THE EFFECTS OF INDUSTRIAL OTOTOXIC AGENTS AND NOISE ON HEARING

PAUL DAMIEN NIALL

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University College London

Institute of Laryngology and Otology

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ABSTRACT

Recent epidemiological and laboratory experimental studies have established that some commonly used organic solvents and heavy metals can be ototoxic. Workers exposed to these substances may also be exposed to high levels of industrial noise.

The possibility of short or long term combined exposure to noise and chemicals poses new questions for hazard risk assessment. This research project reviews aspects of the scientific literature on potential industrial and environmental ototoxic agents and, where possible, on the combined effects of multiple exposures. Multiple exposures may be exposures to mixtures of chemicals, sequential exposures to chemicals or exposures to chemicals and noise sequentially or simultaneously.

Organic solvents implicated are unsaturated aliphatic or aromatic compounds including toluene, styrene, xylene, trichloroethylene and carbon disulphide and the heavy metals include lead, mercury and tin.

Animal and human studies of the effects of these substances on hearing are reviewed. Particular attention is devoted to combined effects. Some data on other possible ototoxic chemicals are reviewed.

The study finds that there are similarities in the effects of various solvents on the cochlea and that there are neurotoxic effects of variable severity. Interactions between noise and chemicals are described.

There is some information on the effects of those agents at the cellular and biochemical level especially for mercury and tin. Generally, however knowledge of the biochemical and pharmacokinetic basis for ototoxicity was found to be quite limited.

Studies of chemicals and noise together in both humans and animals confirm the need for watchfulness in occupational health and safety regulation and rigour in enforcing existing exposure standards for both noise and chemicals especially where there is the possibility of multiple exposures.

CONTENTS

Abbreviations

ABLB	Alternate Binaural Loudness Balance (Test)
ACGIH	American Conference of Governmental Industrial Hygienists
ART	Stapedial Reflex Threshold(s)
BAER	Brainstem Auditory Evoked Response
BERA	Brainstem Evoked Response Audiometry
CAP	Compound Action Potential
CAR	Conditioned Avoidance Response
ceil	ceiling: that concentration of a chemical which should not be exceeded even instantaneously.
CERA	Cortical Evoked Response Audiometry
d	day(s)
d/w	days per week
dB	decibel
dBA	The A-weighted SPL of a sound
dBHL	Sound Pressure Level in dB re audiometric or reference zero
dB L_{eq}	That continuous steady state sound pressure (in dBSPL) which has the same total acoustic energy as the reference sound over a set period of time.
dBSPL	Sound Pressure Level in dB re 20microPascals
DNA	Deoxyribonucleic acid
DPOAE	Distortion Product Otoacoustic Emissions
h	hour(s)
h/d	hours per day
Hg	Mercury
IHC	Inner Hair Cell
IPL	Interpeak Latency
kg	kilogram(s)
kHz	kilohertz

mg	milligram(s)
MRI	Magnetic Resonance Imaging
NIOSH	National Institute for Occupational Safety and Health (United States)
OBN	Octave Band Noise
OHC	Outer Hair Cell
OSHA	Occupational Safety and Health Administration (United States)
Pb	Lead
PB-max	Maximum Speech Discrimination
ppm	Parts per million
PTA	Pure Tone Audiometry
SD	Standard Deviation
SEM	Scanning Electron Microscop(e)(y)
SGC	Spiral Ganglion Cell
SISI	Short Increment Sensitivity Index
SPL	Sound Pressure Level
SRT	Speech Reception Threshold
STEL	Short term exposure limit adopted by ACGIH - the maximum concentration to which workers may be exposed for a period of up to 15 minutes continuously, provided that no more than four excursions per day are permitted, with at least 60 minutes between exposure periods and provided that the daily TLV/TWA is not exceeded.
TEM	Transmission electron microscop(e)(y)
TLV	Threshold Limit Value. The TWA adopted by ACGIH
TMT	Trimethyltin
TWA	Time-weighted average for normal 8 hour working day and 40 hour working week.
w	week(s)

INTRODUCTION

Although it has been known from antiquity that noise could damage hearing, understanding of the role of other factors is much more recent and remains far from complete. The first scientific treatise on noise-induced hearing loss was published in the early eighteenth century (Ramazzini, 1713). It is now known that there are other ototraumatic agents in the workplace including chemicals.

