factors potentially affecting the hearing of petroleum industry workers

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ABSTRACT

This report aims at giving an overview of the various factors that may influence the hearing of petroleum industry workers, including the issue of 'ototoxic' chemical exposure. It also provides guidance for occupational physicians on factors that need to be considered as part of health management programmes.

KEYWORDS

hearing, petroleum industry, hearing loss, audiometry, ototoxicity, chemicals

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SUMMARY

European Directive 2003/10/EC on the minimum health and safety requirements regarding the exposure of workers to the risks arising from noise specifies that the employer shall give particular attention, when carrying out the risk assessment, to, among others, any effects on workers' health and safety resulting from interactions between noise and work-related ototoxic substances.

This report aims at summarising the various factors potentially affecting the hearing of petroleum industry workers: age, occupational and non-occupational noise exposure, diseases and chemical exposures (therapeutic drugs, cigarette smoking and ethanol consumption, industrial chemicals) with special emphasis on gasoline components. Petroleum workers are exposed, among others, to hydrocarbons such as toluene, ethylbenzene, n-hexane and benzene.

In rats, exposure to high concentrations of certain solvents such as toluene, ethylbenzene, and n-hexane produces cochlear lesions, as does noise exposure, but the mechanisms are different. While hearing loss caused by noise results from a mechanical injury to hair cell stereocilia, the damage caused by solvents is mainly due to the destruction of outer hair cells of the Organ of Corti. Moreover, a number of solvents are known neurotoxic substances and a central auditory pathway involvement is also suspected. Toluene-induced vulnerability of the cochlear function appears to be species-dependent, the rat being much more sensitive than the guinea pig or the chinchilla. However, it is not clearly established whether the rat is the most appropriate model to assess the risk in humans. Experimental rat studies have also shown that a) the pattern of exposure is important; b) there is an influence of age on the solvent-induced threshold shift and hair cell loss; c) there is a synergistic or at least additive ototoxic effect of toluene and noise; d) ethanol intake modulates the ototoxic effect of toluene. In mice, there is some indication of a genetic susceptibility to the ototoxic effect of toluene.

The Lowest Observed Adverse Effect Concentrations (LOAECs) reported for auditory impairment in the rat following repeated exposure are in the area of 400 ppm for ethylbenzene, 600 ppm for toluene, 800 ppm for mixed xylenes. Transient auditory system impairment has been observed in the guinea pig exposed at 250 ppm, but this was not reproduced in another study. The No Observed Adverse Effect Concentrations (NOAECs) reported in the rat are 300 ppm for ethylbenzene, 450 ppm for p-xylene and below 600 ppm for toluene.

Data in humans are scarce and equivocal. The association between occupational exposure to some solvents such as toluene and hearing impairment has been suggested only recently. The only study specifically addressing this issue in petrochemical workers does not allow conclusions to be reached on the ototoxic potential of chemicals in these workers.

The ototoxic potential of toluene, xylenes, ethylbenzene, n-hexane and benzene in humans is not well characterised. The assessment of exposure to a single organic solvent is particularly difficult because workers are usually exposed to mixtures of chemicals of highly varying compositions and concentrations and it is difficult to identify workers with exposure to a specific compound only. These studies are not appropriate for determining a LOAEC/NOAEC. A high prevalence of mild high-frequency hearing loss has been described in printing workers exposed to high levels of toluene (higher than the current Occupational Exposure Limits (OELs)) in combination with noise when compared to controls, but a lifetime weighted average exposure to toluene alone at about 50 ppm was not associated with hearing loss. Hence, considering the level of exposure to toluene, in workers handling and exposed to gasoline vapours the risk is probably low. However, these workers are exposed to a mixture of chemicals and further research is certainly needed to better characterise the risk.

While limited data suggest that the risk of ototoxicity associated with exposure to chemicals (alone or in combination with noise) in petrochemical workers is low, it should be recognised that the current scientific literature is too scarce to make specific recommendations for the health surveillance of these workers. The existing scientific data are insufficient to support amending OELs for noise for workers also exposed to solvents, for amending solvent OELs for workers also exposed to noise, or for a combined noise/solvent OEL. Considering, however, that hearing loss is an irreversible process, it appears necessary to stay alert to possible additive, potentiating, or synergistic ototoxic effects in case of combined exposure to several chemicals and in case of combined exposure to noise and chemical substances.

Therefore, the concept of introducing a ‘Noise notation’, in analogy with the established ‘Skin notation’, should be considered. Skin notations are added to OELs for chemical agents where skin absorption can add significantly to the body burden resulting from inhalation. Similarly, the ‘Noise notation’ would serve as an alert for targeted medical surveillance of the hearing function of exposed workers. The ‘Noise notation’ could be added to OELs of certain solvents and other hydrocarbons for which there is significant concern about a possible ototoxic effect, e.g. when experimental data suggest the ototoxic effect is the critical health effect or experimental ototoxic effects occur at a level not much above the lowest level of another effect considered the critical effect for OEL setting.

1. INTRODUCTION

The European Directive 2003/10/EC on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (noise), specifies that the employer shall give particular attention, when carrying out the risk assessment, to, among others, any effects on workers' health and safety resulting from interactions between noise and work-related ototoxic substances, and between noise and vibrations;

A series of reports have appeared in the scientific literature over the last years, suggesting a possible link between combined occupational exposure to noise and to organic solvents, including gasoline components (toluene), and an increased risk of hearing loss.

In 2002, CONCAWE published the results of a survey on occupational exposures to gasoline vapour in European Union countries in the period 1999-2001. Full-shift exposure statistics for gasoline vapour were measured and compared with OELs established in European countries (**Table 1**). It was concluded that « Potential non-compliance of gasoline exposure, e.g. in the form of the 90-percentile level or the arithmetic mean, was detected for research laboratory workers when engaged in intermediate quantity blending operations, and for worst-case rail car loading without vapour recovery, though the latter conclusion is based on suspect data. The same conclusion of potential non-compliance was drawn for the benzene exposures in these two scenarios when comparing the measurement results with the OEL. All other data sets for gasoline vapour and for single constituents appeared to be in compliance with limit values » (CONCAWE, 2002).

Table 1 Summary data for occupational exposures to gasoline vapour and selected components

	Average full-shift exposure results range (mg/m^3)	90th percentile full-shift exposure results range (mg/m^3)
gasoline vapour	1.3 to 613	2.8 to 1568
n-hexane	0.1 to 7.0	0.1 to 18
Benzene	0.1 to 4.0	0.1 to 10
Toluene	0.2 to 18	0.2 to 45
Xylenes	0.1 to 6.4	0.1 to 18

In addition to hydrocarbon exposures, some refinery workers may be exposed to mercury for which an ototoxic hazard has also been suggested. The mercury exposure potential is limited to certain crude oils and gas field condensates with elevated mercury content, and exposure usually only occurs when process vessels are opened for major maintenance. As mercury levels in refinery feedstocks are routinely measured, it is possible to establish appropriate worker health protection procedures. Further, some exposure to mercury has been detected in laboratory technicians as a result of breaking thermometers. However, adequate alternative thermometers are now available which prevent any mercury exposure potential.

This report aims at giving an overview of the various factors that may influence the hearing of petroleum industry workers, including the issue of 'ototoxic' chemical exposure. It will also provide guidance for occupational physicians on factors that need to be considered as part of health management programmes.

2. HEARING, MECHANISMS AND TYPES OF HEARING LOSS

2.1. PHYSIOLOGY OF HEARING: HEARING BASICS

The ear is composed of a sound conductor (outer and middle ears) and a sound receptor (inner ear). Sound (air pressure waves) reaches the sound receptor carried by the air and by the bone.

In air conduction, sound waves enter the ear via the external auditory canal and impact on the tympanic membrane (eardrum) causing it to vibrate. The movement of the tympanic membrane is transmitted to the cochlea through the three auditory ossicles: malleus, incus and stapes (hammer, anvil and stirrup, the smallest bones in the body). Vibrations of the stapes footplate cause the perilymph to form a wave. This wave travels the length of the cochlea. Since the liquid is incompressible, each pressure wave of the stapes footplate at the oval window causes an equivalent outward movement of the round windows. This causes the basilar membrane to move in a wave-like fashion. Due to changes in the mechanical properties of the membrane, the amplitude of vibration changes along the basilar membrane. This explains why sounds of low frequency cause the greatest vibration at the apex while high frequency sounds cause vibration at its base. The stereocilia at the apex of each inner and outer hair cell, which are imbedded in the tectorial membrane undergo a shearing force (i.e. they are bent) triggering a series of mechanical, electrical and biochemical events responsible for mechanical-sensory transduction and initial acoustic signal processing. Electrical pulses travel through the auditory nerve to the brain. The inner hair cells transform signals generated in response to acoustic vibration into electric messages sent to the central nervous system. They are not responsible for the ear's threshold sensitivity and its frequency selectivity. The outer hair cells amplify mechanic-acoustic vibration and so facilitate stimulation of inner hair cells but send no auditory signal to the brain.

In bone conduction, compression waves impact the skull causing the cochlear fluid to deform the round or oval window and a movement of the basilar membrane. Another mechanism involves the movement of the ossicles which induce movement in the scala vestibuli only.

See **Appendix I** for a more detailed description and schemas.

The human ear can perceive a very wide range of sound pressure from 20 µPa to up to 20 or even 2000 Pa. Such a large scale (20-2,000,000,000) is highly inconvenient to use. A simpler way is to use the decibel or dB scale, a logarithmic measurement scale. The hearing threshold of 20 µPa is equivalent to 0 dB. As the sound pressure level (SPL or L_p) increases tenfold, the decibel level increases by 20 dB, in accordance with the following formula:

$$\text{SPL (in dB)} = 20 \log P_x/P_0$$

where P_x = measured sound pressure in Pa

P_0 = reference sound pressure (20 µPa)

As the human ear responds to the logarithmic change in sound level, the decibel scale gives a much better approximation to the human perception of relative loudness than the Pascal scale. The sound pressure level of audible sounds ranges

from 0 dB through 140 dB. The threshold of discomfort is usually noted between 85 and 95 dB and the threshold for pain is between 120 and 140 dB.

The sound pressure level is an objective measure of sound intensity, but is not an accurate measure of what is actually perceived. A normal human ear perceives frequencies from 20 Hz to 20,000 Hz (16 to 25,000 Hz) but the ear is not equally sensitive to all frequencies. The sensitivity of the human ear drops off sharply below about 250-500 Hz and above 4,000 Hz. As the primary concern is the effect on humans, the sound measurements are sometimes compensated by an "A"-weighted filter which attenuates low frequency and very high frequencies, leaving middle frequencies almost unchanged. The dB(A) (dB measured with an A-weighted filter) is often used as it reflects more accurately the frequency response of the human ear.

For short-term and impulsive noises, such as surface blasting, a C-weighted filter is normally used. The C-weighted filter helps to account for the short time period and frequency of impulsive noises.

The Equivalent Continuous Sound Pressure Level (L_{EQ}) is the constant noise level that would result in the same total sound energy being produced over a given period. It is an average noise level.

The Daily Noise Exposure (L_{EX} or $L_{EP,d}$) is the level of the worker's daily exposure to noise in dB(A), averaged over the entire workday and adjusted to an equivalent 8 hour exposure. The L_{EX} would equal the 8-hour L_{EQ} of a worker exposed for 8 hours. A L_{EX} = 90 dB(A) represents a continuous constant level exposure to a noise of 90 dB(A) for 8 hours. L_{EX} is derived from the L_{EQ} measured with a noise dosimeter or integrating sound level meter (SLM) over a sample time; it can be obtained from the measured L_{EQ} by applying a correction factor.

The peak sound level is the maximum instantaneous sound level, in dB(A).

2.2. MECHANISMS AND TYPES OF HEARING LOSS

Hearing loss can result from disorders of the auricle, external auditory canal, middle ear, inner ear, or central auditory pathway. In general, lesions in the auricle, external auditory canal, or middle ear cause conductive hearing losses, whereas lesions in the inner ear or eighth nerve cause sensorineural hearing losses.

2.2.1. Transmission or conduction hearing loss

A pure transmission hearing loss can result from all the conditions hindering the travel of the sound wave through the external ear to the tympanus and its amplification and transmission by the ossicles (middle ear) to the inner ear. Conductive hearing loss usually can be reversed by treating the underlying cause:

- obstruction of the external auditory canal by earwax or a foreign object,
- perforation of the tympanic membrane,
- external otitis or otitis media, tympanosclerosis (thickening and calcification of the tympanic membrane secondary to inflammation or traumatic events with involvement of the ossicular chain).

- cholesteatoma (acquired or congenital: accumulation of squamous epithelium within the middle ear),
- otosclerosis (osteodystrophic disease of the labyrinthine capsule), disruption of the ossicular chain

2.2.2. Sensorineural hearing loss

Sensorineural hearing loss may result from damage occurring at one or more points along the auditory pathway from the cochlea to the primary auditory cortex. Sensorineural hearing impairment can be either unilateral or bilateral, according to the underlying pathology, and in most cases is irreversible.

The main causes of sensorineural hearing loss are:

- congenital (hereditary or acquired)
- presbyacusis (or presbycusis)
- noise
- ototoxic therapeutic drugs
- head trauma: temporal bone fracture, labyrinthine concussion, central damage
- oval or round window rupture
- infections,
- Menière's disease
- idiopathic sudden sensorineural hearing loss (hearing loss of 30 dB within a three-day period)
- cerebellar angle tumours, such as acoustic neuromas, other neoplastic, vascular, traumatic, demyelinating, infectious or degenerative disease affecting the central auditory pathway.

The term *sensorineural* indicates uncertainty as to whether the hearing loss is due to a lesion in the inner ear (cochlea) or in the 8th nerve. The differentiation between sensory (cochlear) and neural (8th nerve) hearing loss is clinically important. Sensory hearing loss results from end-organ lesions (acoustic trauma, viral labyrinthitis, ototoxic drugs, Meniere's disease), which usually are not life threatening. Conversely, neural hearing loss is frequently due to potentially fatal cerebellopontine angle tumours and a wide variety of other neurological disorders (Merck, 2004).

Sensory and neural hearing losses may be differentiated on the basis of tests for speech discrimination, performance-intensity function for phonetically balanced words, recruitment, acoustic reflex decay, pathologic adaptation, otoacoustic emissions, cochlear potentials, and auditory brain stem responses.

Recruitment is the inability to hear quiet sounds coupled with a paradoxical intolerance for loud sounds due to recruitment. An ear with recruitment might well be unable to hear sounds, particularly high frequency sounds, below 50 dB, but find any sounds above 80 dB not only uncomfortable but liable to produce distortion. Hyperacusis differs from recruitment. With recruitment, loud noises are uncomfortable. With hyperacusis, all sounds are too loud.

Mixed hearing loss is a combination of sensorineural and conductive hearing loss.

2.3. EVALUATION OF HEARING LOSS

The evaluation of a patient with auditory complaints aims at determining:

- The nature of the hearing impairment (conductive versus sensorineural)
- The anatomy of the impairment (external ear, middle ear, inner ear, or central auditory pathway pathology)
- The severity of the impairment
- The aetiology.

In any case the assessment of hearing loss is based on a combination of history (personal, social and occupational), clinical examination and audiometric findings.

During the examination, it is crucial to detect: the presence or absence of tinnitus, vertigo, imbalance, aural fullness, otorrhea, headache, facial nerve dysfunction, head trauma, exposure to ototoxins, occupational or recreational noise exposure, family history…

A hearing test takes anywhere from about 15 minutes for a simple screening to hours for a full evaluation. A hearing evaluation should systematically start with a direct inspection of the ears with an otoscope to assess the condition of the ear canal and the tympanum. Pneumatic otoscopy reveals a decrease in the normal mobility of the tympanum.

There are simple tests to screen for hearing loss, such as the whisper test which assesses the ability to hear whispered speech across a short distance and the tuning fork tests (weber's test and rinne's test) which can be used to differentiate conductive from sensorineural hearing loss.

In audiometry**,** hearing loss is quantified. Adequate testing requires correct devices (no cracked or poorly fitting headphones or uncalibrated audiometer), a silent or low-level noisy environment, a competent audiologist, and a cooperative patient.

Pure-tone audiometry is a behavioural test measure used to determine hearing sensitivity. This simple test yields a great deal of information and its primary purpose is to determine the type, degree, and configuration of hearing loss. The conventional frequencies of 250-8000 Hz are used in testing because this range represents most of the speech spectrum. However, many ototoxic agents initially produce hearing loss in the high-frequency range, above the 8000 Hz upper limit of the standard audiogram.

Other types of tests are explained in **Appendix II**.

3. FACTORS AFFECTING HEARING

There is a severe scarcity of accurate, standardised, population based data on the prevalence and causes of deafness and hearing impairment, especially in developing countries.

The most recent estimate by WHO is that 250 million people in the world have disabling hearing impairment (moderate or worse hearing impairment in the better ear). Two-thirds of these people live in developing countries (WHO, 2001).

3.1. AGE

Presbycusis (or presbyacusis) is hearing loss associated with degenerative changes of aging. Hearing loss is a very common problem affecting older adults. Most of the elderly population suffers from progressive hearing loss: 60% of people older than 70 years have hearing loss of at least 25 dB (Gratton & Vazquez, 2003). This type of hearing loss is typically gradual, bilateral, and characterized by difficulty in hearing high frequencies. Presbycusis begins after age 20 but is usually significant only in persons over 65. Men are affected more often and more severely than women. Stiffening of the basilar membrane and deterioration of the hair cells, stria vascularis, ganglion cells, and cochlear nuclei may play a role in pathogenesis.It is also possible that noise exposure contributes, at least in part, to the onset and development of presbycusis. It first affects the highest frequencies (18 to 20 kHz) and gradually affects the lower frequencies. It usually begins to affect the 4 to 8 kHz range by age 55 to 65, although variation is considerable. Some persons are severely handicapped by age 60, and some are essentially untouched at age 90.

A population-based study in Wisconsin (including 3753 adults aged 48 to 92 years) reported that the prevalence of hearing loss was 45.9%. The odds of hearing loss increased with age (OR = 1.88 for 5 years, 95% CI 1.80-1.97) and were greater for men than women (OR = 4.42, 95% CI 3.73-5.24). The male excess of hearing loss remained statistically significant after adjusting for age, education, noise exposure, and occupation (OR = 3.65) (Cruickshanks et al, 1998a).

The interaction between noise-induced hearing loss (NIHL) and age-related hearing loss is difficult to determine. The most commonly accepted assumption is a simple accumulating effect of noise and ageing on the hearing. However, both a less than additive effect as well as a supra-additive effect has been proposed (Rosenhall, 2003). NIHL before old age seems to reduce the effects of ageing at noise-associated frequencies, but accelerates the deterioration of hearing in adjacent frequencies (Rosenhall, 2003).

