15	0.05	0.05	0.05
Blood level (µg Pb/100 ml blood)											
BEL	20	-	-		70	-	-	70	-	60 µg/100 g	30
Action level[b]	-	40	21	17	-	40	50	40	-	-	-
Suspension level	-	-	-	41	-	-	60	-	-	-	-
Mercury (Hg)											
Elemental and inorganic forms[a] (as Hg)											
8-h TWA	0.025	0.05	0.02	0.03	-	0.1	0.025	0.02		0.05 Hg(0)	0.025
STEL	-	-	-	-	-	0.8	-	-	0.1 C	0.1 C	-
Organic forms[a] except alkyl Hg compounds (as Hg)											
8-h TWA	0.05	-	0.02	0.01	-	-	-	-			0.1[c]
STEL	-	-	-	-	-	-	-	-	0.1 C	0.1 C	
Organo alkyl Hg compounds (as Hg)											
8-h TWA	0.01	0.01	0.01	0.01	-	-	0.01	-	0.01	0.01	0.01
STEL	-	-	-	-	-	-	0.03	-	0.04[c]	0.03	0.03
Tin (Sn)											
Organic forms[a] (as Sn)											
8-h TWA	0.1	0.1	0.1	0.1	-	0.1	0.1	-	0.1	0.1	0.1
STEL	-	0.3	-	0.2	-	0.2	0.2	-	-	-	0.2

[a] Definition of "inorganic forms" and "organic forms" may differ between countries.
[b] Most countries have lower levels for women of reproductive capacity and young persons than those given in the table.
[c] Aryl Hg compounds only.
BEL: biological exposure limit, C: ceiling value, STEL: short-term exposure limit (15-min TWA), TWA: time-weighted average (8 hours or for NIOSH up to 10 hours).

Table C. Occupational exposure limits in ppm (mg/m^3) in different countries for the asphyxiants reviewed in this document.

Substance	Denmark (1)	Finland (2)	Norway (3)	Sweden (4)	The Nether-lands (5)	Germany (6)	UK (7)	EU (9-11)	US OSHA (12)	US NIOSH (12)	ACGIH (13)
Carbon monoxide											
8-TWA	25 (29)	30 (35)	25 (29)	35 (40)	25 (29)	30 (35)	30 (35)	-	50 (55)	35 (40)	25 (29)
STEL	50 (58)	75 (87)	100 (120) C	100 (120)	-	30 (35)	200 (232)	-	-	200 (229) C	-
Hydrogen cyanide											
8-TWA	5 (5)	-	-	-	0.9 (1)	1.9 (2.1)	-	-	10 (11)	-	-
STEL	-	10 (11)	5 (5) C	- (5) C	9.1 (10)	3.8 (4.2)	10 (11)	-	-	4.7 (5)	4.7 (5) C
Acrylonitrile											
8-h TWA	2 (4)	2 (4.4)	2 (4)	2 (4.5)	-	30 (35)	2 (4.4)	-	2 (4.3)	1 (2.2)	2 (4.3)
STEL	4 (8)	4 (8.8)	4 (8)	6 (13)	-	30 (35)	-	-	10 (22) C	10 (22) C	-

C: ceiling value, STEL: Short term exposure limit (15-min TWA), TWA: time-weighted average (8 hours or for NIOSH up to 10 hours).

Table D. Occupational exposure limits in mg/m^3 in different countries for polychlorinated biphenyls (PCBs).

Substance	Denmark (1)	Finland (2)	Norway (3)	Sweden (4)	The Nether-lands (5)	Germany (6)	UK (7)	EU (9-11)	US OSHA (12)	US NIOSH (12)	ACGIH (13)
Polychlorinated biphenyls, PCBs											
All											
8-TWA	0.01	0.5	0.01	0.01	-	-	0.1	-	-	0.001	-
STEL	0.02	1.5	0.03	0.03	-	-	-	-	-	-	-
With 42 % chlorine											
8-TWA	-	-	-	-	-	1.1	-	-	1	0.001	1
STEL	-	-	-	-	-	8.8	-	-	-	-	
With 54 % chlorine											
8-TWA	-	-	-	-	-	0.70	-	-	0.5	0.001	0.5
STEL	-	-	-	-	-	5.6	-	-	-	-	

STEL: Short-term exposure limit (15-min TWA), TWA: time-weighted average (8 hours or for NIOSH up to 10 hours).

References for Appendix 1

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Appendix 2. Previous NEG criteria documents

NEG criteria documents published in the scientific serial Arbete och Hälsa (Work and Health):