Heavy metals have poisoned workers for millennia. Early writers on their effects were Hippocrates (370 BC), Nicander (2nd Century BC) and Pliny (1st Century AD) who all wrote on lead (Zenz, 1994). Mercury poisoning was not properly described until the sixteenth century AD and then in connection with iatrogenic rather than occupational disease. The symptoms and signs of heavy metal intoxication are multifarious because of the propensity for bonding with sulphydryl groups. Heavy metals thereby ubiquitously and severely affect enzyme systems and ligands of biochemical importance. These severe multisystem effects have overshadowed the possibility of toxic hearing loss until recent times. There is also the possibility that toxic agents may not penetrate the cochlea as readily as other tissues.

The growth in the use of solvents has accompanied the rise of the chemical industries in the twentieth century and is well documented (Haber, 1978). NIOSH has estimated that 100,000 workers in the United States have had some degree of toluene exposure and about 140,000 have potential xylene exposure (Zenz, 1994). In some industries almost every worker will have had exposure, a situation which may apply to noise exposure as well but to few other hazards. Specific studies of heavy metal ototoxicity followed the mid-century methylmercury disasters in Japan. Studies on lead ototoxicity followed as did reports of manganese and arsenic ototoxicity.

Although solvents had been suspected as ototoxicants from this time firm evidence beyond case reports came early last decade after experiments on rats (Pryor et al., 1983A and B). The investigation of the ototoxic potential of heavy metals and especially of solvents is beginning to

receive greater attention in the scientific literature and by national occupational health and safety administrations.

Investigation of the combined effects of noise and industrial ototoxic agents has been recommended by NIOSH in the United States. The NIOSH Proposed National Strategy for the Prevention of Noise-Induced Hearing Loss gives a research priority to investigations of the degree to which noise interacts with other agents in the workplace including, inter alia, solvents and metals (Franks and Morata, 1996). However, research interest is not restricted to the United States. Work is progressing in Scandinavia, France and other places.

A principal concern must be whether national regulatory frameworks for occupational and environmental chemical exposures are adequate for the protection of hearing. Early investigations of solvents focused on their neurotoxicity. Effects on hearing were not prominent, particularly in high dose acute exposures where central nervous system effects may be dramatic. It is in cases where exposures occur over longer time frames or where central nervous system toxicity is relatively mild that the issue of specific ototoxicity emerges.

Thus, it is plausible and, indeed, likely that exposure standards have not been set with the aim of preserving hearing. This would not matter much if other serious effects (for example, on the non-auditory central nervous system) always predominated over hearing loss at all dose levels and for all dose durations. However, evidence is gradually becoming available to test this point. Further, there is concern that exposures to solvents at otherwise non-ototoxic levels will damage hearing through additive or even synergistic interactions with workplace noise.

There is another reason why knowledge of the effects on hearing of chemicals alone or in combination with noise is important. It is that the mechanisms of damage to the cochlea and auditory pathways may elucidate the fundamental physiology, biochemistry and genetics of hearing.

That drugs could be ototoxic has been known for over one hundred years (Johnson, 1993) and with the introduction of aminoglycoside antibiotics in the 1940s came the pharmaceutical research needed to define their safe use. This has led to a better understanding of cochlear function and biochemistry (Ryback, 1986). Research on industrial chemicals too may contribute to this knowledge.

This project aims to review:

- the modes and sites of action of industrial ototoxic agents.
- the evidence for additive or synergistic interactions between ototoxic chemicals and noise, their possible metabolic basis, and their relevance to understanding of normal function of the auditory system.
- implications for safety practice and areas for future research.

METHODOLOGY

Many chemicals may be potentially ototoxic. In this study the scientific literature on industrial ototoxic agents meeting certain criteria is reviewed. The selection criteria are

1. Auditory pathway neurotoxicity: the agent may damage auditory pathway neurons.
2. Cochleotoxicity: the agent may damage hair cells, supporting cells, the stria vascularis or other cochlear cells or structure.
3. A sufficiency of studies is available for review.
4. There is actual or potential cause for concern at the level of workplace or environmental exposures.
5. There are studies of the putative ototoxic chemical and noise in combination.

The criteria are not rigid and are subjective to some degree. Some latitude is necessary because of the uncertainty which exists. Knowledge is growing so that some chemicals, which would not have met these criteria a few years ago, now do so.