3.2. NOISE

Noise is the most common preventable cause of sensorineural hearing loss.

Noise-induced hearing loss (NIHL) is the result of exposure to high sound levels at all ages: i.e. children noise exposure from toys and games, military service, occupational noise, and recreational activities. In general, it is believed that noise in the military and occupational environments has decreased due to better hearing conservation programmes, improved ear protection, and better compliance to regulations. By contrast, there is much less indication of improvement during

recreational activities, mainly due to lack of regulations and awareness, and poor ear protection (WHO, 1997).

Noise-induced hearing loss develops progressively with a dip at 4-6 kHz, is irreversible and stable, bilateral and symmetric (except in some particular circumstances such as firearms), associated with recruitment and frequently tinnitus.

The pathogenesis of noise-induced hearing loss has been summarized by the WHO (1997). The hair cells in the organ of Corti may be damaged directly by noise, or indirectly by very high levels of continuous sound which cause vasoconstriction of the vessels of the stria vascularis in the cochlea blood supply rendering the hair cells relatively anoxic and secondarily damaged. The amount and type of direct hair cell damage depends on the intensity of the sound. Above a certain minimum exposure of frequency and intensity, the outer hair cells show signs of metabolic exhaustion with drooping of the stereocilia. This correlates with the common phenomenon of temporary threshold shift (TTS), which recovers within a few hours (14-16 hrs). Higher sound levels damage the outer hair cell stereocilia further, including destruction of the inter-cilial bridges, and recovery takes longer. An even higher level of sound leads to collapse of the stereocilia, and the hair cell is eventually phagocytosed. Outer hair cells amplify the movement of the basilar membrane of the cochlea by contracting when stimulated by sound. This increases the stimulus delivered to the inner hair cells which transduce the mechanical movement to trigger a nervous impulse in the afferent nerve endings of the 8th nerve. If the outer hair cells are not functioning, greater stimulation is required to initiate a nervous impulse; thus the threshold sensitivity of the inner hair cells is raised which is perceived as a hearing loss. Hair cells in the basal coil of the cochlea are the most sensitive to noise damage; they are responsible for transducing higher frequencies and this accounts for the high frequency hearing loss found in noise-damaged ears (WHO, 1997).

Occupational exposure to noise

Mandatory directives in European industries have reduced noise emission levels over the last two decades and reduced the risk of damage to hearing by providing hearing protection for workers.

The exposure limit values and exposure action values in respect of the daily noise exposure levels and peak sound pressure are fixed, by Directive 2003/10/EC at:

(a) exposure limit values: $L_{EX,8h}$ = 87 dB(A) and ppeak = 200 Pa [(1)] respectively;

(b) upper exposure action values: $L_{EX,8h}$ = 85 dB(A) and ppeak = 140 Pa [(2)] respectively;

(c) lower exposure action values: $L_{EX,8h}$ = 80 dB(A) and ppeak = 112 Pa [(3)] respectively.

[(1)] 140 dB (C) in relation to 20 µPa.

[(2)] 137 dB (C) in relation to 20 µPa.

[(3)] 135 dB (C) in relation to 20 µPa.

$L_{EX,8h}$ daily noise exposure level : time-weighted average of the noise exposure levels for a nominal eight-hour working day. It covers all noises present at work, including impulsive noise;

$L_{EX,8h}$ weekly noise exposure level: time-weighted average of the daily noise exposure levels for a nominal week of five eight-hour working days the health and safety protection of workers.

ppeak: peak sound pressure maximum value of the 'C'-frequency weighted instantaneous noise pressure.

The Directive specifies that when applying the exposure limit values, the determination of the worker's effective exposure shall take account of the attenuation provided by the individual hearing protectors worn by the worker. The exposure action values shall not take account of the effect of any such protectors.

The daily noise exposure – not taking account of any hearing protection – for several of refining and some non-refining job activities associated with the downstream oil industry is likely to exceed 85 dB(A) $L_{EP,d}$ and, sometimes 90 dB(A) $L_{EP,d}$ (CONCAWE, 2001), see **Figure 1**.

REFINERY DATA

- On-site operators: 40% of the results below 85 dB(A) LEQ,d and 20% in excess of 90 dB(A) LEQ,d
- Off-site operators: 80% of the results below 85 dB(A) LEQ,d and 10% in excess of 90 dB(A) LEQ,d
- Maintenance workers: 65% of the results below 85 dB(A) LEQ,d and 15% in excess of 90 dB(A) LEQ,d
- With the exception of laboratory technicians, less than 10% of the results for all job groups were below 75 dB(A) LEQ,d

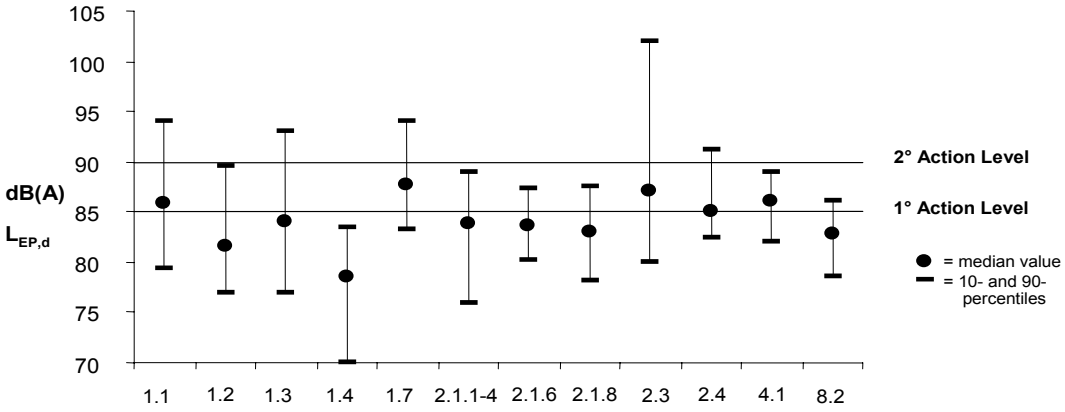
NON-REFINERY DATA

- Distribution terminals, particularly road tanker drivers for whom 40% of the results exceeded 85 dB(A) LEQ,d
- Lube blending facilities, for which most of the results were below 85 dB(A) LEQ,d
- Airport operators, for which about 70% of the results exceeded 85 dB(A) LEQ,d
- Marine activities, for which more than 60% of the results exceeded 85 dB(A) LEQ,d
- LPG bottling, for which over 50% of the results exceeded 85 dB(A) LEQ,d

The change of hearing thresholds was assessed in a study population of over 1000 oil refinery workers subject to noise at work regulations in Member States of the European Union. Audiometric data covering a period of approximately 12 years were retrieved from refinery occupational health departments. This study did not identify any noise-induced hearing loss from occupational noise exposure in workers drawn from 10 European oil refineries, exposed to noise at work in the period from the mid 1980's to the late 1990's. This conclusion was reached using measured hearing threshold data, corrected for the effect of natural ageing according to guidelines issued by the International Organization for Standardization. The same

conclusion was also reached when using a modified age correction proposed in the scientific literature (CONCAWE, 2000).

Figure 1 Personal daily noise exposures for selected job titles in European downstream oil industry operations, 1989-1999



1.1	Refinery on-site operators	2.1.6	Road distribution terminal workers
1.2	Refinery off-site operators	2.1.8	Road distribution terminal maintenance
1.3	Refinery maintenance workers	2.3	Ship distribution personnel
1.4	Refinery lab workers	2.4	LPG bottling operators
1.7	Refinery utilities workers	4.1	Airport refuelling operators
2.1.1-4	Road tanker drivers	8.2	Lubes packaging operators

Environmental and leisure noise: 'socioacusis'

Controversy exists regarding what percentage of age-related hearing loss (presbycusis) is a consequence of a lifetime of socioacusis and how much is due solely to the physiologic aging process.

Environmental noise including that from traffic and recreational activities has been increasing, as have the number of complaints from the public. Noise pollution has had a much lower priority than air and water pollution. In developed countries, the risk from social noise is increasing for young people (WHO, 1997).

No consensus exists regarding the risks associated with leisure noise, in particular, personal listening devices, in causing noise-induced hearing loss (NIHL) (e.g. Rice et al, 1987; Meyer-Bisch, 1996; Dalton et al, 2001). Personal listening devices can produce sound levels greater than 100 dB(A), but there are at least two main differences in comparison to workplace noise:

1) Intermittency of exposure: NIHL is strongly related to noise dose which is determined by level and duration of exposure; the typical intermittency of leisure noise exposure allows the ear to recover between each loud music encounter;

2) The spectrum of amplitude: amplified music is more centred to low frequencies.

The prevalence of sensorineural hearing loss in young military conscripts was 36.7 per 1,000 (95% CI 24.8-51.9). Relative risk of hearing loss was higher in subjects with frequent discotheque visits compared to those who never did so (RR 2.72,

95% CI 1.09-6.76). However, no difference was shown for those with infrequent discotheque visits (RR 0.85, 95% CI 0.38-1.92); frequent personal stereo use (RR 1.08, 95% CI 0.28-4.08) and infrequent stereo use (RR 1.27, 95% CI 0.38-4.20) (Toh et al, 2002). No significant effect of frequent use of personal stereo players or regular attendance at discotheques or rock concerts could be demonstrated on the hearing threshold in a Norwegian study comprising 51,975 participants (Tambs et al, 2003).

3.3. DISEASES

Many diseases are associated with hearing loss; this is not intended to be an exhaustive review of the problem but highlights the importance of this factor in the development of hearing impairment.

Given the unique biological requirements of sound transduction and the selective advantage conferred upon a species capable of sensitive sound detection, it is not surprising that up to 1% of the approximately 30,000 or more human genes are necessary for hearing. There are hundreds of monogenic disorders for which hearing loss is one manifestation of a syndrome or the only disorder (Friedman & Griffith, 2003).

The most common cause of conduction hearing loss, chronic otitis media, may also lead to profound sensorineural hearing loss (e.g. Papp et al, 2003, El-Kashlan et al, 2002; MacAndie & O'Reilly, 1999; Tambs et al, 2003).

Recent studies have associated autoimmune diseases to otological disorders. Sensorineural hearing loss in rheumatoid arthritis is reported to be the result of the extra-articular manifestation of the disease. Patients affected by rheumatoid arthritis show an impairment of auditory threshold (e.g. Salvinelli et al, 2004; Kastanioudakis et al, 1995; Raut et al, 2001). Sensorineural hearing loss appears to significantly correlate with active disease and with the presence of rheumatoid factor (Magaro et al, 1990). A high prevalence of sensorineural hearing loss is also reported in patients suffering from Sjogren's syndrome (e.g. Ziavra et al, 2000; Tumiati et al 1997) or following radiotherapy (Wang et al, 2003).

Although the animal studies and pathology series suggest a plausible biological basis for an association between diabetes and hearing loss, the results of clinical studies and population-based study are equivocal, but there is a tendency to show a positive association. Of 3,571 participants, in the Epidemiology of Hearing Loss Study, 344 (age 69.6 $\pm$ 9.5 years) were classified as having NIDDM (non-insulin dependent diabetes mellitus). Subjects with NIDDM were more likely to have a hearing loss than were subjects without diabetes (59 vs. 44%). However, after results were adjusted for age, this difference was no longer statistically significant. After individuals with hearing loss patterns inconsistent with presbyacusis were excluded, there was an association between NIDDM and hearing loss when controlling for potential confounders (OR 1.41; 95% CI 1.05–1.88). There was no association between duration of diabetes or glycaemic control and hearing loss. Individuals with NIDDM and nephropathy were more likely to have a hearing loss than were those with NIDDM without nephropathy (OR 2.28, 95% CI 1.04–5.00). A person with NIDDM who is also occupationally noise-exposed is more likely to develop severe NIHL than those without NIDDM. History of non-insulin dependent diabetes mellitus (NIDDM) was reported by 16.4% of the men with severe NIHL compared to 4.8% of the 83 men without severe NIHL (OR 3.9, 95% CI 1.2-11.9, P = 0.05) (Ishii et al, 1992).

Epidemiological and experimental studies suggest that hypercholesterolemia promotes the development of sensorineural hearing loss, especially presbycusis and noise-induced hearing loss (Preyer et al, 2001). Observations on guinea pigs have shown that hypercholesterolemia alone may cause auditory dysfunction if dietary cholesterol is kept at a high level for a long time. Alterations attributed to hypercholesterolemia begin in the stria vascularis and then spread over the outer hair cells, mainly in the basal turn (Satar et al, 2001).

3.4. CHEMICAL EXPOSURE

Hearing impairment due to the cochlear toxicity of drugs is well documented. The interest in auditory effects of chemicals is recent and has focused mainly on solvents. Heavy metals and chemicals inducing hypoxia/anoxia have also been studied.

This review aims to focus mainly on the solvent issue as these are the chemicals most likely to be encountered in the petroleum industry.

3.4.1. Therapeutic drugs

A review of the literature on drug-induced ototoxicity found 414 published articles (Palomar Garcia et al, 2001), see **Table 2**. The authors came to several conclusions, among which:

- the number of therapeutic substances that can cause a greater or lesser degree of ototoxicity is very large. As many as 130 commonly-used drugs are reported;
- not all ototoxic medications have the same sites of action in the inner ear;
- the dose and dosing interval are important as they determine whether toxic serum levels are reached;
- not all humans are equally susceptible to ototoxic drugs;
- high-frequency audiometry, transient evoked otoacoustic emissions and distortion-product otoacoustic emissions are highly sensitive for early detection of the ototoxic effects of drugs.

Table 2 Main classes of therapeutic drugs associated with hearing loss

drugs	hearing loss: general characteristics

aminoglycoside antibiotics
Streptomycin, gentamicin, amikacin, neomycin, dihydrostreptomycin, kanamycin
	- systemic and topical administration
	- cochleotoxicity and vestibulotoxicity
	- irreversible - destruction of outer hair cells (OHCs)
	- genetic/familial predisposition
	- synergistic ototoxic effect described in case of co administration of other ototoxic agents:
	  - loop diuretics
	  - noise
	- detected in the cochlea months after final dose administration which may account for delayed onset of hearing loss and prolonged susceptibility to noise-induced hearing loss (observed for several months following therapy discontinuation)

Loop diuretics ethacrynic acid, furosemide, bumetanide	- mainly cochleotoxicity - rapid onset - usually moderate and reversible in adults - co administration of other ototoxic agents: - amino glycoside
non steroidal anti-inflammatory drugs (NSAIDs) salicylates	- mainly cochleotoxicity - rapid onset, + tinnitus - usually moderate, reversible
antimalarial drugs quinine, chloroquine, quinidine	- usually reversible but irreversible hearing loss reported with quinine. - rapid onset, sometimes + tinnitus
antineoplastic agents cisplatin, carboplatin.	- irreversible (destruction of OHCs) - rapid or delayed onset. Cisplatin irreversibly binds to plasma proteins and can be detected months after completion of therapy. Carboplatin is more readily cleared by the kidneys.

3.4.2. Cigarette smoking and ethanol consumption

Cigarette smoking. When considering possible hearing impairment due to exposure to ototoxic substances, it is important to be aware of the likely confounding effect of smoking habits. Smoking may affect hearing through an ischaemic mechanism - both by reducing blood flow and by increasing carboxyhaemoglobin – and by a central mechanism, due to a nicotinic effect.

Several recent studies (a.o., Barone et al, 1987; Virokannas et al, 1995; Cruickshanks et al, 1998b; Stark et al, 1999; Nakanishi et al, 2000; Itoh et al, 2001; Sharabi et al, 2002; Mizoue et al, 2003; Palmer et al, 2003) have demonstrated that smokers are at increased risk of noise-induced hearing loss.

The Epidemiology of Hearing Loss Study concluded that current smokers were 1.69 times (after adjusting for other factors) as likely to have a hearing loss as non-smokers (95% CI 1.31-2.17). This relationship remained for those without a history of occupational noise exposure and in analyses excluding those with non-age-related hearing loss. There was weak evidence of a dose-response effect. According to the study, 25.9% of smokers in the youngest age group - 48 to

59 years of age - were suffering from hearing loss compared to 16.1% among non-smokers. 22.7% of ex-smokers were suffering from hearing loss. The same trend was found in the older age groups (Cruickshanks et al, 1998b).

Ethanol consumption. In addition to the well known metabolic interactions at the cytochrome P450-level, ethanol can either induce or inhibit the biotransformation of organic solvents depending upon the level of alcohol intake – ethanol causes brain damage that may influence the central auditory tract. Experimental studies suggest that alcohol is acting centrally, at the level of mechanisms involved in the temporal and binaural summation of auditory signals, rather than influencing peripheral structures (Pearson et al, 1999).

A decrease in sensitivity of the acoustic reflex occurs after alcohol ingestion (Robinette & Brey, 1978; Bauch & Robinette, 1978). According to Murata et al (2001), drinking extra small amounts of alcohol induces the reduction of auditory threshold. They observed that the auditory threshold was significantly reduced within 30 min after the ingestion of 250 and 500 ml of beer, occurring on and after peak blood alcohol concentration. The reducing effect disappeared 480 min after ingestion. Smith and Riechelmann (2004) have demonstrated that chronic alcohol consumption leads to damage in the brainstem, as shown by a significant delay of the latency I–V at the Brainstem Auditory Evoked Potentials. Hwang et al (2003) identified a temporary reduction in DPOAE's after acute alcohol consumption to the intoxication level, without affecting auditory threshold.

However, the Epidemiology of Hearing Loss Study (EHLS), cited above, concluded a modest protective association of alcohol consumption and hearing loss. In multiple logistic regression analyses controlling for potential confounders, moderate alcohol consumption (>140 g/week) was inversely associated with hearing loss (OR 0.71, 95% CI 0.52-0.97). Alcohol consumption was associated inversely with the odds of having a low frequency hearing loss (OR 0.61) or a high frequency hearing loss (OR 0.60). These findings did not vary significantly by age or gender. There was an increase in the odds of having a high frequency hearing loss (OR 1.35, 95% CI 1.04-1.75), in those with a history of heavy drinking (≥4 drinks/day) (Popelka et al, 2000).

3.4.3. Hydrocarbons

The ototoxicity of many individual solvents or mixtures of solvents have been investigated in animal experiments and/or human studies; mainly toluene, styrene (a.o. Muijser et al, 1988; Möller et al, 1990; Sass-Kortsak et al, 1995; Campo et al, 2001, 2003; Lataye et al, 2000; Loquet et al, 2000; Morata et al 2002; Makitie et al, 2003; Sliwinska-Kowalska et al, 2003), trichloroethylene (a.o. Rebert et al, 1991; Crofton et Zhao, 1993, 1997; Crofton et al, 1994; Fechter et al, 1998; Muijser et al, 2000; Yamamura et al, 1983), n-hexane, ethylbenzene, and carbon disulfide (Rebert & Becker, 1986; Clerici & Fechter 1991; Sulkowski, 1979; Morata, 1989; Hirata et al, 1992; Kowalska et al, 2000; Chang et al, 2003).