Substance/Agent	*Arbete och Hälsa issue*
Acetonitrile	1989:22, 1989:37*
Acid aerosols, inorganic	1992:33, 1993:1*
Acrylonitrile	1985:4
Allyl alcohol	1986:8
Aluminium	1992:45, 1993:1*
Ammonia	1986:31, 2005:13*
Antimony	1998:11*
Arsenic, inorganic	1981:22, 1991:9, 1991:50*
Arsine	1986:41
Asbestos	1982:29
Benomyl	1984:28
Benzene	1981:11
1,2,3-Benzotriazole	2000:24*D
Boric acid, Borax	1980:13
1,3-Butadiene	1994:36*, 1994:42
1-Butanol	1980:20
γ-Butyrolactone	2004:7*D
Cadmium	1981:29, 1992:26, 1993:1*
7/8 Carbon chain aliphatic monoketones	1990:2*D
Carbon monoxide	1980:8
Ceramic Fibres, Refractory	1996:30*, 1998:20
Chlorine, Chlorine dioxide	1980:6
Chloromequat chloride	1984:36
4-Chloro-2-methylphenoxy acetic acid	1981:14
Chlorophenols	1984:46
Chlorotrimethylsilane	2002:2
Chromium	1979:33
Cobalt	1982:16, 1994:39*, 1994:42
Copper	1980:21
Creosote	1988:13, 1988:33*
Cyanoacrylates	1995:25*, 1995:27
Cyclic acid anhydrides	2004:15*D
Cyclohexanone, Cyclopentanone	1985:42
n-Decane	1987:25, 1987:40*
Deodorized kerosene	1985:24
Diacetone alcohol	1989:4, 1989:37*
Dichlorobenzenes	1998:4*, 1998:20
Diesel exhaust	1993:34, 1993:35*
Diethylamine	1994:23*, 1994:42
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Diethylenetriamine	1994:23*, 1994:42
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Dimethylamine	1994:23*, 1994:42
Dimethyldithiocarbamates	1990:26, 1991:2*
Dimethylethylamine	1991:26, 1991:50*
Dimethylformamide	1983:28
Dimethylsulfoxide	1991:37, 1991:50*
Dioxane	1982:6
Enzymes, industrial	1994:28*, 1994:42
Epichlorohydrin	1981:10
Ethyl acetate	1990:35*

Substance/Agent	*Arbete och Hälsa issue*
Ethylbenzene	1986:19
Ethylenediamine	1994:23*, 1994:42
Ethylenebisdithiocarbamates and Ethylenethiourea	1993:24, 1993:35*
Ethylene glycol	1980:14
Ethylene glycol monoalkyl ethers	1985:34
Ethylene oxide	1982:7
Ethyl ether	1992:30* N
2-Ethylhexanoic acid	1994:31*, 1994:42
Flour dust	1996:27*, 1998:20
Formaldehyde	1978:21, 1982:27, 2003:11*D
Fungal spores	2006:21*
Furfuryl alcohol	1984:24
Gasoline	1984:7
Glutaraldehyde	1997:20*D, 1998:20
Glyoxal	1995:2*, 1995:27
Halothane	1984:17
n-Hexane	1980:19, 1986:20
Hydrazine, Hydrazine salts	1985:6
Hydrogen fluoride	1983:7
Hydrogen sulphide	1982:31, 2001:14*D
Hydroquinone	1989:15, 1989:37*
Industrial enzymes	1994:28*
Isoflurane, sevoflurane and desflurane	2009;43(9)*
Isophorone	1991:14, 1991:50*
Isopropanol	1980:18
Lead, inorganic	1979:24, 1992:43, 1993:1*
Limonene	1993:14, 1993:35*
Lithium and lithium compounds	2002:16*
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Mercury, inorganic	1985:20
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Methyl chloride	1992:27*D
Methyl chloroform	1981:12
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Methyl ethyl ketone	1983:25
Methyl formate	1989:29, 1989:37*
Methyl isobutyl ketone	1988:20, 1988:33*
Methyl methacrylate	1991:36*D
N-Methyl-2-pyrrolidone	1994:40*, 1994:42
Methyl-tert-butyl ether	1994:22*D
Microbial volatile organic compounds (MVOCs)	2006:13*
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Penicillins	2004:6*
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Substance/Agent	*Arbete och Hälsa issue*
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Phthalate esters	1982:12
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Polyethylene,	1998:12*
Polypropylene, Thermal degradation products in the processing of plastics	1998:12*
Polystyrene, Thermal degradation products in the processing of plastics	1998:12*
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Polytetrafluoroethylene, Thermal degradation products in the processing of plastics	1998:12*
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Propylene glycol ethers and their acetates	1990:32*N
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Refined petroleum solvents	1982:21
Refractory Ceramic Fibres	1996:30*
Selenium	1992:35, 1993:1*
Silica, crystalline	1993:2, 1993:35*
Styrene	1979:14, 1990:49*, 1991:2
Sulphur dioxide	1984:18
Sulphuric, hydrochloric, nitric and phosphoric acids	2009;43(7)*
Synthetic pyretroids	1982:22
Tetrachloroethane	1996:28*D
Tetrachloroethylene	1979:25, 2003:14*D
Thermal degradation products of plastics	1998:12*
Thiurams	1990:26, 1991:2*
Tin and inorganic tin compounds	2002:10*D
Toluene	1979:5, 1989:3, 1989:37*, 2000:19*
1,1,1-Trichloroethane	1981:12
Trichloroethylene	1979:13, 1991:43, 1991:50*
Triglycidyl isocyanurate	2001:18*
n-Undecane	1987:25, 1987:40*
Vanadium	1982:18
Vinyl acetate	1988:26, 1988:33*
Vinyl chloride	1986:17
Welding gases and fumes	1990:28, 1991:2*
White spirit	1986:1
Wood dust	1987:36
Xylene	1979:35
Zinc	1981:13

* in English, remaining documents are in a Scandinavian language.
D = collaboration with the Dutch Expert Committee on Occupational Safety (DECOS).
N = collaboration with the US National Institute for Occupational Safety and Health (NIOSH).

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