Chemicals were included in this study if they met at least four of the five criteria. Those which did are trichloroethylene, toluene, styrene, xylene, carbon disulphide, lead, mercury and trimethyltin. A Table of Human Exposure limits setting out a sample of mandated and recommended exposure limits for these chemicals is given as an Appendix.

Some chemicals which did not meet four criteria are nevertheless discussed briefly in a section on miscellaneous agents. These agents mostly did not meet the third criterion but available studies are in included for completeness. Some of these agents are possible subjects for future research.

The selected agents fall naturally into two groups - the organic solvents and the heavy metals. Each agent is briefly introduced as to its chemical nature, industrial uses, metabolism and toxic potential. Human and animal studies are reviewed separately. Where appropriate, this is followed by a

summary and discussion taking the human and animal studies together. Studies reporting chemical exposures concurrently or sequentially with noise are not reported separately except, in the case of toluene, where it is justified by the volume of material available. Studies of mixed solvent exposures with or without noise are considered in a separate section. Finally, a synthesis is attempted in a summary and conclusions section. Summary tables are presented where necessary.

The approach taken is to examine the evidence for the agent acting alone and then, where possible, in combination with other chemical agents. In the case of some solvents there are studies of interactions with noise.

This study examines effects on hearing and not just on the ear. Thus the concept of ototoxicity as used needs to be clear. Strictly, ototoxicity refers to toxic damage to the sensory or secretory epithelia of the labyrinth and perhaps the auditory nerve - that is, to pathology within the temporal bone. Toxicity in the central auditory pathways is referred to as neurotoxicity. Toxicity affecting the VIIIth nerve may be referred to as neurotoxicity or cranial nerve toxicity.

However, ototoxicity is implicitly used by some authors to include any toxicity in the auditory system whether involving the cochlear sensory structures (hair cells, supporting cells, stria vascularis) or the neurons of the acoustic nerve and more central auditory pathways.

One reason for this is that there is a need for a term which will encompass all toxicity in the auditory system without implying restriction of damage to part of the system. However there is no such widely accepted term.

Secondly, some agents were known or suspected of causing hearing loss before the site or sites of action were known but were nevertheless designated as ototoxic. Often the site of action is still unknown, incompletely known, controversial or possibly different between animals and humans.

One example should suffice. In BAER studies without supporting psychoacoustic or pathological data, Wave I abnormality does not clearly distinguish cranial nerve toxicity from cochlear damage. Of course they may coexist. Several such BAER studies are reviewed.

The state of knowledge as to sites of action is in flux and was so during the period in which the scientific literature, reviewed here, was published. This means that it is not always practical and may be anachronistic to apply the definitions of ototoxicity and neurotoxicity strictly to describe agents whose status was or remains uncertain. However in this project the strict definitions of ototoxicity and neurotoxicity used as far as possible. One issue which remains is the definitional status of acoustic nerve toxicity and in particular of the afferent nerve terminals in the cochlea. The acoustic nerve courses through the temporal bone and its nerve endings are within the cochlea. Thus, where precision is required this overlap may be confusing. To avoid this problem the term, cochleotoxicity is used in this study where appropriate. It encompasses epithelial damage but not nerve ending damage within the cochlea.

The term, ototraumatic, is applied inclusively by some authors to all drugs, other chemicals and physical agents such as noise which may damage the ear or hearing. It is used sparingly in this study because it is at odds with the traditional medical definition of trauma which implies a wound. Also, it is insufficiently specific as to location of damage.

Interactions

This study examines interactions between (a) individual solvents and noise and (b) mixtures of solvents, in their effects on hearing,. Interactions are of different types and some confusion exists because of different terminology. Many terms such as augmentation, enhancement, multiplication etc. are used but are defined differently or not at all by authors. The nomenclature of interactions in this study is based on Berenbaum's (1989) definitions with the rubric of synergism being further divided as described by Nylén (1996).

Thus there are three classes of interactions; firstly, zero interaction in which the effect of a combination of agents is that expected from their dose-response relationship. In the literature on ototoxicity common synonyms for this are addition or additiveness. Secondly, antagonism is when the effect is less than expected from the dose-response relationship. Thirdly, synergism is when it is greater.

Synergism is divided into coalism, potentiation and co-synergism. Coalism is where the agents alone are without effect but together produce an effect greater than that seen in controls. Potentiation is where only one agent has an effect on its own but together a greater effect occurs than the dose-response relationship would suggest. Co-synergism is where each agent has an effect on its own but where the combined effect is greater than that expected from dose-response relationship.