The most relevant of these solvents to the petroleum industry are toluene and xylenes as gasoline contains typically about 10% of both compounds. Other compounds of less importance are ethylbenzene and n-hexane, representing about 2 and 1.3% of gasoline content (CONCAWE, 2002). **Table 3** lists current OELs.

Table 3 Selected occupational exposure limits for gasoline components in some EU countries and US (8-hours time weighted average)

	ACGIH	EU	Belgium	France	D	NL	Poland	UK	Sweden
toluene									
mg/m³	*190*	192	191	375	190	150	100	191	200
(ppm)	(50)	(50)	(50)	(100)	(50)	*(40)*	(27)	(50)	(50)
xylenes									
mg/m³	*440*	221	440	435	440	210	100	220	200
(ppm)	(100)	(50)	(100)	(100)	(100)	*(50)*	(23)	(50)	(50)
ethyl-benzene									
mg/m³	*440*	442	440	435	-	215	100	441	200
(ppm)	(100)	(100)	(100)	(100)		(49)	(23)	(100)	(50)
n-hexane									
mg/m³	*175*	72	179	170	180	90	100	72	90
(ppm)	(50)	(20)	(50)	(50)	(50)	*(25)*	(27)	(20)	(25)

in italic: calculated level (toluene: 190 mg/m³ ≈ 50 ppm; xylenes: 440 mg/m³ ≈ 100 ppm; ethylbenzene: 440 mg/m³ ≈ 100 ppm; n-hexane: 180 mg/m³ ≈ 50 ppm).

3.4.3.1. Animal experiments

- **toluene**

There is clear evidence that inhalation of high levels of toluene disrupts the auditory system and cause a permanent elevation of the auditory thresholds in experimental animals (mainly rats) (Pryor et al, 1983, 1984a, 1984b; Pryor & Howd, 1986; Rebert et al, 1983; Johnson and Canlo 1994a, 1994b; Campo et al, 1997, 1999; Lataye et al 2003).

Toluene ototoxicity occurs at relatively intense schedules of exposure. In rats, for instance, 400 and 700 ppm were without effect even after 16 weeks of exposure. Two weeks of exposure to 1,000 ppm toluene 14 hours/day caused hearing loss. Three-day exposures to 1,500 ppm for 14 hours/day or to 2,000 ppm for 8 hours/day were ototoxic. Single exposures to 4,000 ppm for 4 hours or to 2,000 ppm for 8 hours were without effect. Intermittent exposure to 3,000 ppm for 30 minutes every hour for 8 hours/day caused hearing loss within 2 weeks, but a similar exposure schedule for 4 hours/day was ineffective even after 9 weeks (Pryor et al, 1984). The main experimental studies are summarized in **Appendix III, Table 1**. It can be concluded that repeated exposure to toluene in concentrations ranging from 600 ppm to 1500 ppm, depending on the auditory tests used, appears to be necessary to cause ototoxicity in rats. There is some indication that this ototoxicity is a long-lasting irreversible effect: even after one year hearing function had not recovered (Nylen et al, 1994).

With increasing age, toluene exposure (1000 ppm, 12 h/day, and 7 days) appears to have little effect on the aging process of the auditory system in CBA mice but accelerates age-related hearing loss in C57 mice. This observation indicates that toluene aggravates auditory deterioration only in mice with a strong genetic predisposition to spontaneous age-related hearing loss (Li et al, 1992).

Electrophysiological data have demonstrated that in the rat toluene-induced hearing loss is primarily located in the mid-frequency area (16–20 kHz) (Campo et al, 1997; Johnson & Canlon, 1994; Crofton & Rebert, 1994). The auditory threshold shifts increase significantly as a function of the concentrations of the solvent.

A slight frequency shift exists between the electrophysiological data and histological data showing that along the organ of Corti, toluene induces a trauma not only in the middle (mid-frequency area: 16-20 KHz) but also in the mid-apical (mid to low frequency area: 4-5 kHz) turns of the cochlea (Campo et al, 1997; Lataye et al, 1999). However, the electrocochleographic approach (auditory-evoked potentials directly from the round window of the cochlea) showed that toluene-induced hearing deficits are positioned not only in the mid-frequency region, but also in the mid–low frequency region (Lataye et al, 1999). This suggests that a broader range of frequencies than that usually reported by the literature could be damaged by solvents (Lataye et al, 1999).

It is well-established that noise-induced hearing loss is due primarily to a stereocilia pathology (Lataye & Campo, 1997), whereas toluene-induced hearing loss is caused by its toxic effect on the organ of Corti. It is likely that solvent contamination of the organ of Corti is different from that caused by antibiotics. The solvent-induced hearing loss would not be caused by fluid contamination, but by a tissue intoxication involving the outer sulcus rather than the auditory nerve. Cerebrospinal and inner ear fluids were shown to be free from detectable solvents, whereas the organ of Corti, the nerves, and the brain were contaminated (Campo et al, 1999). Disorganization of the membranous structures could be the starting point for the cochlear injury. The pathological events could be due to an easy penetration of lipophilic solvents into the phospholipid layers, modifying the membrane fluidity and structure. The membranous impairments induced by solvents might also have mechanical consequences on the organ of Corti (Campo et al, 1999). The histological findings demonstrate that supporting cells are the first targets of the solvent. Then, the outer hair cells of the third row (OHC3) are disrupted, followed successively by OHC2 and OHC1 from the basal (20 kHz) to the upper turn (4 kHz) of the cochlea (Loquet et al, 1999; Campo et al, 1997, 1998, Johnson & Canlon, 1994a, 1994b; Sullivan et al, 1988). This chemical insult leads to the destruction of outer hair cells whereas the inner hair cells seem to be well preserved (Campo et al, 2001).

Toluene-induced vulnerability of the cochlear function appears to be **species**-dependent. Indeed, most published data have been obtained in the rat, which developed cochlear deficits, whereas the guinea pig did not show any permanent hearing loss after solvent exposure. Actually, concentrations as low as 250-ppm toluene (8 h/day, 5 days/week for 1 week) impaired the auditory function, measured as depression of distortion product otoacoustic emissions (DPOAEs) acutely after exposure. However, the impairment measured with DPOAEs was reversible and toluene concentrations up to 1000 ppm did not cause permanent hearing loss (McWilliams et al, 2000). In a comparative study by Lataye et al (2003) the rat model showed severe disruption of auditory function and cochlear pathology following exposure to high levels of toluene (600 ppm) whereas the guinea pig had no disruption of DPOAE or cochlear pathological alterations. No explanation could be given for the difference observed between this study and the study by McWilliams. Chinchillas are widely used for studying noise effects on the cochlea. However, this species seems to be markedly less susceptible to the ototoxic effect of toluene than rats (Davis et al, 2002).

These differences could be explained by the metabolism pathways of solvents, by differences in enzyme activities in the liver and in the cochlear sensory epithelium or by the morphological differences of the membranes of the hair cells according to the species (Lataye et al, 2003). Hepatic microsomes from chinchillas were found to contain more of the P450 enzymes CYP2E1 and CYP2B than rats or humans. In addition, the P450 enzymes were shown to be more active in chinchillas than in rats and humans, suggesting greater detoxifying properties. The rate of conversion of toluene to benzyl alcohol, a more water soluble product easier to eliminate via the kidney, was found to be almost 3 times faster in chinchillas than in rats or in humans (Davis et al, 2002). Hence, the rat was considered by these researchers to be a more appropriate model than the chinchilla to assess human toluene ototoxicity. Lataye et al (2003) showed a difference in the toxicokinetics of toluene between the rat and the guinea pig, and suggested that the rat was a more appropriate model; however no direct comparison with humans was made. The essential question of the best animal model for human risk assessment still needs to be addressed.

Both noise and toluene each can cause permanent threshold shifts, but the mechanisms of cochlear damage are different. As already mentioned, noise-induced hearing loss is mainly related to injury of the OHCs stereocilia, whereas toluene-induced hearing loss is related to outer hair cell losses. In the rat, there is evidence that exposure to high concentrations of toluene, in combination with exposure to noise, can cause a permanent synergistic or at least additive loss of auditory sensitivity (Johnson et al, 1988; Lataye & Campo, 1997; Brandt-Lassen et al, 2000*).* Simultaneous exposure to high levels of toluene and noise (Lataye and Campo, 1997) or sequential exposures to toluene followed by noise (Johnson et al, 1988; Brandt-Lassen et al, 2000) produced hearing losses which were greater than the summated loss caused by toluene and noise alone. The study by Brandt-Lassen et al (2000) suggests a synergistic interaction between toluene and noise at a toluene concentration (1000 ppm) that in itself did not seem to cause any significant threshold shift. In contrast, if the hearing loss after exposure to noise followed by toluene was greater than that recorded after exposure to noise alone or toluene alone, it did not exceed the summated loss caused by noise alone and toluene alone. Hence, the exposure sequence can determine the extent of auditory impairment (Johnson et al, 1990).

The similarity of effect via subcutaneous and gavage administration to that observed following inhalation exposure indicates that direct penetration of the toluene vapours through the external ear structure, as might occur during inhalation exposure, is not a necessary condition for inducing the hearing loss (Pryor & Howd, 1986; Sullivan et al, 1988). Moreover these studies show that noise from the inhalation system used to generate the test atmosphere was not a major contributing factor to the hearing loss.

There is strong evidence that toluene itself is responsible for the auditory dysfunction (Pryor et al, 1991). Indirect evidence is given by the observation that, acute doses of ethanol administered by gavage, large enough to inhibit the microsomal cytochrome P450 system and slow down toluene biotransformation, caused a significant enhancement of the toluene ototoxicity (Campo et al, 1998). While the rat auditory function was not affected by a daily acute exposure to ethanol alone, hearing and outer hair cell losses were larger after exposure to both ethanol and toluene than those induced by toluene alone, indicating a potentiation of solvent ototoxicity by ethanol. The hair cell losses increased dramatically (almost the totality of OHC3 had disappeared) when a high dose of ethanol was added daily to the solvent exposure (Campo et al, 1998). In the study by Nylen et al (1995), ethanol administered at lower doses in drinking water, antagonized toluene-induced loss of

auditory sensitivity. It can be hypothesised that in this experimental protocol, ethanol produced an induction of the microsomal cytochrome P450 system, leading to lower toluene concentration in blood.

A synergistic enhancement of loss of auditory sensitivity was observed in rats exposed to toluene (1000 ppm) and n-hexane (1000 ppm) (21 h/d, 7 d/w, 28 d) 3 months, but not 1 year after exposure (Nylen et al, 1994).

It has also been shown that acetyl salicylic acid given by gavage to rats permanently potentiates toluene-induced loss of auditory sensitivity (Johnson, 1992).

The EU has recently conducted a risk assessment for toluene (EU, 2003). The conclusion in regard with the auditory effect in experimental animals was the following: “The evidence points to long-lasting irreversible ototoxicity of toluene. It is likely that the rat must be exposed to a certain minimum concentration of toluene for a certain minimum of time before ototoxicity will develop. The size of this minimum concentration is not known; nor has it been sufficiently documented that a certain low concentration will not cause ototoxicity after long-term exposure. The study with the longest exposure period is the study by Pryor (1984) in which 700 ppm (2,625 mg/m³) toluene for up to 16 weeks was a NOAEC with a 14-hour daily exposure duration. In the same study, the LOAEC was 1,000 ppm (3,750 mg/m³) (14 hours/day). At 1,000 ppm an exposure duration of only 2 weeks was associated with hearing loss. In this study auditory function was evaluated by estimation of auditory sensitivity, which is not the most sensitive method. It is known that damage to the auditory system can be present without being detected by estimation of auditory sensitivity. In the Campo et al (1997) study loss of hair cells was detected down to 1,000 ppm, while auditory thresholds were only significantly changed at 1,500 ppm and above. In the McWilliams et al (2000) study, hearing function was evaluated via distortion product otoacoustic emission. This revealed transient auditory system impairment at a toluene concentration of 250 ppm i.e., one fourth of the LOAEC from the Pryor et al (1984) study determined via the brainstem auditory evoked response. Thus, it is possible that effects would have been detected at 700 ppm if more sensitive measurement methods had been employed, such as morphological examination, or auditory acuity measurement. The rapporteur is of the opinion that there can be no doubt that toluene is ototoxic in rats, and that the effect is chronic.”

Actually in a further study conducted by Campo’s team (Lataye et al, 2003), disruption of auditory function (as measured by DPOAE) and cochlear pathology (as measured by histology) were observed in rats 4 weeks after exposure to 600 ppm, 6 hours per day during 5 days. In the guinea pig, no such effect was observed not even temporarily. Hence, a precise NOAEC can not be established on the available scientific literature but is lower than initially thought and could lie around 400–500 ppm.

- **xylene, ethylbenzene and n-hexane**

Repeated inhalation exposure to high levels of mixed xylenes resulted in hearing dysfunction in the frequency range 4-24 kHz in rats (Pryor et al, 1987; Crofton et al, 1994; Nylen et al, 1994; Gagnaire et al, 2001). The auditory threshold shift did not reverse after 8 weeks recovery (Gagnaire et al, 2001). No hearing impairment developed in rats exposed once to 1700 ppm for 4 hours (Pryor et al, 1987).

A study comparing the ototoxicity of the three isomers showed that para-xylene produced moderate to severe increase in auditory thresholds and OHC losses in rats repeatedly exposed to ≥900 and 1800 ppm, respectively. The no-observed

effect level was 450 ppm. No effect was observed in animals exposed up to 1800 ppm ortho-xylene and meta-xylene (Gagnaire et al, 2001).

Ethylbenzene has also been shown to induce hearing loss in the rat. The mid-frequency region appears to be affected after exposure to levels of ethylbenzene only 3–4 times the threshold limit value. Histological examination of the cochlea demonstrated OHC loss, especially in the upper basal and lower middle turns (Cappaert et al, 1999) showing that the pattern of OHC loss is similar to that observed with toluene.

The effects after mild ethylbenzene exposure can be characterised as severe where OHC loss is concerned and minor with respect to physiological deterioration. Apparently, there can be a relatively large loss of OHCs, especially in the third row, without any functional consequences. With increasing ethylbenzene concentration, the affected region broadened, the loss became larger and the loss expanded to the second and first row of OHCs (Cappaert et al, 2001) and in a first study, Cappaert et al (2000) established a NOAEL and LOAEL at 300 ppm and 400 ppm, respectively. But in a further study, OHCs losses were already observed at 300 ppm (Cappaert et al, 2001).

Guinea pigs, in contrast to rats, are not susceptible to the ototoxic action of ethylbenzene. In a comparative study, rats showed deteriorated auditory thresholds in the mid-frequency range, based on electrocochleography, after 550 ppm ethylbenzene (8 h/day, 5 days). Outer Hair Cell loss was found in the corresponding cochlear regions. In contrast, guinea pigs showed no threshold shifts and no OHC loss after exposure to much higher ethylbenzene levels (2500 ppm, 6 h/day, 5 days). The difference in susceptibility between the species is suggested to be related to the ethylbenzene toxicokinetics. Ethylbenzene concentration in blood was more than 8 times higher at the end of the first day of exposure in the rat than in the guinea-pig (Cappaert et al, 2002).

Some perturbations in brainstem auditory-evoked responses (BAERs) have been observed in rats repeatedly exposed to high levels of n-hexane (1000 ppm) (Rebert et al, 1982). Rats were not as severely affected by repeated, brief (10 minutes) exposures to 24,000 or 48,000 ppm hexane 6 or 12 times per day; only the amplitude of the fifth BAER component was affected by the 48,000 ppm exposure (Rebert et al, 1982).

The main experimental studies are summarized in **Appendix III, Table 1**.

3.4.3.2. Human studies

Most investigations addressing the auditory toxicity of solvents in humans relate to exposure with mixtures of organic solvents alone or in combination with noise. None concerns exposure to toluene or other solvents of interest alone. However, some studies have investigated the effect of combined exposure to toluene and noise, and to mixtures of solvents including toluene, xylene and ethylbenzene.

Sensorineural hearing loss has been reported in solvent abusers inhaling large amounts of solvents (primarily toluene) and showing multifocal central nervous system damage (i.e. Metrick & Brenner, 1982; Ehyai & Freemon, 1983; Williams, 1988; Cavanagh, 1983; Fornazzi et al, 1983; Lazar et al, 1983; Hormes et al, 1986). In three of four patients with a history of chronic solvent vapour (primarily toluene) abuse for 2 or more years, BAERs were still abnormal after an abstinence period of at least 4 weeks (Hormes et al, 1986).

♦ **petrochemical workers**

Morata et al (1997a) explored the occurrence of hearing loss among 438 South American refinery workers exposed to various low levels of hydrocarbons (benzene, toluene, xylene, ethylbenzene, cyclohexane) and noise. Several groups of workers were selected from different departments:

1) controls selected from the warehouse and health clinic;
2) workers from the aromatics and paraffin plants (as the occasions when workers spent 3 h or more exposed to noise were rare and the use of hearing protectors was enforced, the noise dose was not considered to represent a health risk);
3) workers from the shipping (considering that the workers spent up to 3 h on alternate days exposed to noise and that the use of hearing protection was enforced, their noise dose was not considered to represent a health risk);
4) workers from a maintenance crew assigned to the aromatics and alkylation plant exposed to noise;
5) workers from the quality control laboratory;
6) workers who in the past worked in the aromatics department and were transferred to other departments: they were neither exposed to noise, nor to solvents.

Personal exposures and area concentrations of benzene toluene, xylene, ethylbenzene and cyclohexane were measured during the investigation but the sampling methods and measurements are not detailed. The exposure levels were well below the exposure limits recommended by the US National Institute for Occupational Safety and Health (0.1 ppm), except for benzene levels which exceeded the recommended level in several instances (**Table 4**). Only concentration ranges are provided. The exposure level of the workers previously exposed to aromatics was unknown.

The majority of workers had no previous industrial employment and lived in the refinery area. Previous exposure to noise and to chemicals, medical and audiological histories (ear infections and surgery, history of hearing loss in the family, use of ototoxic medications, tinnitus, high fever, measles, mumps, diabetes, high blood pressure), hobby history and prior military service were assessed. The standard deviations of these variables were particularly wide.

Otoscopy, immittance measurements and pure tone audiometry (0.5–8 kHz) were performed. PTA was preceded by a period of at least 14 h without occupational exposure to noise. Audiograms were classified as normal if no single threshold exceeded 25 dB.

The average thresholds are not provided but it is reported that the prevalence for hearing loss in the hydro/noise-carbon exposed group ranged from 42 to 50%, thereby exceeding the prevalence observed in the non-exposed group (warehouse and health clinic and laboratory workers) (15-30%) (ANOVA, $p < 0.005$). However, the 95% CI of the different groups overlapped.