It should be noted, however, that this division of synergism is not used by all authors (Humes, 1984). Further, it is possible for there to be different interactions to the same chemical with respect to effects on different (or even the same) systems in the organism (Nylén, 1996). That is, for example, synergism and antagonism of agents could coexist. This possibility would only be relevant in this study if different effects of combinations of agents occurred at different levels in the auditory system.

Methods of Testing

The Conditioned Avoidance Response (CAR) technique allows an animal to avoid an aversive electrical current by detecting the auditory (or other) test stimulus. A training procedure is required (Pryor et al., 1987).

The accuracy of behavioural audiometry in animals using CAR is limited by the animal's ability to move in a sound field during and between trials. However the technique reliably reflects auditory function and has proven useful in determining the effects of impairing agents. It should be remembered that these techniques are usually based on pure tones. They are relatively sensitive to cochlear pathology but because of increasing neural redundancy centrally they are progressively insensitive to retrocochlear pathology. A detailed review of CAR and other audiometric techniques for the assessment of ototoxicity in animals is available (Young and Fechter, 1983).

Brainstem evoked auditory response (BAER) techniques in animals use subcutaneous electrodes in rats which have been anaesthetised or restrained to reduce sound field variation (Rebert et al., 1983). It is also possible to use permanently implanted electrodes which make frequent testing more feasible although the mortality rate may be higher.

The main response parameters of value are the latency and amplitude of the peaks in the waveform. Wave I, III and V latencies are measured in milliseconds. Interpeak latencies I-V, III-V, I-III and sometimes I-II are used. Typical amplitude measurements recorded (in microvolts) are I, V and V/I. The exact origin of the wave potentials is uncertain (Prasher, 1982). The possibilities are the synapses in auditory pathway nuclei or the axons of the pathways. Wave I is thought to originate primarily in the auditory nerve, Wave II in the cochlear nucleus, wave III in the superior olivary complex, Wave IV in the pre-olivary and lateral lemniscal nuclei and Wave V in the inferior colliculus. Thus Wave I latency increase or amplitude decrease indicates cochlear or acoustic nerve pathology assuming stimulus and acquisition factors are constant. Wave III and Wave V latency increases or amplitude reductions imply caudal and rostral brainstem pathology respectively if earlier waves are normal. I-V interpeak latency increase is a most sensitive indicator of acoustic nerve or brainstem pathology. Isolated III-V interpeak latency delay implies intra-axial brainstem pathology.

The clearest waveforms allow the most accurate measurements of these parameters. However such waveforms are best generated by a short duration click causing a synchronous neural discharge. Such clicks have very limited frequency specificity.

Thus at a cost of waveform clarity more highly specific frequency responses can be obtained by the use of tone pips. Compared with pure tone audiometry BAER is more sensitive to brainstem neurotoxicity but less frequency specific as to cochlear pathology. This must be understood when interpreting the results of studies of noise and ototoxic chemicals.

Some experiments reported here have used recordings of the cochlear microphonic. This occurs simultaneously with basilar membrane movement (Prasher, 1982) and reflects intact outer hair cell function.

Finally, some studies have used the acoustic middle ear reflex (Borg, 1973) to localise dysfunction to the brainstem at the level of the cochlear nucleus and the superior olivary complex.

TRICHLOROETHYLENE

Introduction

Trichloroethylene is a volatile, chlorinated, unsaturated aliphatic hydrocarbon solvent with a variety of uses and potential human exposures (Crofton and Zhao, 1997). Trichloroethylene is used where solvent properties and non-inflammability are required. Examples of its use are degreasing in the metal industries, extraction of oils and fats, dry cleaning of clothes and organic synthesis. It is also used in industry as a solvent for chemicals and polymers. Mixed exposure to the solvent and noise is common. Exposure limits vary from 50-150ppm.

Acutely, trichloroethylene primarily has a narcotic effect. Chronic intoxication may produce hepatic or myocardial effects but the central nervous system is the main target organ. Confusion, drowsiness and euphoria can occur, the latter being the basis of abuse.

It is detoxified in the liver via conversion to chloral hydrate (an hypnotic agent) and trichloroethanol. This metabolic pathway interferes with that of ethyl alcohol which causes a synergistic effect between the two. World production has been as high as one million tons and NIOSH estimates that 3.5 million workers have some exposure to trichloroethylene and that 100,000 are exposed full-time (Ryback, 1992).

Only very limited epidemiological data concerning hearing effects are available in respect of human populations exposed to trichloroethylene. Studies in rats have revealed that trichloroethylene exposure leads to a mid-frequency hearing loss.

Human Studies

Ryback (1992) and Franks and Morata (1996) discussed a study in which 40 workers exposed to concentrations then above international recommended values underwent pure tone audiometry. Possible confounding effects of noise were avoided by excluding from study persons with previous

or current noise exposure history. Twenty-six of the 40 workers were found to have hearing losses which were sensorineural, bilateral and with a dip beginning from 2 or 3 kHz. Longer exposure was associated with greater loss.

Franks and Morata (1996) also cite data from the United States National Exposure Registry showing significant increase in reported hearing losses in children in the 0-9 years age range who had been exposed to trichloroethylene through contaminated water.

Animal Studies

Studies have been made on the effects of trichloroethylene in rats (Rebert et al., 1991; Jaspers et al., 1993; Crofton and Zhao, 1993). These have concerned the detection of BAER changes or modification of auditory evoked startle following a range of doses and time exposures.

Rats were exposed to concentrations of 1600ppm or 3200ppm, (12h/d, 13 w) (Rebert et al., 1991). All BAER amplitudes were depressed in the high dose group, more so for tone pips than for clicks. The latencies of components to clicks was unaffected in both groups but significant increases were noted to tone pips in the high dose group for Wave V latency and both I-V and III-V interpeak latencies. Additionally, tone pip tests were done across a range of frequencies and intensities. Amplitudes were reduced across the intensity range but the effects were confined to the high dose group and were largest for the 16kHz stimulus, which is a mid-range frequency in the rat (Müller, 1991). No histopathological examinations of the cochlea were reported. These results led the authors to suggest that threshold concentration for ototoxicity is around 2000ppm for 12h/d exposures.

Jaspers et al. (1993) also reported a mid-frequency hearing loss in rats. They used inhalation exposures of 0, 1500 or 3000ppm (18h/d, 5d/w, 3w). Raised hearing thresholds were again only demonstrated in their high dose group at the selected mid-range frequency of 20kHz.

Crofton and Zhao (1993) used concentrations of 1000, 2000 or 4000ppm (6h/d, 5d). They, too, found effects in the mid-frequency range (specifically at 8, 16 and 24 kHz) for their high dose group only. The time course pattern for the hearing loss was of a rapid onset and persisting essentially unchanged thereafter. The magnitude of the threshold shift at 16kHz was 40 to 45dB.

A more recent study (Crofton and Zhao, 1997) examined the adequacy of high-concentration, short term exposures to trichloroethylene for predicting effects of longer duration exposures. It was shown that the ototoxicity of trichloroethylene was not proportional to the product of concentration and time. That is, Haber's Law* (Zenz, 1994) does not predict the ototoxic effect. When extrapolating from short duration to long duration exposure data trichloroethylene ototoxicity is less than such a simple model would imply.

Summary and Further Discussion

Although Crofton and Zhao (1993) concede that further work is needed to determine the relationship between concentration, exposure duration and the upper and lower limits of hearing loss, the occurrence of an effect on hearing in rats is established. The value of the information obtained from high concentration experiments in rats lies in its contribution to understanding auditory function. Its relevance for human occupational exposures is uncertain. Also, human exposures are of a longer-term, lower concentration nature. At this time neither trichloroethylene ototoxicity nor auditory pathway neurotoxicity is confirmed in humans. There are neither animal nor human studies of any interactions with noise. BAER studies have established moderate auditory brainstem toxicity in rats exposed in high doses. Trichloroethylene-induced Wave I amplitude reduction and latency increase indicate that there is either acoustic nerve damage or cochlear damage or both. Further research is required to investigate cochleotoxicity. In particular, surface preparation and histopathological studies are needed.

* Haber's Law- The effect of a toxic gas exposure is proportional to the product of concentration and time of exposure.

TOLUENE

Toluene is benzene in which a single methyl group has been substituted for a hydrogen atom. It is used in industry both as a solvent and a raw material in the production of many other chemicals. It is in common use in the painting, paper milling and printing industries; and may be abused for its inebriative effects or in industrial plants as an unauthorised cleaning agent (Morata et al., 1997). World production is about five to ten million tons annually. Threshold limit values vary from 50-200ppm (Johnson, 1993).