The bilateral high-frequency sensorineural hearing losses were examined as a binary outcome variable (normal hearing vs high-frequency hearing loss) using multiple logistic regression. Conductive and unilateral hearing losses were entered as normal hearing. The independent variables considered for inclusion were age, exposure group, tenure, previous occupational exposure to noise or chemicals,

exposure to non-occupational noise and medical history (including smoking and alcohol consumption). Age and department were the only variables that met the significance level criterion for inclusion in the regression model. The age-adjusted OR estimates were 2.4 (95% CI 1.0-5.7, ns) for the aromatics & paraffins group; 3.0 (95% CI 1.3-6.9, p <0.05) for the maintenance group; 1.8 (95% CI 0.6-4.9, p <0.05) for the shipping group and 0.5 (95% CI 0.2-14; ns) for the laboratory group when compared to the group from the warehouse and the health clinic. The maintenance group represented combined exposure to noise and to low levels of hydrocarbons. No stratification in function of the exposure level was done.

Concerning the acoustic reflex measurements, multivariate analysis of variance (including age, group, ear, frequency of the stimulus and stimulus presentation) did not show any significant difference between the groups. Subsequent ANOVAs indicated that the percentage of reflex decay was significantly higher in the aromatics group and a subgroup of the maintenance workers (pipe-filters), and higher when the stimulus was presented ipsilaterally.

Table 4 Characteristics of South-American refinery workers study (Morata et al, 1997a)

	"warehouse & health clinic" n=41 men	**"aromatics & paraffins" n=89 men**	**"shipping" n=40 men**	**"maintenance" n=180 men**	**"laboratory " n=69 men**
noise (dB(A))	<85	85 ("aromatics": 78-101, "paraffins":71-98, 2 h/day) hearing protection	<85 (82-104 at selected points, up to 3 h on alternate days) hearing protection	**≥85** 89; 78-101	<85
Hydro-carbons range (ppm)	ND	**toluene : ND-13.2** **xylene : ND-2.6** **ethyl-benzene : ND-0.6** **benzene : ND-15** *cyclo hexane : ND-13.6*	**toluene : ND-18.4** **xylene : ND-1.2** **ethyl-benzene : ND-0.3** **benzene : ND-0.12** *cyclo hexane : ND-0.6*	**toluene : ND-11** **xylene : ND-5.1** **ethyl-benzene : ND-1.1** **benzene : ND-32** *cyclo hexane : ND-1.3*	**toluene : ND-0.3** **xylene : ND-0.3** **ethyl-benzene : ND-0.1** **benzene : 0.01-0.87** *cyclo hexane : ND-0.3*
tenure mean (SD) (years)	18.4 (4.8)	16.6 (7)	16 (6.5)	18.6 (4.5)	16.3 (5.6)
age mean (SD) (years)	44 (0.9)	40.4 (0.6)	41.5 (0.9)	43.9 (0.4)	40.7 (0.7)
hearing loss*	30% (upper 95% CI: 44%)	49% (upper 95% CI: 58%)	42% (upper 95% CI: 57%)	50% (upper 95% CI: 57%)	15% (upper 95% CI: 26%)
OR for hearing loss		2.4 (95% CI 1.0-5.7)	1.8 (95% CI 0.6-4.9)	3.0 (95% CI 1.3-6.9)	0.5 (95% CI 0.2-14)

Hydrocarbon concentrations: measurements performed during the investigation, no detail provided; ND not detected

* prevalence of bilateral high frequency hearing loss

♦ **rotogravure printing plants**

In 1993, Morata and collaborators (Morata et al, 1993) explored the effects of occupational exposure to solvents and noise in 190 Brazilian workers from rotogravure printing and paint manufacturing plants (**Table 5**). The occurrence of hearing disorders was evaluated in groups of workers

(1) not exposed to noise or organic solvent: workers from a rotogravure printing plant involved in set up operations (graphic arts, composition, photocomposition …).

(2) exposed to **noise alone**: workers from a rotogravure printing plant; finishing and binding division.

(3) exposed to **noise + toluene** (98% purity); workers from a rotogravure printing plant. Time-weighted average concentrations of toluene ranged from 140 to 600 ppm (even up to 1860 ppm) in 1978. In 1980, a ventilation system was installed; TWA concentrations were 150-370 ppm, concentrations above 1000 ppm were observed. The length of employment was 8.1 years (SD 6.2), hence most of the examined workers were not exposed to such high levels. In 1990, the TWA concentrations ranged from 75 to 365 ppm. It is not known whether respiratory or skin (toluene is absorbed via the skin) protection were used.

(4) exposed to a **solvent mixture including toluene**: these workers came from a paint manufacturing plant, filling division.

Subjects were interviewed with regard to health history, work history and solvent and noise exposure. The study population was characterized (mean, SD) in terms of age, length of employment, previous noise exposure, previous chemical exposure, diabetes, HTA, ear infection, ototoxic medication, hunting, shooting, motor sports, amplified music, power tools, military services were taken into account. Ethanol consumption and smoking habits were not taken into account.

Otoscopy, pure tone audiometry (0.5, 1, 2, 3, 4, 6 and 8 kHz) and immittance audiometry were performed by audiologists. It is not known whether PTA was performed after a period free of noise exposure. Audiograms were classified as normal if no single threshold exceeded 25 dB.

The audiometric threshold averages indicated that the groups exposed to noise alone or noise + toluene had significantly poorer hearing thresholds than both the non-exposed group and the solvent group. However, it was not possible to discriminate between the effects of noise or toluene. No association between mixed-solvent exposure and hearing loss was noted (Morata, 1998). The high-frequency hearing losses were then scaled from mild to profound and further classified as either unilateral or bilateral. No statistically significant differences were observed between groups for moderate and profound hearing losses. The prevalence of mild high-frequency bilateral hearing loss (30-40 dB at 3-8 kHz) was 53% in the group exposed to noise and toluene simultaneously, 8% in the unexposed group, 26% in the noise-only group and 18% in the solvent-exposed group (95% CI not provided). When subjects with unilateral and conductive hearing losses were excluded, the prevalence did not significantly change (58%, 10%, 33%, 20%, respectively).

Although SDs were particularly large for many variables, a multiple logistic regression was done. Conductive and unilateral hearing losses were considered as normal hearing and hearing loss was considered as a dichotomous variable (normal hearing vs high-frequency hearing loss). The independent variables considered for

inclusion in the model were the exposure group, the length of employment, previous occupational exposure to noise or chemicals, exposure to non occupational noise. The age was not included as a risk factor due to the similarities of the group (mean age ranging from 32 to 36 years). The only variable that met the significance level criterion, besides exposure group, was length of employment (RR 1.1; 95% 1.0-1.1). The adjusted relative risk estimates of hearing loss were calculated to be 4 times greater for the noise group, 11 times greater for the noise + toluene group, and 5 times greater for the solvent-mixture group. However, the confidence intervals overlapped and the difference can not be considered as significant (95% CI 1.4-12.2; 95% CI 4.1-28.9; 95% CI 1.4-17.5, respectively). Not further stratification was done according to the level of exposure to toluene.

The noise + toluene group had a significantly greater percentage of cases of reflex decay than the other groups ($p < 0.001$), this percentage was significantly higher for contra lateral stimulation than for ipsilateral stimulation and it was higher at 2 kHz than at 0.5 and 1 kHz and it was suggested that there might be a central auditory pathway involvement in the hearing loss observed.

In conclusion this study showed that the groups exposed to noise alone or noise + toluene had significantly poorer average hearing thresholds than both the non-exposed group and the solvent group. No significant difference was observed between the noise alone group and the noise + toluene group. However, an increased prevalence of mild high frequency hearing loss in male rotogravure printing workers exposed to noise + toluene was detected. The toluene exposure levels were well above 100 ppm. The study design was not appropriate to establish a NOAEC or a LOAEC.

Table 5 Characteristics of Brazilian rotogravure printing and paint manufacturing workers study (Morata et al, 1993)

	"unexposed" n = 50 male	**noise n = 50 male**	**noise + toluene n = 51 male**	**solvent mixture n = 39 male**
noise level (dB(A))	<85	88-97 [a]	88-98[a]	<85
previous noise exposure mean (SD) (years)	0.5 (1.5)	1.2 (2.5)	1.2 (2.2)	1.3 (2.6)
solvent level TWA (ppm)			**75–600 (peak 1860)** [b]	**toluene : 10–70** [c] **xylene : 12-40** [c] *MEK : 0-32* [c] *MIBK : 0-20* [c] *ethanol : 0-16* [c]
previous solvent exposure mean (SD) (years)	1.6 (2.7)	1.2 (2.5)	1.2 (2.2)	1.3 (2.6)
length of employment mean (SD) (years)	13.1 (7.6)	11.6 (7.8)	8.1 (6.2)	5.6 (3.7)
age mean (SD) (years)	34.7 (9.8)	36.1 (8.2)	32.5 (7.9)	31.7 (7.2)
hearing loss*	10%	33%	58%	20%
RR hearing loss		4.1 (95% CI 1.4-12.2)	10.9 (95% CI 4.1-28.9)	5.0 (95% 1.5-17.5)

[a] no hearing protection was used
[b] range of TWA concentrations measured in 1978, 1979, 1980 and 1990
[c] range of TWA concentrations in 1990
* bilateral high frequency hearing loss

In 1997, the same team reported a similar study on 124 male rotogravure printing workers exposed to various levels of noise and an organic solvent mixture composed of predominantly toluene, ethanol, ethyl acetate (Morata et al, 1997b). The different tasks covered in the study ranged from printing, paint preparation, engraving and lamination to colour proofing etc. The characteristics of the study population are presented in **Table 6**.

Sound pressure measurement indicated continuous noise levels in the range of 71-93 dB(A). 60% of the population was exposed to noise doses considered to be high enough to cause hearing loss (>85 dB(A) and about 18% were exposed to ≥91 dB(A). 11% of the workers exposed to noise >85 dB(A) reported using hearing protection during 100% of the time when noise-exposed. Personal full-shift, TWA exposure evaluation was conducted for all the subjects. Only range levels were provided. Biological monitoring was performed on 109 subjects: urine samples were collected immediately after the end of the workday.

As in the previous study, an exposure index was calculated for each solvent by dividing the observed air concentration by the corresponding exposure limit (190 mg/m³ for toluene; 1090 mg/m³ for ethyl acetate; 1480 mg/m³ for ethanol). 12% of the workers were exposed to >50 ppm toluene. The fractions for the 3 solvents were summed in order to obtain the exposure index for the mixture and exposure to the mixture was considered to be exceeded when the sum was greater than unity. 27% of the workers were considered to be overexposed on this basis.

Otoscopy, pure-tone audiometry and immitance audiometry were performed to assess the hearing status. PTA was preceded by a period of at least 14 hours without occupational noise exposure.

The prevalence of bilateral high frequency sensorineural hearing loss was 49.2% which cannot be compared to an “unexposed” group.

Although the study population was characterized by variables with large SDs (cfr **Table 6**), suggesting a non-Gaussian distribution, a multiple logistic regression was performed for the estimation of odds ratio and the testing of interactions. From the numerous variables that were analyzed, by stepwise logistic regression, for their contribution to the development of hearing loss (age, tenure, noise dose, use of hearing protection, solvent concentrations in air, biological marker for toluene, job category, work and medical history, medications, smoking, alcohol consumption, work perception scores, non occupational exposures, previous occupational exposure to noise), age and hippuric acid in urine were the only variables that met the significance level in the final multiple logistic regression model. The odds ratio 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 only 1.00 for the noise dose (95% CI 1.00-1.01; p 0.94). An OR of 1.76 for each gram of hippuric acid per gram of creatinine (95% CI 1.00-2.98; p 0.0338). Toluene concentration in air was not found to be significantly associated with hearing loss. It is possible that significant skin absorption of toluene may have occured and that the biological monitoring reflected total exposure rather than ambient monitoring; unfortunately dermal exposure was not monitored in this study. Hippuric acid in urine is a non-specific biologic marker for toluene. It is a common urinary constituent originating mainly from food but also from salicylic acid. The background level in this study was probably low as 52% of the workers had hippuric acid levels below 0.5 g/g creatinine and 75% had <1 g/g creatinine. 17% of the workers had levels of urinary hippuric acid exceeding 1.5 g/g creatinine (1.6 g/g creatinine being the BEI corresponding to the TLV-TWA of 50 ppm). 0.02% (of 109 subjects) of the workers had levels between 4.2 and 5.5 g/g

creatinine. However, one might question the relevance of such parameter in this type of study, the half life of hippuric acid in urine is less than 5 hours and hence it only reflects the exposure level of the day.

No significant interactions (enhanced hearing loss) were noted between the solvents, the solvent mixture and noise, or each individual solvent and noise.

Table 6 Characteristics of Brazilian rotogravure printing workers study (Morata et al, 1997b)

noise level (dB(A))	75.5-92.8	
length of noise exposure mean (SD ; range) (years)	7.7 (6.0 ; 0-25)	
previous noise exposure mean (SD ; range) (years)	2.2 (4.3 ; 0-26)	
solvent level (breathing zone)	**toluene** *ethyl acetate* *ethanol*	: 0.14-919 mg/m³ (0.04-242 ppm) *: 1.1-2635 mg/m³* *0.3-703 ppm* *: <0.25-1240 mg/m³* *<0.13-646 ppm*
length of solvent exposure mean (SD ; range) (years)	6.5 (6.0; 0-25)	
previous solvent exposure mean (SD ; range) (years)	1.8 (4.2; 0-22)	
age mean (SD ; range) (years)	33.8 (8.5; 21-58)	
hearing loss*	49.2%	

exposure evaluation (solvents & noise) conducted during the study
*bilateral high frequency hearing loss

In conclusion, this study showed a high prevalence of bilateral high frequency hearing loss in rotogravure printing workers exposed to noise and toluene (at exposure levels ranging from very low to 240 ppm) among other solvents. No conclusion can be drawn about the potential ototoxicity of toluene.

In 2003, Schäper and collaborators reported a longitudinal study over 5 years with four repeated examinations in 333 German male workers from 14 rotogravure printing plants exposed to **toluene (Tables 7a** and **7b)**. The sample size went down from 333 to 278 (83.5%) in examination 2, 241 (72.4%) in examination 3 and only 216 (64.9%) in examination 4. For 192 participants, a complete data set was available to fit the follow-up design. The mean (SD) age and exposure duration were 38.1 (9.8) years and 13.4 (9.7) years.

Otoscopy, pure-tone audiometry (0.125, 0.250, 0.5, 0.750, 1, 1.5, 2, 3, 4, 6, 8 and 12 kHz) and tympanometry (examination 4) were performed to assess the hearing status. PTA was preceded by a period of at least 3 hours of exposure free time. 28 persons were categorized as suffering from non-occupationally induced hearing defects. As the numbers did not differ between the groups, these subjects were not excluded from the analysis.

Depending on the intensity of exposure (high vs low) and on job tenure (short = about 6 years vs long = about 21.5 years), four groups were considered: “Short High” (n = 90), “Long High” (n = 91), “Short Low” (n = 86), “Long Low” (n = 66). A third stratification factor, i.e. intensity of current noise exposure (high vs low) was introduced with a cut-off point at the median noise data 82 dB(A).

Current and lifetime weighted average exposures (LWAEs) to toluene and noise were considered. The current individual toluene and noise exposures were measured for each participant twice a year. Across the whole study the average (SD) current exposure level for toluene in breathing zone was 25.7 (20.1) ppm in the printing area, and 3.2 (3.1) ppm in the end-processing area, revealing an exposure relation between the groups of 8:1 (ratio of means). The relation between the groups "high" vs "low" amounted to about 5:1 with respect to lifetime exposure to toluene. In the group “Long & High” exposure to toluene, the LWAE to toluene was 57.1 (13.4) ppm for the “Low noise” group and 61.7 (14) ppm for the “High noise” group. During examination period 2, hippuric acid and o-cresol were measured in after-shift urine samples (n 80). The mean (SD; range) levels were 1.8 g/l urine (1.6; 0.1-8.9) for hippuric acid and 1.0 mg/l urine (1.2; 0-6.0) for o-cresol.

For the whole study the mean current noise exposure was 81.1 (3.5) dB(A) in the printing area and 81.6 (4.2) in the end-processing area. For the two groups dependent on the intensity of actual noise exposure were 79 (3) dB(A) vs 84 (1) dB(A). The percentage of subjects declaring “always wearing ear protection” was 28% at the onset of the study, and 22% at the end of the study.

The prevalence of bilateral high frequency hearing loss in the whole group was 36%. The logistic regression did not reveal any significant effect of toluene level or exposure duration on the auditory threshold, nor did it reveal any interactions between toluene and noise on the auditory threshold. The stratification dependent on noise intensity itself (79 $\pm$ 3 vs 84 $\pm$ 1 dB(A)) was significantly associated with the auditory thresholds. A general change in the auditory thresholds took place during the 5-year of the study. A stepwise regression analyzing the data of a sub sample (n = 80) revealed that only age significantly elevated the risk of bilateral high frequency hearing loss.

In conclusion, this study showed that combined long (about 22 years) exposure to a current mean exposure level of 25 ppm (LWAE about 60 ppm) and a mean current noise level of 81 dB(A) (LWAE 86 dB(A)) was not associated with hearing loss. Hence, the threshold level for developing a hearing loss due to toluene exposure is probably above 50 ppm toluene.

Table 7a Mean toluene and noise exposures in German rotogravure printing workers study (Schäper et al, 2003)

	toluene ppm mean (SD)		**noise dB(A) mean (SD)**	
	Current	LWAE	Current	LWAE
processing (n = 86 men) = low toluene exposure	3.2 (3.1)	9.5 (7.3)	81.6 (4.2)	81.8 (4.1)
printing (n = 106 men) = high toluene exposure	25.7 (20.1)	44.7 (17.1)	81.1 (3.5)	81.9 (7.1)

LWAE: lifetime weighted average

Table 7b Past and current toluene exposure (ppm: mean ± SD) in German rotogravure printing workers study during the four examination periods (Schäper et al, 2003)

Toluene exposure	**noise exposure**	**current exposure**				**LWAE**
short low	low noise	2.6 ± 4.1	2.9 ± 4.7	3.6 ± 4.1	5.0 ± 6.9	5.8 ± 1.1
	high noise	2.0 ± 1.2	2.8 ± 1.7	3.1 ± 1.9	3.2 ± 2.2	6.1 ± 1.2
long low	low noise	2.6 ± 2.7	2.5 ± 2.0	4.2 ± 5.2	4.9 ± 4.3	13.5 ± 12.4
	high noise	2.7 ± 1.6	3.2 ± 1.1	3.6 ± 1.8	3.2 ± 1.5	13.8 ± 5.9
short high	low noise	27.4 ± 17.0	24.1 ± 15.6	27.0 ± 17.5	34.9 ± 31.8	32.7 ± 9.3
	high noise	24.9 ± 25.0	22.1 ± 21.7	27.4 ± 18.2	26.8 ± 20.2	34.5 ± 10.7
long high	low noise	25.6 ± 16.8	28.6 ± 29.7	25.3 ± 18.4	21.8 ± 13.6	57.1 ± 13.4
	high noise	25.2 ± 13.9	22.3 ± 13.7	20.5 ± 1.7	18.7 ± 12.9	61.7 ± 14.0

LWAE: lifetime weighted average
low noise vs high noise: cut off point at median noise data 82 dB(A).

Vrca et al, (1996) examined the brainstem auditory evoked potentials (BAEPs) in 49 workers (46 men/3 women; age: 42.3 (6.8; 24-55) years; tenure: 21.4 (7.4; 4-30) years) employed in a printing press with exposure to low concentrations of toluene, and in 59 control subjects (54 men/5 women; age: 43.0 (7.2; 23-55) years; tenure: 20.6 (7.7; 4-32) years).

No significant differences were found with regard to age, work service, education, smoking habit, coffee and alcohol consumption, history of head injury. Workers had been asked not to take any medications for 5 days before BAEPs examination which was performed on a Monday morning.

The level of exposure to toluene was assessed by measuring toluene concentration in blood and hippuric acid and o-cresol in urine before the work shift (**Table 8**). The mean toluene, hippuric acid and o-cresol concentration were respectively 0.036 mg/l, 0.426 g/g creatinine and 0.211 g/g creatinine in the exposed subjects (n=36). The corresponding values in the control group (n=27) were 0.00096 mg/l, 0.338 g/g creatinine and 0.474 g/g creatinine, respectively. The concentrations of hippuric acid and o-cresol were also measured in an end-of-shift urine sample: 0.485 g/g creatinine and 0.276 g/g creatinine in the exposed and 0.223 g/g creatinine and 0.383 in the controls. The workers in the printing press had used toluene exclusively for at least 30 years during which period technology, workshops, ventilation, number and type of place had not changed significantly.

In the group of exposed workers, a significant decrease was found in all wave amplitudes examined (P1, P2, P3, P4, P5). The interval latency of P3-P4 and every other inter-peak latency that contained inter-peak latencies P3-P4 (P1-P4; P1-P5, P3-P5) were also significantly longer suggesting an effect at the extramedullary and high medullary part of the auditory pathway. These results are however difficult to interpret with regard to toluene exposure as the metabolite levels indicated a very low exposure to toluene; most values being in the range of the values observed in the « general » population.

Table 8 Toluene exposures in printing press workers study (Vrca et al, 1996)

	exposed (n = 36) mean ± SD (range)	non exposed (n = 27) mean ± SD (range)	p value*
Before the work shift, Monday			
toluene in blood (mg/l)	0.036 ± 0.025 (0.002-0.094)	0.00096 ± 0.0037 (0.0-0.019)	<0.0001
hippuric acid in urine (g/g creatinine)	0.426 ± 0.262 (0.13-1.25)	0.338 ± 0.224 (0.02-0.82)	<0.05
o-cresol in urine (g/g creatinine)	0.211 ± 0.624 (0.0-3.760)	0.474 ± 0.860 (0.0-3.980)	ns
after the work shift, Monday			
hippuric acid in urine (g/g creatinine)	0.485 ± 0.261 (0.122-1.10)	0.223 ± 0.1207 (0.020-0.41)	<0.001
o-cresol in urine (g/g creatinine)	0.276 ± 0.409 (0.0-1.850)	0.383 ± 0.652 (0.0-2.570)	ns

* Mann-Whitney test

- **paint and lacquer enterprises**

Sliwinska-Kowalska et al (2001) evaluated the hearing effects of a mixture of organic solvents (toluene, xylene, ethylbenzene, *ethyl acetate, white spirit, butyl acetate*) alone or in combination with noise in Polish workers from four paint and lacquer enterprises (**Table 9**). 517 subjects were divided into three groups: subjects with no risk due to noise or organic solvent exposure at the workplace, workers exposed to organic solvents only, and workers exposed to both organic solvents and noise. The workers exposed to noise wore hearing protection regularly. There was no group exposed to noise only.

The inclusion criteria were: at least 6 months of exposure to solvents, no history of middle-ear diseases, normal tympanic membrane appearance at otoscopy, no air-bone gap in audiometric tests, type A tympanogram, and present ipsilateral stapedius reflex. The subjects' previous occupational or non-occupational exposure to noise and chemicals, lifestyle (including alcohol and tobacco consumption), hobbies, prior military service were assessed in detail in an interview based on a questionnaire. The interview protocol also included question about the medical and audiological history.

Otoscopy, immittance audiometry and PTA (1, 2, 3, 4, 6, 8 kHz), after at least 14 h without occupational exposure to noise, were performed. Audiograms were classified as normal if none of the single hearing thresholds exceeded hearing loss of 25 dB for either ear.

Personal monitoring was used for air sampling. The subjects were exposed to a solvent mixture with mixed xylene isomers as the predominant ingredient. Low percentages of toluene, ethylbenzene, ethyl acetate, white spirit, butyl acetate were also present. In 1999, xylene air concentration ranged from 0 to 290 mg/m³, but all mean air concentrations of solvents were below the current Polish occupational exposure limits (100 mg/m³ for xylene, toluene, ethylbenzene, butyl acetate; 200 mg/m³ for ethyl acetate; 300 mg/m³ for white spirit). The exposure index for the mixture, calculated as the sum of the fractions (concentration of given solvent by its normative limit) of all compounds, did not exceed the limit (value >1 indicating overexposure). The lifetime average exposures to solvents were also below the OEL. However, in the solvent-only group 4% of the workers were exposed to xylene

air concentration >100 mg/m³ for a mean duration of 20 years, while in the solvent + noise group all the workers had exposure below 100 mg/m³. 28% of the workers in the solvent-only group had an exposure index for the mixture >1 for a mean duration of 12.4 years and 9% of the workers in the solvent + noise group had an overexposure for a mean duration of 4.7 years.

The prevalence of hearing loss in both of the solvent exposed groups was significantly increased when compared to that of the controls (61.5% in the solvent + noise group; 57.5% in the solvent group and 36% in the controls). The group exposed to solvents and noise included more men, a medical history of acoustic trauma was more common in the group exposed to solvents only, and head trauma was more common in both groups exposed to solvents.

The relative risk (RR) of hearing loss in the two groups exposed to solvents was significantly higher than in the reference group (RR 2.8; 95% CI 1.8-4.3 and RR 2.8; 95% CI 1.6-4.9, respectively). Strangely, the probability of hearing loss in a subgroup of solvent-only exposed subjects, whose occupational exposure to noise was ≤80 dB(A) was 4.4 times higher (95% CI 2.3-8.1) than the noise-matched reference subgroup. It is not clear whether these RR concern only bilateral hearing loss. Hearing thresholds were significantly poorer in a wide range of frequencies (2-8 kHz) for both groups exposed to solvents, when compared with the reference group. The hearing loss was essentially poorer in the solvent + noise group than in the solvent-only group, matching significance for frequencies 3 and 4 KHz for both ears.

A multiple logistic regression model was used to identify any relation between solvent exposure (air concentration, exposure indices) and the probability of hearing loss. A multiple linear regression model was used to determine the relation between exposure and hearing loss.

There was no linear correlation between the risk of hearing loss and the extent of solvent exposure. However, a linear correlation was found between some exposure indices and the hearing thresholds at single frequencies only, including the mean toluene concentration and hearing loss in the right ear at frequencies 4 and 6 kHz, the mean toluene concentration and hearing loss in the left ear at frequencies 3, 4 and 6 kHz, and the mean xylene concentration and hearing loss in the right ear at frequencies at 3 kHz. The relevance of these observations should be questioned however since ototoxicity is considered to be bilateral and grossly symmetrical.

In conclusion, this study has shown a high incidence of hearing loss (bilateral only?) in workers exposed to a mixture of organic solvents alone or in combination with noise. The solvents mixture included toluene, ethylbenzene and xylene, the level of exposure were considered as being moderate but 28% of the workers had been “overexposed”, according to the Polish OELs, for a mean duration of 12.4 years.

Table 9 Characteristics of Polish paint workers study (Sliwinska-Kowalska et al, 2001)

	« unexposed » n = 214 113 men-101 women	**solvent n = 207 121 men-86 women**	**solvent + noise n = 96 77 men-19 women**
noise level (dB(A))	≤80 (n = 174) 80-85 (n = 40)	≤80 (n = 104) 80-85 (n = 103)	>85 86-90: 78% around 100 : 2%
solvent level (breathing zone) mean (SD; range) (mg/m³)		**toluene** : 8.4 (10.4; 0.0-92.5) **ethyl-benzene** : 7.7 (10.8; 0.0-65.6) **xylene** : 28.7 (22.3; 1.0-110) *ethyl-acetate : 11.5 (10.8-61.6)* *white spirit : 11.7 (0.0-563)* *butyl-acetate : 8.3 (0.0-285.5)*	**toluene** : 5.8 (7.9; 0.0-48) **ethyl-benzene** : 7.9 (4.4; 0.3-65.6) **xylene** : 28.3 (18.7; 1.0-86.4) *ethyl-acetate : 7.7 (0.0-120)* *white spirit : 7.0 (0.0-64.4)* *butyl-acetate : 1.8 (0.0-16.6)*
tenure mean (SD; range) (years)		12.8 (8.2; 0.5-39)	12.2 (8.5; 0.5-39)
age mean (SD; range) (years)	38.5 (10.6; 19-72)	39.5 (9.3; 22-63)	38.4 (9.1; 20-58)
Hearing loss*	36%	57.5%	61.5%
RR of hearing loss		2.8 (95% CI 1.8-4.3)	2.8 (95% CI 1.6-4.9)

lifetime average exposure

*prevalence of hearing loss (audiograms were classified as normal if none of the single hearing thresholds exceeded hearing loss of 25 dB for either ear)

- **dockyard workers**

In a similar study, Sliwinska-Kowalska et al (2004) assessed the hearing loss associated with exposure to a mixture of organic solvents in 701 noise-exposed Polish dockyard workers (517 noise + organic solvent mixture-exposed, 184 noise-only-exposed, 205 control subjects not exposed to either noise or solvents) (**Table 10**). For the noise and solvent-exposed group, the composition of solvent was quite similar over the last 20 years, with the main constituent being xylene, with a mean workplace concentration of 245 mg/m³. Higher xylene concentrations were measured in the past. In the early 1980's, the concentration of toluene occasionally reached 481 mg/m³. Among other compounds there were minor concentrations of ethylbenzene, ethyl acetate, butyl acetate, n-butanol, white spirit and toluene. To assess the exposure to all chemical compounds, an exposure index was calculated. It was defined as the sum of the fractions (average concentration of a given chemical divided by its admissible level [see § 3.4.3. admissible levels in Poland]) of all compounds in the mixture. Current measurements showed that the mean exposure index of the mixture exceeded the admissible value of 1 by more than 6 times. The average (range) current and lifetime xylene exposures were 245.2 mg/m³ and 3025.2 mg/m³ x year (1.2–15750.1). The mean (range) current and lifetime toluene exposures were 28.9 mg/m³ (0–225) and 762.3 mg/m³ x year (0–9341.1). The levels of exposure were highly variable. The lifetime noise exposure exceeded 90 dB(A) and was higher in the noise + solvents group as compared with the noise-only group. The workers exposed to noise were required to wear personal hearing protectors.

The prevalence of hearing loss in both of the noise-exposed groups was significantly increased when compared to that of the controls at almost all frequencies tested (67.5% in the solvent + noise group; 64.7% in the noise group and as high as 39.5% in the controls). The prevalence of male, smokers, solvent and noise exposures in the past, exposure to noise during army, noise exposure during leisure time was highest in the solvent and noise-exposed group. The prevalence of treatment with amino glycoside and middle ear inflammation in anamnesis was highest in the control group.

The final multiple logistic regression showed that the odds ratio (OR) of hearing loss in both noise-exposed groups was significantly higher than in the reference group. The age-adjusted OR was not significantly higher in the noise + solvent group as compared to the noise-only group (3.34; 95% CI 2.06-5.43 in the noise-only group and 4.88; 95% CI 3.09-7.68 in the noise + solvent group). ORs for hearing loss were 1.12 (95% CI 1.10-1.14; $p < 0.001$) for each increment of 1 year of age, 1.07 (95% CI 1.04-1.09; $p < 0.001$) for every decibel of lifetime noise exposure, and only 1.004 (95% CI 1.00-1.01; $p < 0.05$) for each increment of the index of lifetime exposure to solvents.

Age, gender, noise and current solvents exposure and past exposure to noise were the variables that met the significance level criterion in the multiple linear regression analysis in the entire group of subjects. A significant positive linear relationship was found between age and hearing loss at almost all frequencies tested; gender and hearing loss at the frequencies 3 to 8 kHz; and lifetime noise exposure and hearing loss at the frequency range from 2-3 to 8 kHz. A positive relationship was also found between noise in the past and hearing threshold at 3-4 kHz. Moreover, the authors reported that a) at the single frequency of 8 kHz, in both ears the mean hearing thresholds adjusted for age and gender were worse in the noise + solvents group as compared with the noise-only group; b) a linear relationship was found between the lifetime cumulative exposure to mixture of solvents and the profoundness of hearing loss at this particular frequency. These statements are not further explained (no mean hearing thresholds, no 95% CI, the multiple regression analysis for hearing loss at 8 kHz gives a partial regression coefficient of only 0.024 for the lifetime exposure index for mixture of solvents).

In conclusion this study showed a significantly higher permanent threshold shift in both noise-exposed group and noise and solvent-exposed group than in the unexposed group and it covered almost the entire region of frequencies tested (2-8 kHz). A non significant difference was observed between the noise + solvent group and noise-only group. The only hearing threshold that seemed to be affected by solvents + noise, was associated at the frequency of 8 kHz.

Table 10 Characteristics of Polish dockyard workers study (Sliwinska-Kowalska et al, 2004**)**

	« unexposed » **n = 205** ***170 men/53 women (?)***	**noise** **n = 184** ***? men/ ?women***	**solvent + noise** **n = 517** ***? men/ ? women***
current noise level (SD ; range) dB(A)	70-85	90.1 (4.1; 85-100)	93.1 (3.3; 85-102)
lifetime noise exposure mean (SD; range) dB(A)	74.1 (5.1; 70-85)	90.3 (3.8; 85.1-100.1)	94.2 (3.4; 85.1-100)
current solvent level (breathing zone) mean (SD; range) mg/m³			**Toluene** : 28.9 (53.8; 0.0-225) [7.6; 0.00-59.2 ppm] **Xylene** : 245.2 (235.4; 0.1-1815.3) [56; 0.02-412.5 ppm] exposure index for the mixture : 6.3 (3.0; 0.8-23.2)
lifetime solvent level mean (SD; range) mg/m³			**Toluene** : 762.3 (1740.9; 0.0-9341) **Xylene** : 3025.2 (3412.1; 1.2-15750.1) exposure index for the mixture : 66.6 (72.6; 0.1-346.5)
tenure mean (SD; range) yrs		12.8 (8.2; 0.5-39)	12.2 (8.5; 0.5-39)
age mean (SD; range) yrs	39.8 (9.3; 21-61)	42.2 (9.3; 21-61)	37.4 (9.2; 20-66)
hearing loss*	39.5%	64.7%	67.5%
OR hearing loss		3.34 (95% CI 2.06-5.43)	4.88 (95% CI 3.09-7.68)

* prevalence of “abnormal” audiograms: audiograms classified as normal if none of the single hearing thresholds exceeded hearing loss of 25 dB for either ear.

One should notice that the prevalence rates of hearing loss in the control groups ranged from 8% to 39.5% across these studies (**Tables 4, 5, 9 & 10**).

Based on the available studies on rotogravure printing workers (Morata et al, 1993, 1997; and the first results of the German study [not completed at the time of the risk assessment]), the conclusion of the recent EU (EU, 2003) risk assessment for toluene in regard with its potential auditory toxicity in humans was the following: « Auditory toxicity has also been studied in humans. The Morata studies indicate that occupational exposure to toluene at high concentrations may increase the risk of developing mild high-frequency hearing loss, especially in noisy environments. However, the studies are not appropriate for determining a LOAEC/NOAEC. The preliminary conclusion of the ongoing German study investigating effects in relation to low exposure levels, is that no association between toluene exposure intensity and age-corrected auditory thresholds could be found, but that the observed interaction between duration and intensity of exposure needed further evaluation. » This last study was published in 2003 (Shäper et al, 2003).

Discussion:

Several organic solvents commonly used in the industry such as toluene, styrene, ethylbenzene, p-xylene, trichloroethylene have been shown to impair hearing in animals when applied repeatedly in high concentrations. Of these solvents only toluene, xylene, n-hexane and ethylbenzene are of particular interest in the petroleum industry.

In rats, solvent exposure produces cochlear lesions, as does noise exposure, but the mechanisms are different. Hearing loss caused by noise results from a mechanical injury to hair cell stereocilia. For chemicals reaching the cochlea through tissue intoxication, the lowest turn of the cochlea and the OHC3 cells are the most sensitive. Moreover, solvents are known neurotoxic substances and apart from causing a cochlear toxicity, a central auditory pathway involvement is also suspected.

The pattern of solvent exposure is important. For instance exposure to 700 ppm toluene did not cause impairment even after 16 week-exposure; 1000 ppm for 14 h/d during 2 weeks, 1500 ppm for 14 h/d or 2000 ppm for 8 h/d during 3 days did induce hearing loss; single 4-h exposure to 4000 ppm or single 8-h exposure to 2000 ppm were without effect; intermittent exposure to 3000 ppm for 30 min/h, 8 h/d, during 2 weeks caused hearing loss while intermittent exposure to 3000 ppm for 4 h/d during 9 weeks was not ototoxic (Pryor et al, 1984).

The LOAECs reported for auditory impairment in the rat following repeated exposure are in the area of 400 ppm for ethylbenzene, 600 ppm for toluene, 800 ppm for mixed xylenes. Transient auditory system impairment has been observed in the guinea pig exposed at 250 ppm, but this was not reproduced in another study. The NOAECs reported in the rat are 300 ppm for ethylbenzene and 450 ppm for p-xylene. Considering the study of Lataye et al (2003) it is not possible to establish a precise NOAEC for toluene which is below 600 ppm.

There is an influence of age on solvent-induced threshold shift and hair cell loss in the rat and there is some indication of a genetic susceptibility in mice. A synergistic or at least additive ototoxic effect of toluene and noise has been demonstrated in rats. It has also been shown that ethanol modulates the ototoxic effect of toluene.