Tissue uptake is via the lungs and skin. Metabolism is mainly via the cytochrome P-450 system with hydroxylation of the methyl group and oxidation to benzoic acid (Johnson, 1993; Zenz, 1994). Eighty per cent is conjugated with glycine to hippuric acid which, although non-specific to toluene, is the best marker of exposure and is measured in the urine. Twenty per cent is expired unmetabolised. It is predominantly neurotoxic but also has hepatic, renal, cardiac and hematological toxic effects. Possible ototoxic effects were first reported by Pryor et al. (1983A and B) in rats.

Human Studies

Information on the human effects of toluene has been obtained from case studies in occupational situations, from accident reports and from consequences seen in abusers. Concentration data and periods of exposure may be poorly known if at all. In both occupational and abuse studies actual exposure to mixtures of solvents or other factors including noise may make conclusions less certain.

A sample of 40 normal hearing rotogravure workers underwent electrophysiological and acoustic reflex testing (Abbate et al., 1993). Toluene exposures were known at a group level only. Workers were said to have been exposed to an average of 97ppm toluene over 12-14 years. A control group not exposed to toluene or noise was used.

The authors found that BAERs were significantly affected by toluene exposure. Interpeak latencies were significantly longer (I-III, I-V and III-V) indicating effects throughout the auditory brainstem.

However the most pronounced alterations were in respect of wave I indicating cochlear or acoustic nerve lesions in the presence of normal hearing to pure tones. Thus the technique was seen to have potential for early detection of toxicity.

An abuse study found that pathology was indicated both by BAER and MRI (Rosenberg et al., 1988). Typically the BAER showed loss of wave definition with marked prolongation of Wave III and V latencies and of III-V and I-V interpeak latencies. However normal Wave I latency was found indicating cochlear sparing or BAER insensitivity to mild ototoxicity. MRI showed cerebral atrophy and loss of grey/white differentiation. However not all abusers were shown to have pathological MRI lesions or abnormal BAERs.

One of the most detailed studies of the effects of organic solvents and noise was carried out by Morata et al. (1993) in Brazil. The 190 printers and paint manufacturing workers were assigned to either an unexposed group, a group exposed to noise only (88-97dBA), a group exposed to toluene vapour only (100-365ppm) or a noise and toluene group. The paint workers were assigned to a mixed solvent exposure group where plant ventilation had been poor and sampling showed exposures to toluene and xylene. However, the toluene concentrations were much lower than for the printing workers. Noise levels were below 85dBA.

Compared with the not-exposed group the noise only group had a central estimate of relative risk of 4, the noise and toluene group of 11, and the solvent only group of 5. It was concluded that an interaction between noise and toluene had occurred. However, there was no group available with a toluene only exposure and no firm statements could be made about interactions. Because of differences in solvent exposure levels the solvent only group cannot be compared with the noise and toluene group to draw conclusions about noise-toluene interactions.

However, the solvent only group relative risk is a cause of concern because it demonstrates the ototoxic potential of relatively low does of solvent in the absence of noise, a concern for regulatory attention. However, an alternative focus would be the poor ventilation. From an occupational health viewpoint a simpler and cheaper preventive action such as proper ventilation may be more likely to be carried out by managers.

The noise and toluene group had a significantly greater prevalence of reflex decay and the pattern suggested an intraaxial brainstem site of lesion. This finding does not exclude a cochlear damage component. Cary et al. (1997) point out that it is plausible that retrocochlear damage could reduce the effectiveness of the acoustic reflex and hence expose the cochlea to greater noise energy. The non-availability of a toluene alone group was the critical difficulty in this study.

The evidence for toluene induced hearing loss in humans was strengthened in a study (Morata et al., 1997) which was able to assess both total noise and total toluene exposures on an individual basis in 124 Brazilian printing workers. The separate individual observations were able to produce a stronger data set than had been possible in previous studies.

An odds ratio estimate for hearing loss of 1.76 was obtained for each increment of one gram of urinary hippuric acid per gram of creatinine in urine. By contrast, the odds estimate for age was 1.07 per annum. The odds ratio for noise was 1.00. The authors point out that workers in the study had a relatively short noise exposure time. However the 60% of workers in the study with estimated exposures at hazardous levels (taken as >85dBA) had mean exposures of about seven years and this result is a little surprising and may be attributable to overly strict classification rules of hearing loss at the extreme of normality. No statistical interactions between noise and toluene were found.

The authors question the appropriateness of current exposure standards for toluene given that hearing loss risk is significant even at exposure levels below those standards.