The association between occupational exposure to some solvents such as toluene and hearing impairment has been suggested only recently: data are scarce and equivocal. The ototoxicity of these compounds in humans is not well characterized. The assessment of exposure to a single organic solvent is particularly difficult because workers are usually exposed to mixtures of chemicals of highly varying compositions and concentrations and it is difficult to identify workers with exposure to a specific compound only. Only few studies have addressed the problem of hearing impairment in humans exposed to a specific solvent without any significant noise exposure.

Moreover, it is well known that a period of at least 14 hours without noise exposure has to precede the audiometry to avoid confusion with TTS. One might expect that a period of solvent free exposure, depending on its retention time, should also precede such test, but this has not been clearly demonstrated.

Several workplace studies are of particular interest in the context of this report. The hearing ability of groups of workers exposed to noise, toluene in combination with noise or not or to a mixture of solvents including toluene has been assessed by pure

tone audiometry and immittance audiometry. Such studies are difficult to conduct and have several shortcomings:

- a major difficulty in epidemiology is to obtain homogenous populations. It is essential to properly characterize the populations and to take into account the factors that could alter auditory function such as: age, length of employment, length of previous noise exposure, length of previous chemical exposure, diabetes, hypertension, history of ear infection or trauma, use of ototoxic medication, ethanol consumption and smoking habits, hunting/shooting, motor sports, amplified music, power tools, military services,….
- industrial workers often face combined exposures to mixture of chemicals and noise. A history of exposure to noise in former occupational activities or in non-occupational activities is certainly not a rare event but is not always easy to detect;
- in some studies, the solvent and noise exposure levels are poorly characterized: only ranges are provided, only current levels of exposure are considered;
- statistical analyses are not always satisfactory; for instance, some considered variables have very large SDs, implying non-Gaussian distribution of the studied populations which is not always taken into account in the statistical analyses;

In conclusion, data are scarce and the currently available scientific literature does not establish a causal relationship between the occupational activity in the petroleum sector and hearing loss. The only study specifically addressing this issue does not allow conclusions to be reached on the ototoxic potential of chemicals in petrochemical industry workers.

Several studies have investigated hydrocarbon solvents of interest for the oil industry (**Table 11**). These studies are not appropriate for determining a LOAEC/NOAEC. A high prevalence of mild high-frequency hearing loss has been described in printing workers exposed to high levels of toluene (higher than the current OELs) in combination with noise when compared to controls, but a lifetime weighted average exposure to toluene at about 50 ppm was not associated with hearing loss. Hence, considering the level of exposure to toluene, in workers handling and exposed to gasoline vapours, the risk is low. However, these workers are exposed to a mixture of chemicals and further research is certainly needed to better characterize the risk.

Table 11 Summary overview of available ototoxicity data and exposure levels for gasoline vapour components

	experimental data: rat		OELs	exposure to gasoline vapour P90 full-shift(*)	main relevant human data
	NOAEC	**LOAEC**			
toluene	Nd	600 ppm	27-100 ppm (100–375 mg/m³)	*~0.05-10.5 ppm* (0.2–40 mg/m³)	75-600 ppm + noise (88-98 dB(A), during ~8 years: associated with a higher prevalence of hearing loss when compared to noise-only exposure (Morata et al, 1993)
xylenes	450 ppm (p xylene)	800 ppm (mixed xylenes)	23-100 ppm (100–440 mg/m³)	*~0.045-4 ppm* (0.2-18 mg/m³)	lifetime weighted average exposure to 50 ppm toluene + noise (86 dB(A) : not associated with hearing loss (Schäper et al, 2003)
ethyl-benzene	300 ppm	550 ppm	23-100 ppm (100–441 mg/m³)	Nd	some indication of high prevalence of hearing loss in workers exposed to a solvents mixture including toluene, xylene, ethylbenzene at levels <100 mg/m³ (Sliwinska-Kowalska et al, 2001)
n-hexane	Nd	1000 ppm	25-50 ppm (72-180 mg/m³)	0.03-5 ppm (0.1-18 mg/m³)	Nd

(*) full-shift exposure results from gasoline vapour, 90th percentile (CONCAWE, 2002);
nd: no data

3.4.4. Metals

Studies of adults, children, and laboratory animals suggest an association between lead exposure and hearing loss (a.o. Forst et al, 1997; Araki et al, 2000; Wu et al, 2000; Bleecker et al, 2003; Zou et al, 2003).

Trimethyltin (TMT) and triethyltin (TET) are considered to disrupt auditory function in rats and guinea pigs at doses below those shown to be neurotoxic (Besser et al, 1987; Eastman et al, 1987; Liu & Fechter, 1996; Clerici et al, 1993; Fechter et al, 1992; Hoeffding & Fechter, 1991; Crofton et al, 1990).

Atoxyl, an arsenic compound (sodium-p-amino-phenyl arsenate), once used for the treatment of trypanosomiasis is a recognised ototoxic drug. Hearing loss in humans exposed to arsenic pollution has been reported (EHP, 1994; Tchounwou et al, 2004).

Mercury might be of concern for the petroleum industry.

Hearing loss is a known possible consequence of methylmercury intoxication. In 1953, a severe neurological disorder was recognized among persons living in the vicinity of Minimata, Japan, where methylmercury-containing effluent flowing from a chemical manufacturing plant into the local bay contaminated shellfish. Deterioration in hearing and deafness were reported among other neurological symptoms. Findings consistent with Minimata disease have been reported in other instances of

accidental organic mercury intoxication in Japan and Iraq. Early stages of poisoning may result in cochlear lesions, whereas hearing loss in the late stages of intoxication may result from neurological damage (EHP, 1994). Both cochlear and postcochlear damage have been involved in methylmercury poisoning (Oyanagi et al, 1989). In a case of acute fatal dimethyl mercury poisoning, the patient demonstrated an inability to understand speech, yet with relatively good hearing sensitivity for pure tones bilaterally. Distortion product otoacoustic emissions showed only minimal deficits in each ear while auditory brain stem response was abnormal bilaterally. Dimethylmercury poisoning, in this case, resulted in compromise of the auditory neural system with little effect on the sensory (cochlea) mechanism (Musiek & Hanlon, 1999).

A few individuals have also noted hearing loss following acute inhalation of high concentrations of elemental mercury vapour (ATSDR). Prolongation of brainstem auditory-evoked potentials was observed in workers exposed to mercury vapours (Chang et al, 1995; Discalzi et al, 1993) with urinary mercury levels of 325 µg/g creatinine (Discalzi et al, 1993).

An in vitro study as shown that $HgCl_2$ has a more toxic effect on auditory networks of mouse embryo when exposed chronically, and the levels of mercury showing toxic effects on auditory cortex networks are within the dose range shown to cause neurological symptoms in humans (Gopal, 2003).

It has been suggested that high-dose mercuric sulfide or methyl mercury intoxication is associated with a decrease in functional $Na^{(+)}/K^{(+)}$-ATPase activity in the brainstem of affected animals, this presumably arising via excessive nitric oxide production, and suggesting that brainstem damage may play a role in mercury-induced hearing loss (Chuu et al, 2001).

3.4.5. Asphyxiants

The mammalian cochlea represents a metabolically highly active structure that is vulnerable to the effects of hypoxia and chemical asphyxiants. Disruption of blood supply (ischemia) and reduction in available oxygen levels (hypoxia) have been suggested to be fundamental mechanisms that are responsible for many forms of sudden hearing loss and drug ototoxicity. Hypoxic hypoxia disrupts cochlear function in laboratory animals. Acute exposure to carbon monoxide (CO) can yield profound hearing loss in humans and laboratory animals (Tawckoli et al, 2001).

Fechter and collaborators have shown that a) CO by itself has no permanent effect on auditory sensitivity b) the potentiation of NIHL by CO increases as a function of CO concentration at levels of 500 ppm and above, but that the extent of potentiation shows a non-linear relationship to total noise energy with the greatest potentiation shown at moderate noise exposures that produce limited permanent threshold shifts; c) the potentiation of NIHL by CO appears to saturate as noise severity is increased such that at the most severe conditions used, the effects of CO on NIHL are obscured totally by the noise effect; d) CO is able to impair the recovery of NIHL that normally occurs when periods of silence are interspersed within noise exposure (Fechter et al, 2000, 2002). Similarly, by itself, HCN had minimal auditory effects even at 50 ppm, the highest dose investigated. However, in combination with noise exposure, low concentrations of HCN potentiate noise-induced hearing loss.

The authors did a series of calculations using a benchmark dose approach for risk assessment analysis. For a benchmark response corresponding to a 5 dB increase

in auditory threshold above the effect of noise alone, the lower bound on the 95% confidence interval for the benchmark dose was 9 ppm HCN or 320 ppm CO. The benchmark doses that impaired auditory threshold 10% above the effect of noise alone had a lower bound of 2 ppm HCN or 194 ppm CO (Fechter et al, 2000, 2002).

4. CONCLUSION

While occupational physicians managing the issue of hearing conservation in the workplace are well aware of the relationship between excessive noise exposure and hearing loss, they should keep the following concerns in mind:

- Although noise-induced occupational hearing loss is the most common occupational disease, it is often underestimated or neglected because the affection is particularly insidious and occurs without pain or obvious physical abnormalities. One should not forget that noise hazards are also found in the environment, at home and when participating in recreational activities (traffic, gardening, car racing, home improvement, pubs, discotheques…).
- The relationship between specific noise exposure patterns and risk of hearing loss, including impact noise, fluctuating noise is not fully understood. However, it is clear that the noise does not have to be constant to cause damage. Impulsive noises are harmful.
- While noise-induced hearing loss is typically bilateral and roughly symmetric, asymmetric sources of noise (such as sirens or gunshots) lead to asymmetric losses.
- Over a period of years of prolonged noise exposure, hearing loss due to noise expands to involve additional frequencies. Hence, in older individuals, the effects of noise may be difficult to distinguish from presbycusis without access to previous audiograms.
- There are wide individual variations in the susceptibility of humans to noise-induced hearing loss, which justifies surveillance programmes to detect the most sensitive individuals.

There is currently very little awareness in the occupational health community of the potential chemical hazards to hearing. Standard hearing conservation practices focus entirely on noise and do not take into account the potential risk to hearing posed by chemical exposures.

- There is a large array of ototoxic therapeutic drugs, some of which having a long half-life in the cochlea. A synergistic ototoxic action with noise exposure is possible.
- Some industrial chemicals are clearly ototoxic as shown by experimental rat studies; a.o. toluene, ethylbenzene, xylenes, styrene. However, there are species differences: the guinea pig and the chinchillas being much less sensitive than the rat. Hence, if the rat is an appropriate model for human risk assessment, which has currently not been clearly demonstrated, it is plausible to expect that if workers are exposed to these agents at high enough concentrations, they are at risk of developing hearing loss. There is a growing body of epidemiological literature on the ototoxic effect of solvents. However, data in humans are still equivocal, no dose-response or dose-effect relationship can currently be established, and the risk associated with exposure to such agents remains poorly understood. Occupational exposure to gasoline vapour entails, among others, exposure to toluene. Combined exposure to toluene and noise at lifetime weighted average exposure of 50 ppm (190 mg/m³) and 86 dB(A) was not associated with hearing loss. A European survey has shown that the 90P full-shift exposure for toluene range from 0.2 to 40 mg/m³ and one might conclude that the risk for these workers is very low. However, these workers are also exposed to other hydrocarbon solvents and chemical agents

and a high prevalence of hearing loss has been reported in workers from other industry sectors concurrently exposed to toluene, ethylbenzene and xylene at levels below the current OELs.

- A synergistic ototoxic action between noise and solvents such as toluene has been shown in experimental animal investigations. The daily noise exposure – not taking account of any hearing protection – for several of refining and some non-refining job activities associated with the downstream oil industry is likely to exceed 85 dB(A) LEP,d and, sometimes 90 dB(A) LEP,d (CONCAWE, 2001b). In a study on rotogravure printing workers, a higher prevalence of bilateral hearing loss was reported in the group of workers exposed to noise and toluene at very high levels when compared to a group of workers exposed to noise only. The adjusted relative risk estimates of hearing loss were calculated to be 4 times greater for the noise group, 11 times greater for the noise + toluene group, but the difference could not be considered as significant. One study on dockyard workers did not report a significantly higher age-adjusted OR in the noise + mixed-solvent group (including mainly toluene and xylene at “moderate” levels) as compared to the noise-only group.

  Pure-tone audiometry, generally used to detect hearing loss in humans, cannot distinguish the chemical from the acoustic trauma, both entailing a scotome around the same frequencies area. Regardless of its spectrum, noise is known to produce a dip in the hearing threshold, predominantly in the region around 4 kHz in humans. The only hearing threshold that seemed to be affected by solvents, in addition to noise, was associated with a frequency of 8 kHz. Given that solvents such as toluene affect the outer hair cells, otoacoustic emissions (OAEs) might be recommended.

- Some petrochemical workers are exposed to mercury and lead. The ototoxicity of methyl mercury is established but there is some indication that inorganic and metallic mercury might also be ototoxic. Lead exposure may also be associated with hearing deficits. It is however impossible to assess the risk in petroleum workers on the basis of the available scientific literature.
- Individual susceptibility to the auditory effects of solvents and of combined exposure to noise and solvents is probable.

Hearing loss is a very common problem affecting older adults. This type of hearing loss is typically gradual, bilateral, and characterized by difficulty in hearing high frequencies. Presbycusis begins after age 20 but is usually significant only in persons over 65. Men are affected more often and more severely than women.

Moreover, some diseases are associated with a high prevalence of auditory impairment. Smoking may be a risk factor for high frequency hearing loss, its combined effect on hearing with exposure to occupational noise appears to be additive. Hearing loss has also been associated with ethanol consumption and one should remember its modulating role in the biotransformation of solvents and hence the possible modulation of their toxic effects.

Finally, one should not exclude the possibility of an interaction between noise and other physical factors such as vibrations or heat (not considered in this report).

In conclusion, further research is needed to better characterize the risk. Once the hearing loss is incurred, it is irreversible and one should be alert to possible additive, potentiating, or synergistic ototoxic effects in case of combined exposure to several chemicals and in case of combined exposure to noise and chemical substances.

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GLOSSARY

ABR	auditory brainstem response
BAEP	brainstem auditory evoked potential
BAER	brainstem auditory evoked response
CAP	Compound Action Potential
CAR	Conditioned Avoidance Response
DPOAE	distortion product of OAEs
EcochG	electrocochleography
IHC	inner hair cell
LOAEC	lowest observed adverse effect concentration
NIDDM	non-insulin dependent diabetes mellitus
NIHL	noise induced hearing loss
NOAEC	no observed adverse effect concentration
OAE	otoacoustic emission
OEL	occupational exposure limit
OHC	outer hair cell
OR	odds ratio
PTA	pure tone audiometry
RMA	Reflex Modification Audiometry
RR	relative risk
SD	standard deviation
SPL	sound pressure level
TTS	temporary threshold shift

APPENDIX I PHYSIOLOGY OF HEARING

Sound consists of alternating compression and depression waves carrying mechanical energy through an elastic medium. Sound is first captured by the external ear, and then transmitted to the sensorineural receptor where mechanical energy is converted in electrical signal suitable for elaboration in the central nervous system. Hearing begins with this mechanical-electrical transduction.

- **'Capture' and transmission of the sound: external and middle ear**

Sound reaches the sensorineural receptor (*cochlea,* from the Greek word for snail) carried by the *air* and by the *bon*e.

In air conduction, sound waves enter the external auditory canal and cause alternating increases and decreases of pressure across the tympanic membrane which in turn starts to vibrate with a specific frequency and intensity. The vibrations are transmitted to the cochlea thanks to the movement of the ossicular chain in the middle ear: as the footplate of the stapes moves within the oval window, a pressure change in the fluid-filled inner ear elicits a travelling wave in the basilar membrane of the cochlea. The 22:1 ratio between the area of the tympanic membrane and the area of the oval window and the lever action created by the ossicular chain minimizes the loss of energy due to the reflection of the sound at transition from the air to a fluid medium. The middle ear acts as an impedance matching mechanism.

Sound can be transmitted to the cochlea through head bones too: such vibrations are transferred to endocochlear fluid-filling and cause the travelling waves in the basilar membrane.

- **From mechanical input to electrical signal: the inner ear and the cochlea**

The inner ear's receptor is the organ of Corti, a neuroepithelial strip lying onto the basilar membrane. It is placed into the scala media of the cochlea and it contains two families of receptor cells: a single row of inner hair cells (IHCs) and three rows of outer hair cells (OHCs).

Hair cells are innervated by nerve fibres that send auditory signals into the brainstem (afferent neurons) and to other nerve cells that carry signals from the brain into the ear and influence cochlear function in a feed back loop (efferent neurons). IHCs provide 95% of the afferent input of the cochlear nerve: a single IHC is, in fact, associated to many afferent fibres and receive little lateral olivo–cochlear (LOC) efferent connections from the brainstem. LOC fibres can have both excitatory and inhibitory actions on auditory nerve post-synaptic terminals.

On the opposite, information from several OHCs is carried by one afferent axon (with a 10:1 ratio), while they receive large myelinated efferent input from superior olivar complex. In particular, the medial olivo–cochlear (MOC) efferent cholinergic system make multiple inhibiting connections to the OHC base. In addition to a hyper polarization of the OHCs, acetylcholine can also influence the OHCs motility by modulating their axial stiffness. MOC may act in a reflex fashion by changing the cochlear amplifier as a consequence of the amount of auditory pathway activity and may also provide protection from over stimulation by noise. The MOC processes are both crossed and uncrossed innervating both ipsilateral and contra lateral cochleae.

Their mechanically sensitive hair bundles (stereocils) protrude into the endolymph, the fluid filling the scala media; the hair bundles of the outer hair cells are tightly bound to the lower surface of the membrana tectoria, a gelatinous matter present along the full length of the basilar membrane. The travelling wave causes an up-and-down motion of the basilar membrane with the greater amplitude at a point determined by the frequency of the stimulating tone. The "tonotopic" organization of the basilar membrane is due to its not uniform mechanical properties: it decreases in width and becomes thicker from the apex towards the base. This explains why the

basal portion is more sensitive to high frequencies tones while the apex is more sensitive to lower ones. The frequency range for hearing varies greatly between individuals. The complete audible range is 17-20,000 Hz, but a healthy young person usually can only hear up 17,000–18,000 Hz. The human ear is most sensitive to frequencies in the range of 2,000-5,000 Hz. The frequencies of normal conversation in a quiet place are 500 to 2,000 Hz. Frequencies below 30 Hz are hard to distinguish.

The displacement of the basilar membrane causes the mechanical deflection of the hair bundle onto the hairy cells and a subsequent change of the cationic flow through the cell membrane which leads to depolarization: the mechanical input is finally translated into an electrical signal.

- **From the organ of Corti to the primary auditory cortex: the central auditory pathways**

The 8th cranial nerve carries information from both the cochlea and the vestibular apparatus. The cochlear nerve is formed by afferent and efferent axons. The afferent component is formed by centrifugal axons of the bipolar neurons constituting the spiral ganglion. Their centripetal axons enter the brainstem at the medullo-pontine junction and synapse with the postsynaptic neurons of the cochlear nuclei. The activation of neurons constituting the cochlear nerve is frequency specific. This tonotopic organization is maintained at each point of the central auditory pathway.

Three main pathways originate from the post-synaptic neurons of the cochlear nuclei:

1. The dorsal acoustic stria: it projects to the controlateral nucleus of the *lateral lemniscus* (pons) and to the controlateral *inferior colliculus* (midbrain);
2. The intermediated *acoustic stria*: it projects to the controlateral *inferior colliculus*;
3. The trapezoidal body: it projects to both ipsilateral and controlateral superior olivar nucleus (pons); post synaptic neurons, then, project to *inferior colliculus* (midbrain).

Summarizing, information from the cochlear nucleus to the *inferior colliculus* are carried by the *lateral lemniscus* which contains axons relaying inputs from both ears.

Finally, post-synaptic neurons of the *inferior colliculus* project to the medial *geniculate nucleus* of the thalamus; the geniculate axons terminate in the primary auditory cortex, a part of the *superior temporal gyrus*.

As previously described, the efferent component consists in the lateral and in medial olivo–cochlear bundles, originating from the superior olivary complex and regulating IHCs and OHCs respectively. It influences the activity of the organ of Corti both in a qualitative and in a quantitative way. It is suggested that the main role of the olivo–cochlear bundle is to protect the inner ear from acoustic over stimulation. The olivo–cochlear activity could also make more accurate the encoding of the signals in noisy backgrounds, leading to better detection and discrimination threshold in noise. Within the cochlea, the olivo–cochlear bundle would modulate the *compression* which is a basic determinant of intensity encoding by the peripheral auditory system, affecting then intensity perception.

In short, sound waves cause vibrations of the tympanum which are conducted and amplified by the auditory ossicles through the tympanic cavity to the oval window. Sound wave energy is then transmitted to the fluid of the cochlea and converted by the hair cells of the organ of Corti into nerve impulses that are transmitted via the auditory nerve to the brain.

APPENDIX II HEARING LOSS EVALUATION

1. WORKER'S INTERVIEW

Proper assessment of workers with suspected hearing loss begins by collecting personal clinical history, stressing those conditions directly or indirectly linked to deafness.

The following list aims to highlight main factors which can be involved in hearing impairment:

1.1 Occurrence of the hearing impairment

- Does the hearing difficulty involve one or both ears?
- When was the hearing loss first noticed?
- The onset was sudden or gradual?
- Which are the most penalized activities? (TV, telephone, one-to-one conversation, in group conversation…)
- Is there tinnitus, vertigo, pain, headache*?*

1.2 Medical history (personal & family)

- Any previous ear disease, ear surgery, brain surgery, head injury?
- Frequent episodes of otitis media during the childhood?
- Has the worker been treated with potentially ototoxic therapeutic drugs, such as aminoglycosides, loop diuretics, acetylsalicylic acid…?
- Is the worker suffering from diabetes, hypertension, and other diseases?
- Cigarette smoking, ethanol consumption?
- Is there any deaf from birth in the family?
- Is there any other family component who complains hearing loss?

1.3 Non-occupational exposure to noise

- Has the worker done the military service?
- Is there a history of recreational shooting, motorcycling, discoteque, loud music listening (personal listening devices intense use), playing musical instruments?

1.4 Occupational history: exposure to noise and chemicals

With regard to employment (current and previous), the worker should indicate:

- the sector of production, length of employment, specific task carried out
- duration of the work shift
- environmental noise & chemical levels (if unknown, even personal guesstimate is useful)
- personal protection devices use, which kind?
- any prevention programme?
- any medical surveillance? The workers should provide (if possible) previous audiograms. The examination of previous audiograms before going on with clinical evaluation is of prime importance.

2. CLINICAL EXAMINATION

The clinical examination has to be performed before any audiometric test such as pure tone audiometry, immitance audiometry, brainstem auditory response, etc…

Clinical examination should include:

- Accurate inspection of the external ear and of the mastoid area, in order to individuate scares, for instance, due to previous operations;
- Otoscopy with particular attention to:
  - external auditory tract: the presence of wax could distort the audiology findings, leading to a false high-frequency hearing loss (6–8 kHz);
  - eardrum: the full tympanic membrane should be visualised in order to establish if there is evidence of chronic (perforations, retractions) or acute (mucous membrane congestion) middle ear pathology;

During clinical examination it's also possible to screen the auditory ability of the subject by the following simple tests:

- whisper test: simple screening test that assesses the ability to hear whispered speech across a short distance.
- tuning fork tests: these easy tests can provide valuable information about the type of hearing loss that may be present; they are used to differentiate conductive from sensorineural hearing loss.
  - Weber's test: This test is used to classify unilateral hearing loss. The stem of a vibrating tuning fork is placed on middle of the forehead, and the subject indicates whether the tone is louder in his left ear, louder in his right ear, or equally loud in both ears. A subject with a unilateral conductive hearing loss hears the tone louder in the affected ear. Subjects with a unilateral sensorineural hearing loss hear the tone louder in the normal ear.
  - Rinne's test: the stem of a vibrating tuning fork is placed in contact with the mastoid process (for bone conduction) and near the pinna (for air conduction). 256, 512 and 1024 Hz forks are most useful. Forks of lower frequency may be felt as vibration, while those with higher frequency are heard by air conduction when bone conduction is tested. In normal (positive Rinne) response, air conduction is heard approximately twice as long as is bone conduction; moreover, the stimulus is heard louder by air conduction than by bone conduction. In case of transmission hearing impairment bone conduction is heard longer that air conduction (negative Rinne). In case of sensorineural hearing loss, both air and bone conductions are reduced, but the ratio remains the same as that for normal hearing.

3. AUDIOMETRIC TESTS

3.1 Pure tone audiometry PTA

Pure tone audiometry is a behavioural test used to assess hearing impairment. It's a 'subjective' test: it's based on the patient's response and it's of first importance that the examiner well explains both purpose and instructions. With a pure tone audiometry it's possible to perform both liminal (auditory threshold) and supraliminal (easy audibility) tests. Auditory threshold is considered 'normal' when heightened of 25 dB or less, while the 'easy audibility' level is comprised between 30 and 40 dB.

Pure tone threshold – the lowest sound able to elicit an auditory sensation in the 50% of the cases - is tested both by air and by bone conduction for 0.25, 0.5, 1, 2, 3, 4 kHz and only by air conduction for 6 and 8 kHz.

Pure tone audiometry has to be performed in a soundproof room after a 14–16 hours noise free period to let the temporary threshold shift exhaust.

3.1.1 Air conduction

Pure tones are given through headphones to the right and to the left ear in subsequent, separate moments at a known (calibrated) intensity level (from -10 dB to 120 dBHL).The subject is asked to indicate not only when but also where – in the left or in the right ear - the tone is heard. Firstly, a trial is performed with a supraliminal stimulus at 1 kHz to let the patient know the kind of sound employed. Each tone is given firstly at very low intensity (0 dB); if it's not heard after 4 or 5 times, the intensity is increased by 5 dB until the patient can hear the tone. The threshold is the lowest intensity (dB) able to elicit an auditory sensation. The sequence is performed for each frequency for both the ears.

3.1.2 Bone conduction

Pure tones are given through a vibrator placed onto the mastoid process and they reach the cochlea by-passing the middle ear air conduction. Frequencies tested are 0.25, 0.5, 1, 2, 3 and 4 kHz.

In case air conduction test shows a consistent difference between the right and left thresholds, masking during bone conduction is compulsory in order to prevent the best ear hearing the tone even when given to the contra lateral one. A white sound (multi-frequency) is given - through earphones - to the best ear while pure tones are given to the other one by bone conduction. The masking noise (white noise) has to be greater than the testing pure tone, since the head has an attenuating power of about 40 dB: in other words, a sound given to the left ear reach the right one decreased in its intensity for about 40 dB.

In case of middle ear pathology, typically bone conduction threshold is lower (better) than air conduction one.

The results of PTA are plotted on a graph (audiogram): the intensity of sound is plotted on the y-axis as dB Hearing Level and the frequency is along the x-axis with increasing frequency from left to right. For air conduction (AC) of the right ear the symbol is an "O", while for left ear it is an "X". A triangle represents bone conduction (BC). Open brackets represent masked BC thresholds: a bracket open to the right corresponds to the response of the right ear, a bracket open to the left represents the response from the left ear. If no response is obtained either for AC or BC stimulus at the maximum level available on the audiometer then an arrow is drawn pointing downward, indicating that the threshold is worse than the maximum level available.

3.2 Automatic audiometry

Automatic audiometry is a pure tone audiometry where the tone – given by earphones or by vibrator – is changed in its intensity without the operator's intervention. Firstly, a *continuous* pure tone is given to the patient with increasing intensities (starting from lowest one); the patient is asked to push a button when he can hear the tone which, in turn, starts decreasing gradually. The button has to be pushed until the tone is heard and it has to be released when it's no more perceived.

Hearing threshold for each frequency is expressed as a mean value obtained from 5 or 6 trials, so that the variability of the threshold is low.

This kind of audiometry does not need a highly trained technician and it's independent from the examiner's subjectivity, but some disadvantages can be listed:

- It's a time consuming test, even in case of healthy subjects;
- The examiner can't take part in the test by correcting and encouraging the patient;
- It requires the subject more concentration, resulting in a definitely demanding test;

3.3 Vocal audiometry

Vocal audiometry is a very useful completion of the pure tone audiometry, since it highlights impairments of central auditory pathway responsible for hearing impairment even if the pure tone threshold is normal.

The examined subject is in a soundproof room and he's asked to repeat vocal messages with different degrees of complexity (meaningless or meaningful syllables, simple words, simple phrases, complexes phrases…) given him through earphones. A phonetic composition of the messages in conformity with the local language is of basic importance or the reliability of the test.

Three thresholds are obtained:

- Detection threshold: the subject has perceived an auditory sensation but he can't repeat the message;
- Perception threshold: the subject can repeat the 50% of the message;
- Intelligibility threshold: the subject repeat correctly the 100% of the message;

Very poor results, out of proportion to PTA, suggest probable retro cochlear cause of hearing loss. The results of speech and word tests may not be accurate if the person being tested has language problems.

The vocal audiometry is also useful to assess the ability of the patient to understand the conversation voice and to evaluate the necessity of an auditory prosthesis.

3.4 Immitance audiometry

It evaluates the resistance to movement of the middle ear conducting system against to the sound stimulus presented via a probe placed in the outer ear canal and measures the sounds reflected from the tympanic membrane.

- Tympanometry: measures the impedance of the middle ear to acoustic energy. While the patient remains quiet, a probe containing a sound source and microphone is placed in the ear canal to measure how much acoustic energy is absorbed (passes through) or is reflected by the middle ear. Normally, maximal compliance of the middle ear occurs when the pressure in the ear canal equals atmospheric pressure. Increasing or decreasing pressure in the ear canal demonstrates various patterns of compliance.
- Acoustic reflex testing: acoustic reflex testing is completed with the same equipment used during tympanometry. A sound stimulus of strong intensity (about 85 dB) induces the reflex contraction of the stapedius muscle, aiming – according to some authors - at protecting the inner ear from sound traumatisms. Acoustic reflex test can detect changes in compliance produced by reflex contraction of the stapedius muscle. The reflex is determined by the *auditory sensation* elicited more than by the intensity of the sound itself and that's why it's so useful in assessing noise induced hearing losses; in fact, as previously mentioned, NIHL is always accompanied by *recruitment,* the pathological loss of proportionality between the intensity of the sound (SPL) and the auditory sensation perceived due to lesion of the OHCs. In the healthy subject, the reflex usually appears at 70–80 dB above the auditory threshold while, in patients with NIHL – and recruitment – it's evoked by sensibly lower intensities, such as 20–30 dB above the auditory threshold. Moreover, the presence or absence of this reflex is important in the topographic diagnosis of middle ear functioning, facial nerve paralysis, or to detect simulators. Decay is absent or mild in sensory hearing loss and severe in neural hearing loss.

In the interpretation of the results, it's very important to take into account the crossed nature of the reflex: a high intensity sound given to the left ear elicits the stapedial reflex in the right ear too.

3.5 Auditory brainstem evoked potential

Evoked Response Audiometry measures electrical potentials generated by sound stimulation of the cochlea (i.e. evoked response) provides information concerning the cochlea (i.e. electrocochleography), the cochlear nerve and brainstem (i.e. auditory brainstem response or ABR) and higher cortical auditory pathways (i.e. threshold evoked potentials, cortical evoked response audiometry).

The brainstem auditory evoked potential (BAEP) (or Auditory Brain Response ABR, or brainstem auditory evoked response, BAER) consists in stimulating hearing by clicking noises or tones through earphones; the electrical response in the brainstem is recorded by using electrodes placed on the scalp and earlobes. The ABR is a non-invasive, objective test, which does not require the subject's active participation, though a good compliance – in terms of remaining quiet, not chewing, not speaking…- is needed.

A 3-frequency audiogram (1 kHz, 2 kHz, and 3 kHz) is obtained for each ear. The degree and type of hearing loss can be determined from the presence or absence of waves, the time (latency) at which certain waves occur, and the time interval between different waves. This technique allows for differentiating sensory from neural hearing loss. Five distinct electric waveforms generated in the 8^{th} nerve, brain stem, and other regions in response to acoustic stimulation are examined. They can be recorded using a computer to average responses to many stimuli. Each waveform probably emanates from a distinct structure in the auditory pathway, such as the 8^{th} nerve, cochlear nuclei, superior olivary complex, lateral lemniscus, and inferior colliculus. With lesions of the 8^{th} nerve, one or more waveforms may be lost, the latency of the waveforms from the onset of the acoustic stimuli may be increased, and the interwave latencies may be prolonged. With cochlear lesions, the waveforms are easily recognized, and the latency relationships remain normal.

- I and II are generated by different sections of the VIII cranial nerve;
- III arises from the cochlear nucleus;
- IV may receive contribution from the superior olivary nucleus, cochlear nucleus, lateral lemniscus;
- V: lateral lemniscus as it enters the inferior colliculus.

3.6 Electrocochleography

Electrocochleography (EcochG) is an ABR where the electrode is placed closed to the tympanic membrane or to the cochlea. The stimulus is a click or a tone burst presented at slow rate which elicits a frequency-specific response. The EcochG components are:

- Compound Action Potential (CAP): the complex sum of spike activity across a large number of fibres of the 8th cranial nerve stimulated by 'clicks'.
- Microphonic cochlear (MC): generated by Outer Hairy Cells
- Summating potential (SP): response from the Organ of Corti Inner Hair Cells

MC and SP are the 'receptor potentials'.

In case of lesions of the acoustic nerve, while OAEs are preserved because of the integrity of OHCs, CAP can be altered in threshold and in morphology because of the desynchronized neural activation. It's not correlated with pure tone threshold since the desynchronization may not prevent auditory sensation. It has a better signal-to-noise ratio than ABR. Absent CAP with normal SP and MC suggest VIII cranial nerve pathology. In case of desynchronization of neural discharge due to demyelinization, for instance, CAP and ABR can be absent, with normal SP and MC, normal pure tone audiometry and severe impairment of speech perception (accurate temporal encoding of acoustic signals is requested for normal ABR and speech comprehension). Usually tympanometry is normal, but acoustic reflexes are absent.

3.7 Otoacoustic emissions (OAE) testing

This non invasive, objective test does not require the subject's active participation.

The primary purpose of otoacoustic emission (OAE) tests is to determine cochlear status, specifically hair cell function. The normal cochlea does not just receive sound; it also produces low-intensity sounds called OAEs. These sounds are produced most probably, by the cochlear outer hair cells as they expand and contract, either spontaneously or in response to sound (Spontaneous Otoacoustic Emissions and Evoked Otoacoustic Emissions). Consequently, they can be detected only when the middle ear is operating normally. OAEs can be captured in the external auditory canal by a probe and monitored by a computer. These otoemissions represent an objective evidence of the normal functioning of the cochlea. Absence of otoacoustic emissions indicates damage in the cochlea. If otoacoustic emissions are present, the cochlea is intact. If the loss is sensorineural and otoacoustic emissions are present, the damage is in the 8^{th} nerve. Middle ear diseases, such as otitis media, eliminate otoacoustic emissions. Special information from different parts of cochlea may be obtained because of its frequency specific properties. If moderate or advanced hearing losses are detected in pure-tone audiometry with normal OAEs, simulation should be considered.

Distortion product of OAEs (DPOAEs): are sounds emitted in response to 2 simultaneous tones of different frequencies. DPOAEs emitted by the cochlea at 2f1-f2 in response to pairs of pure tones at f1 and f2 form a class of otoacoustic emissions and as such, are viewed as a reliable tool for screening outer hair cell (OHC) dysfunctions. The lower frequency pure-tone stimulus is called the f1 primary, and the higher frequency stimulus is called the f2 primary. DPOAEs are generated in the tail of the basilar membrane displacement of f1, that is the place where travelling waves elicited by f1 and f2 overlap.