The study successfully addressed some of the difficulties in epidemiological studies of solvents and noise. Efforts were made to obtain good estimates of noise exposure (with dosimetry) and toluene (metabolic rather than air concentration data) and to do so individually with each worker.

The results of the human studies together support the following statements:

- Toluene does cause hearing loss in sufficiently exposed humans.
- The interactive status between noise and toluene in humans remains uncertain.
- The site or sites of damage in humans are not clear but there is evidence for both cochleotoxicity and central neurotoxicity.
- Toluene may be dangerous to hearing at or below accepted solvent exposure standards.

The difficulties of controlling for variables in human toxicity studies are very great. Nevertheless where there are possibilities for ethical studies the opportunity to make further advances in knowledge should be taken. Individual exposure estimates which are accurate for the period of hearing loss risk are important. Although much is still uncertain the studies so far have generated important data on which to found changes to standards and better enforcement in many countries.

Animal Studies

A long series of animal experiments, mostly in rats, has been carried out. Several reviews have been published in recent years (Ryback, 1992; Morata et al., 1995; Johnson and Nylén, 1995). The effects of toluene alone or with other chemical agents or with noise have been studied. Thus the ototoxic effects of toluene, in concert or alone are the best understood of any of the organic solvents.

Many of the early studies were carried out by Pryor and his colleagues (Pryor et al., 1983A and B; Rebert et al., 1983; Pryor et al., 1984A and B; Pryor and Howd, 1986; Pryor et al., 1991). They first reported rat ototoxicity in studies of weanling rats (Pryor et al., 1983A and B). Many further experiments have since been carried out by them and others. The different factors involved in these

experiments included variations in animals and animal strains, concentration, daily hours of exposure, periods of exposure, methods of exposure; as well as combinations with other solvents, combinations with noise and with acetylsalyclic acid and post-exposure testing protocols. Effects have been studied using CAR, BAER, electrocochleography, tone discrimination, otoacoustic emissions and microscopy.

Commonly, experimental designs included dosing by inhalation. The equipment for this necessarily produces noise. The level of noise was usually but not always measured. Often, efforts were made to reduce it to levels not expected to cause any possible potentiation of solvent ototoxicity. In at least one experiment (Pryor and Howd, 1986) noise was excluded by the use of injected solvent. The main results of these experiments are presented and discussed below.

In a BAER study (1200 or 1400ppm, 14h/d, 4 or 5w) auditory thresholds were raised by 13-27dB (Rebert et al., 1983). Wave I was significantly delayed consistent with cochlear and/or acoustic nerve damage confirming an associated behavioural study (Pryor et al., 1983A) reported simultaneously.

The permanence of the effect and its frequency specificity between 8 and 20kHz was established (Pryor et al., 1983B). A greater effect at 20kHz was found in weanling rats than in young adult rats on a particular regime (1200ppm, 14h/d, 7d/w, 5w) (Pryor et al., 1984A). Scanning electron microscopy showed patchy unquantified IHC and OHC stereocilial loss, fusion or disorientation in the basal turn of the cochlea.

In the next experiments (Pryor et al., 1984B) the conditions under which ototoxicity occurs were further defined. Extended exposure times at concentrations below 1000ppm or four-hour exposures at 4000ppm were not ototoxic. However concentrations of 2000 to 4000ppm were ototoxic after a few days. Thus the authors were able to speculate that if these data were applicable to humans the

OSHA TWA of 200ppm should not cause hearing loss in exposed workers. However, abuse especially in young persons or accidental large occupational exposures would be a different matter.

Laboratory equipment noise was excluded as a possible confounding factor when ototoxic effects over a similar frequency range were found in rats after subcutaneous injections were used rather than an inhalation system (Pryor and Howd, 1986). Ethyl alcohol in drinking water was found to potentiate toluene induced hearing loss in rats (Pryor et al., 1985). It had no effect alone.

Specific evidence for the cochleotoxicity of toluene was the demonstration of hair cell loss by phase-contrast microscopy (Sullivan et al., 1989). Cytocochleograms showed gross losses in the middle turn of the rat cochlea, worst in the outermost row and least in the innermost row of OHC. Inner hair cells were spared. This OHC damage corresponded to 2-8kHz where, in this experiment, the greatest losses to BAER were also evident. The difference from the earlier experiments could have been due to exposure route, strain of rat or noise level differences.