APPENDIX III TABLE SUMMARIZING THE MAIN EXPERIMENTAL STUDIES ON TOLUENE, XYLENE, ETHYLBENZENE AND N-HEXANE

Table AIII-1: main experimental studies with toluene, xylene, ethylbenzene and n-hexane

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Toluene				
Pryor et al, 1983a, 1983b, 1984a Rebert et al, 1983	male Fischer 344 rats	- 0, 900, 1200 or 1400 ppm - 14 h/d, 7 d/w, 5 or 14 w	- CAR (4–20 kHz) (hours, last w of exposure; 1-3 w, 2 months after exposure) - BA (4, 8, 12, 16, 20 kHz) - BAER (2 w to 2.5 months after exposure) - histology (basal turn of the cochlea)	- irreversible high frequency hearing loss : CAR acquisition impaired along with the acquisition of a tone-intensity discrimination task (at 4 kHz) when tested within hours after the daily exposure ended; lack of response to sound (a 20 kHz tone), last week of exposure and again 2 months after the last exposure; hearing unimpaired at 4 kHz, slightly impaired at 8 kHz, and markedly impaired at 12, 16, 20 kHz 2.5 months after the last exposure - young and pre-pubertal rats more severely affected than older rats.
Pryor et al, 1984b	Male Fisher 344 rats	- seven experiments: 400 to 4000 ppm, - varying schedules	- CAR (4–20 kHz) (weekly or biweekly after the exposures started, 2 h after the daily exposures ended) - BA (4, 8, 12, 16, 20 kHz) - BAER (4, 8, 16 kHz) (weekly, 2 h after the daily exposures ended & 3 w after exposure)	- 400, 700 ppm: no effect even after 16 week-exposure - 1000 ppm 14 h/d, 2 weeks: hearing loss - 1500 ppm (14 h/d, 3 d) or 2000 ppm (8 h/d, 3 d): hearing loss - 4000 ppm (single 4 h exposure) or 2000 ppm (single 8 h exposure: no effect - intermittent exposure to 3000 ppm (30 min/h, 8 h/d, 2 w) : hearing loss - intermittent exposure to 3000 ppm (4 h/d, 9 w): no effect.
Crofton et al, 1994	Male Long Evans rats	- 2500 ppm - 8 h/d, 5 d	- reflex modification audiometry (RMA) (0.5–40 kHz) (5 to 8 w after exposure)	- increased thresholds at 8-24 kHz.
Pryor & Howd, 1986	Male Fischer 344 rats	- PEG-300 (control), toluene 1.5 or 1.7 g/kg bw by SC injection - 2/d, 7 d	- CAR (4, 8, 12, 16, 20 kHz) - BAER (4, 8, 16 kHz)	- dose-related hearing loss at 8 kHz and above, no hearing loss at 4 kHz - (no effect on CAR in response to light, nonaversive foot shock).
Sullivan et al, 1989	Male Sprague Dawley rats	- corn oil (control), toluene: 0.5-1.0 ml/kg bw/d in corn oil by gavage - 8 w	- BAER (0.5, 1, 2, 4, 8, 16, 32 kHz) (before exposure, 1 d after last dose) - histology (phase-contrast microscopy)	- greatest threshold elevation at 2-8 kHz - loss of OHCs in the middle and basal turns of the organ of Corti, mainly in the third row and progressively less in the second and first rows.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Johnson & Canlo, 1994 a, b	Male Sprague Dawley rats	- 1400 ppm - 16 h/d, 8 days - (noise <50 dB(A))	- BAER (during exposure: 12.5 kHz; after 1.6, 3.15, 6.3, 12.5, 20 kHz) - DPOEs (3, 4, 5, 6.3, 8, 9, 11.4, 14.3, 17.9 kHz) (before exposure, 3 & 5 d after the start of exposure, 4 d after exposure) - histology: light microscopy and scanning electron microscopy (3 & 5 d after the start of exposure, 4 d & 6 w after exposure)	- after 3 d exposure: tendency to lower DPOE amplitudes and elevate BAER thresholds, no loss of hair cells - after 5 d: significantly lower DPOE amplitudes at most frequencies along with elevated ABR thresholds, slight loss in the third row OHCs - 4 d after the 8 d exposure: DPOE amplitudes greatly diminished at all frequencies, BAER thresholds raised by about 40 dB ≥1.6 kHz, loss of OHCs in all 3 rows, mainly in the middle and upper turns of the cochlea - 6 w post-exposure: damage on the hair cells progressed towards the basal part of the cochlea, 50-100% loss of OHCs and some loss of IHCs.
Campo et al, 1997, Loquet et al, 1999	male Long-Evans rats	- 0, 1000, 1250, 1500, 1750, 2000 ppm - 6 h/d, 5 d/w, 4 w - (noise <52–66 dB SPL)	- BAER (inf colliculus) (2, 4, 6, 8, 10, 12, 16, 20, 24, 32 kHz) (before exposure, 24-32 h & 6 w after exposure) - histology: optical & scanning electron microscopy (7-8 after exposure)	- ≥1500 ppm: significant auditory threshold shifts, hearing deficit in the 8–24 kHz frequency range, no effect at 32 Hz - ≥1000 ppm: OHC loss (OHC3 >OHC2 >OHC1) (peaks at 4 and 20 Hz): at 1500 ppm, almost all OHC3 swept off.
Campo et al, 1999	male Long-Evans rats	- 0, 1750 ppm - 10 h (6 consecutive h + 4 h the following d)	- gas chromatography: rates of solvent uptake in blood, brain, auditory nerves, the organ of Corti, CSF, and IEF (1 h after exposure)	- toluene detected in blood, brain, auditory nerve, cochlea - CSF (cerebrospinal) and IEF (inner ear fluid): free from detectable solvents.
McWilliams et al, 2000	male pigmented guinea pigs	- 0, 250, 500 and 1000 ppm - 8 h/d, 5 d/w, 1 & 4 w	- DPOAE (6, 8, 12, 16, 20, 24 kHz) (before, 1 & 4 w after exposure) - succinate dehydrogenase (SDH) staining density in hair cells	- ≥250 ppm: dose-dependent temporary reduction of DPOAE (2F1-F2) amplitude - SDH staining suggests that reduced enzyme activity in the mid frequency region of the cochlea occurs acutely following toluene exposure - progression of auditory dysfunction between 1 and 4 w, but no permanent loss and no hair cell death.
Lataye et al, 2003	- male Long-Evans rats - male pigmented guinea pigs	- 600 ppm - 6 h/d, 5 d	- DPOAE (2, 3, 4, 5, 6, 8, 10, 12, 16 kHz) (before, 20 min, 2 & 4 w after exposure) - histology (4 w after exposure) - hippuric acid in urine (during 17.5 h period)	- rat: severe disruption of auditory function and cochlear pathology. - guinea pig : no effect - increase in hippuric acid excretion higher for the guinea pig than for the rat.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Toluene + ethanol				
Campo et al, 1998	male Long-Evans rats	- ambient air - toluene alone: 1750 ppm, - ethanol alone: 4 g/kg (gastric intubation), 5 d/w, 4 w - toluene + ethanol - 6 h/d, 5 d/w, 4 w	- BAER (inferior colliculus) (2, 4, 6, 8, 10, 12, 16, 20, 24, 32 kHz) (at the beginning & 6 w after exposure) - histology: optical & scanning electron microscopy (6-7 w after exposure) - hippuric acid in urine	- higher excretion of hippuric acid in group toluene alone compared to group toluene + ethanol - toluene alone: OHC loss from the third to the first row - ethanol alone: no effect - toluene + ethanol: permanent threshold shifts not larger than toluene-induced ones - toluene + ethanol: loss of OHC significantly larger than that induced by exposure to toluene alone.
Nylen et al, 1995	male Long-Evans rats	- ambient air - toluene alone: 1000 ppm, 21 h/d - ethanol alone : 5.7-8.0% in drinking water, - toluene + ethanol - 8 w - (noise: 76-78 dB SPL)	- BAER (1.6, 3.15, 6.3, 12.5, 20 kHz) (1 w after exposure)	- auditory sensitivity reduced after exposures including toluene at all frequencies tested - ethanol antagonized toluene-induced loss of auditory sensitivity at 20 kHz - flash evoked potentials not affected by toluene.
Toluene + Hexane				
Pryor and Rebert, 1992	male Fischer-344 rats	- ambient air - toluene alone: 1200 ppm - n-hexane alone: 4000 ppm, - toluene + n-hexane - 14 h/d, 7d/w, 9 w	- CAR (4 kHz) (12 w after exposure) - BAER (4, 8, 16 kHz) (2 w after exposure) - (tests for motor function)	- hexane alone: slight hearing impairment (decrease BAER amplitude) - toluene alone: hearing loss (clearest at 16 kHz) - toluene + n-hexane: group with the poorer performance on auditory tests - (hexane-induced neurotoxicity greatly reduced by toluene).
Nylen et al 1994	male Sprague-Dawley rats	- ambient air - toluene alone: 1000 ppm - n-hexane alone: 1000 ppm - toluene + n-hexane - 21 h/d, 7 d/w, 28 days - (noise: 76-78 dB SPL)	- BAER (2 d, 3 months & 1 year after exposure)	- reduction in auditory sensitivity 2 d after exposure to toluene alone, or to toluene + n-hexane, not after exposure to n-hexane alone. The reduction lasted one year after the exposure - 3 months post-exposure: synergistic enhancement of hearing loss in the toluene + n-hexane group.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Toluene + Noise				
Johnson et al, 1988	male Sprague-Dawley rats	- ambient air - toluene alone: 1000 ppm 16 h/d, 5 d/w, 2 w - noise alone: 100 dB Leq, 10 h/d, 7 d/w, 4 w - toluene followed by noise.	- BAER (1.6, 3.15, 6.3, 12.5, 20 kHz) (immediately, 1 & 6 month after exposure)	- High-frequency auditory impairment observed after exposure to toluene alone and noise alone with slight recovery 1 and 6 months after the toluene exposure - toluene followed by noise : higher threshold at all frequencies with slight recovery 6 months post-exposure - synergistic effect between toluene and noise, particularly at 3.15 and 6.3 kHz.
Johnson, et al 1990	male Sprague-Dawley rats	- ambient air - toluene alone: 1000 ppm, 16 h/d, 7 d/w, 2 w - noise alone: 100 dB Leq, 10 h/d, 7 d/w, 4 w - noise followed by toluene.	- BAER (1.6, 3.15, 6.3, 12.5, 20 kHz) (1-3 w after exposure)	- auditory loss after all exposures - toluene + noise: hearing loss greater than after single exposure to noise or to toluene but no synergism.
Lataye & Campo, 1997	male Long-Evans rats	- ambient air - toluene alone : 2000 ppm, 6 h/d, 5 d/w, 4 w - noise alone: 92 dB octave band centred at 8 kHz - toluene + noise.	- BAER (inferior colliculus) (2-32 kHz) (before exposure, last d of exposure, 6 w after exposure) - histology: optical & scanning electron microscopy	- toluene: hearing deficit restricted to mid-frequency range (8-24 kHz), losses of OHC - toluene + noise: auditory deficit exceeds the summated losses caused by toluene alone and by noise alone at all frequencies tested - noise: injured stereocilia of OHCs, without cell loss - toluene: massive loss of OHCs.
Brandt-Lassen et al, 2000	male Wistar rats	- ambient air - toluene alone: 0, 500, 1000, 1500, 2000 ppm, 6 h/d, 10 d - noise alone: 96 dB SPL, 2 h - noise (96 dB SPL, 2 h) following daily toluene exposure, 10 d - noise (105 dB SPL, 4 h) following toluene exposure by 30 d	- BAER (4, 8, 12.5, 16, 20, 31.5 kHz) (before exposure, 12 d after exposure)	- ≥1500 toluene alone: mid-frequency ABR threshold shift - 500 ppm toluene + noise: small, but statistically significant threshold shift, equal to the hearing loss in rats exposed to noise only. Synergistic interaction evident at ≥1000 ppm - no further hearing loss at the 2000 ppm than at the 1500 ppm level, indicating that a saturation of the auditory impairment had been reached - acute noise exposure (105 dB SPL, 4 h) following toluene exposure by 30 d: interaction noted at 1500 ppm toluene, not at 1000 ppm.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Davis et al, 2002	- Chinchillas - Sprague Dawley rats	- ambient air - toluene alone: 2000 ppm, 8 h/d or 12 h/d, 10 d - noise alone : 95 dBA at 500 Hz, 8 h/d, 10 d - toluene 8 h/d or 12 h/d + noise 8 h/d, 10 d - toluene 2000 ppm, 8 h/d, 5 d (positive control)	- BAER (0.5, 1.2, 4, 8, 16 kHz) (before exposure, 1, 3, 7, 14 & 30 d after exposure) - liver microsomes: quantification of proteins CYP1A, CYP2E1 & CYP2B	- toluene alone : large threshold shift in rats but no effect in chinchillas - noise alone: threshold shift - no interaction of noise and toluene on the ear in chinchillas - P450 CYP2E1 & CYP2B chinchillas > rats.
Xylene				
Crofton et al, 1994	male Long Evans rats	mixed xylene - 1800 ppm - 8 h/d, 5 d	- reflex modification audiometry (RMA) (0.5 – 40 kHz) (5-8 w after exposure)	- increased thresholds at 8-24 kHz.
Pryor et al, 1987	male Fischer-344 rats	mixed xylenes (10% p-, 80% m-, 10% o-): - 0, 800, 1000, 1200 ppm; 14 h/d, 7 d/w, 6 w - 1700 ppm, 4 h - 1450 ppm, 8 h - 1450 ppm, 8 h/d, 3 d	- behavioural audiometry: CAR (4 kHz) (2 d after exposure) + variation (2-20 kHz) (2 w after exposure) - BAER (4-16 kHz) (2 w after exposure)	- 6 w exposure: - 1200 ppm: CAR slightly impaired - dose-related effect on BAER thresholds; elevated thresholds at frequencies depending on concentration; at 800 ppm,12 and 20 kHz; at 1000 ppm, 8 kHz; at 1200 ppm all frequencies tested. Elevated BAER thresholds: at 16 kHz ≥800 ppm, at 8kHz ≥1000 ppm, at 4kHz at 1200 ppm - acute exposure: 1450 ppm,1 or 3 d: hearing loss 1700 ppm, 4 h: no effect.
Gagnaire et al, 2001	Sprague-Dawley rats	ortho-, meta- or para-xylene : - 450, 900, 1,800 ppm, - 6 h/d, 6 d/w, 13 w	- BAER - histology : light and electron microscopy (8 w post- exposure)	- ortho-, meta-xylene: no effect - para-xylene: - moderate to severe ototoxicity at 900 and 1,800 ppm. Increased auditory thresholds at 2, 4, 8 and 16 kHz in rats exposed to 1800 ppm para-xylene. Irreversible after 8 weeks of recovery - moderate and severe losses of OHCs at 900 and 1800 ppm - no observed effect level of para-xylene : 450 ppm.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Xylene and n-hexane				
Nylen & Hagman, 1994	male Sprague-Dawley rats	- xylene alone: 1000 ppm - n-hexane alone: 1000 ppm - xylene + n-hexane - 18 h/d, 7 d/w, 61 d	- BAER (2 d, 4 & 10 months post-exposure) - flash evoked potentials (2 d & 4 months after exposure) - (nerve & muscle action potentials)	- n-hexane alone, or xylene alone: slight loss of auditory sensitivity (BAER 2 d post-exposure) - n-hexane + xylene: persistent loss of auditory sensitivity (7-17 dB; $P < 0.05$) which was non-additively enhanced ($P < 0.01$).
Hexane				
Rebert et al, 1982	male Fischer rats	- 1000 ppm 24 h/d, 5 d/w, 11 w - 24000 or 48000 ppm - 10 min, 6 or 12 times/d	- BAER	- first component of the BAER slightly affected (indicating that the increased latency of the fifth component reflected a brainstem dysfunction). Latency returned to normal within 5 weeks after exposure, but amplitude not - repeated, brief exposures to 48,000 ppm: only the amplitude of the fifth BAER component affected.
Pryor et al, 1983	male Fisher 344 Rats	- 2000 ppm - 14 h/d, 7 d/w, 14w	- CAR (during 12 through 14h of exposure and 1, 4 & 6 w post-exposure) - BAER (8 kHz) - histology (14 w post-exposure)	- decreased amplitude of the fifth component of BAER - transient impaired CAR.
Ethylbenzene				
Cappaert et al, 1999	male Wistar-derived strain Wag/Rij/Cpb/Hsd	- 0, 800 ppm - 8 h/d, 5 d	- RMA (4, 8, 16, 20, 24 kHz) (before exposure, 1 & 4 w after exposure) - electrocochleography (CAP) (1, 2, 4, 8, 12, 16, 24 kHz) (8-11 w after exposure) - histology	- RMA threshold increased significantly, 1 and 4 w post-exposure, irrespective of the stimulus frequency tested - CAP: threshold increased significantly at all frequencies tested - OHCs loss, especially in the upper basal and lower middle turns to an extent of 65%.
Cappaert et al, 2000	male Wistar-derived strain Wag/Rij/Cpb/Hsd	- 0, 300, 400 and 550 ppm - 8 h/d, 5 d	- CAP (1-24 kHz) (3 to 6 w after exposure) - DPOAEs (4-22.6 kHz) - histology	- at 400 ppm: auditory thresholds increased at 12 and 16 kHz; 25% OHC loss at the 11 and 21 kHz region, respectively - at 550 ppm: auditory thresholds increased at 8, 12, 16 kHz; DPOAE amplitude growth with stimulus level affected after at 5.6, 8, 11.3 kHz; 40% and 75% OHC loss at the 11 and 21 kHz location, respectively - NOAEL: 300 ppm.

References	Species	Exposure protocol	Auditory tests, other measurements	Main results (concerning the ototoxicity)
Cappaert et al 2002	- female albino Wag/Rij rats - female albino guinea pigs	rats: - 0, 550 ppm - 8 h/d, 3 d guinea pigs: - 0, 2500 ppm - 8 h/d 1 d, then 6 h/d, 4 d	- electrocochleography (CAPs) 1, 2, 4, 8, 12, 16, 24 kHz) (4-8 w after exposure) - histology - ethylbenzene blood concentration	- rats: 550 ppm: deteriorated auditory thresholds in the mid-frequency range; OHCs loss in the corresponding cochlear regions - guinea pigs: no threshold shifts, no OHC loss after exposure to 2500 ppm - ethylbenzene blood concentration: after day 1 (500 ppm, 8 h/d, 3 d). 23.2 ± 0.8 µg/ml in rats, 2.8 ± 0.1 µg/ml in guinea pig; after day 3, ethylbenzene concentration in rat blood was still 4.3 times higher.
Ethylbenzene + noise				
Cappaert et al 2001	Albino Wag/Rij Rats	- ethylbenzene alone: 300, 400 ppm - noise 95 or 105dB SPL or background noise at 65 dB_{lin} SPL - ethylbenzene + noise - controls - 8 h/d, 5 d	- DPOAE (3 & 7 w after exposure) - electrocochleography (CAPs) (1, 2, 4, 8, 12, 16, 24 kHz) - histology	- ethylbenzene alone: at 300 and 400 ppm, no significant DPOAE or CAP shifts; OHCs loss after exposure to 300 ppm ethylbenzene located in the third row; at 400 ppm, OHCs loss spread out to the second and first row - 105 dB noise alone, and 105 dB noise + ethylbenzene (from 300 ppm): DPOAEs and CAP affected. Loss for the combined exposure: not greater than the loss for noise alone - noise alone: OHCs count hardly affected (except a minor loss in the first row of OHCs after 105 dB SPL) - noise at 105 dB + ethylbenzene at 300 and 400 ppm: no interactions in DPOAE or CAP but OHCs loss greater than the sum of the losses induced by noise and ethylbenzene alone.

CAR Conditioned Avoidance Response
BAER Brainstem Auditory Evoked Response
CAP Compound Action Potential
DPOAEs 2f1-f2 distortion product otoacoustic emissions
RMA Reflex Modification Audiometry (estimates auditory thresholds by recording the reduction of a noise-evoked startle response when it is preceded by a single-frequency sound stimulus, the pre-pulse)