Since 1990 the investigators have turned increasingly to genetic, biochemical and structural matters underlying toluene ototoxicity.

Pryor et al. (1991) concluded that toluene induced hearing loss is not caused by an intermediate metabolite formed during detoxification. If it were not so this could have indicated a common biochemical basis for ototoxicity for other aromatic hydrocarbons and trichloroethylene which have related and overlapping detoxification pathways. The experimenters induced detoxication pathway activity with phenobarbital and found this reduced toluene levels and protected rats from toluene ototoxicity. They interpreted their results as indicating that ototoxicity was due to toluene itself although other possibilities were considered.

Li et al.(1992) showed that in inbred mice strains with early or late onset hereditary hearing loss, respectively, toluene was able to aggravate loss only in the former group as the animals aged although the youthful sensitivity to toluene in rats (Pryor et al., 1984A) also applied to mice.

To elucidate the nature of ototoxic effects at the cell level Johnson (1992) examined the combined effects on rats of inhaled toluene and water-dissolved acetylsalicylic acid (aspirin). The aspirin potentiated the toluene induced loss. Attention was directed to the possibility that both agents may have an effect on hair cell membranes. The actual mechanism remains speculative, however, but the possibility is raised of human ototoxic effects of combined toluene and aspirin.

Toluene ototoxicity was described with further precision by Johnson and Canlon (1994A and B). BAER and DPOAE were found to be progressively affected in a parallel fashion. A mid-frequency maximal loss were found even if thresholds were elevated at all measured frequencies. DPOAE amplitude decline was most marked in the mid-frequency region. The mid-frequency hearing loss was correlated to histopathological changes of progressive outer hair cell loss in the middle and upper turns of the rat cochlea which increased over the time of the exposure and which confirmed the row effect described by Sullivan et al. (1989). Six weeks post-exposure there was evidence of post-exposure progression apically and basally and with some loss of inner hair cells.
Again, the different results of a predominantly high frequency loss found by Pryor et al. (1983A and B) were noted but not explained. These results showed a site of lesion similar to that found for trichloroethylene ototoxicity (Crofton and Zhao, 1993).

Recently, Campo et al. (1997) expressed the view that the Pryor series of studies did not point to a classical high-frequency loss. They noted the relative lack of ototoxic damage in the hook region of the rat cochlea compared with extensive middle turn pathology and because frequencies higher than 20kHz were not tested. In a meticulous and detailed electrophysiological study they confirmed previous findings of a mid-frequency loss but observed for the first time a bimodal distribution of

this loss with a peak at 4kHz and a smaller peak at 20kHz detectable in each of the differently affected rows of outer hair cells. Furthermore it was noted that Deiter's cells were affected and there was loss of neural fibres of the intraganglionic bundle, probably efferent fibres. There was some BAER insensitivity to this pathology. This could be related to distribution differences between responding nerve fibres in damaged and undamaged animals although other interpretations are possible. Thus, caution is required in interpreting the failure of BAER thresholds to rise after exposure to the lower dose range of ototoxicants as indicating lack of cochlear pathology. Furthermore, but not discussed in their paper, apparent failure of lower doses to cause ototoxicity in rats based only on electrophysiological studies (without histopathological correlation) is an uncertain foundation for discussions of safety limits in human occupational exposures.

Toluene and Noise

A number of animal studies have focused on the effects of combined exposures to toluene and noise (Johnson et al., 1988 and 1990; Johnson, 1993; Lataye and Campo, 1997).

Johnson et al. (1988) exposed rats to toluene (1000ppm, 16h/d, 5d/w, 2w), noise (100dB L_{eq}, 10h/d, 7d/w,4w) or to toluene followed by noise (T+N). Effects on tone pip BAER (1.6, 3.15, 6.3, 12.5 and 20kHz) showed significant broad band threshold elevation especially at 12.5kHz (40dB) for toluene alone with some recovery over one month. Slight, temporary brainstem toxicity was evident (Wave latencies). For noise alone, there were significant losses only at 6.3, 12.5 and 20kHz (maximum at 12.5kHz-50dB, 20kHz-45dB) In the (T+N) group the threshold increase was greater than the summated effects of toluene alone and noise alone (as far as could be observed) with little recovery at six months.

Johnson et al. (1990) found that reversing the order of exposure (noise followed by toluene, N+T) led to threshold elevations less than the summated effects of noise alone and toluene alone.

This phenomenon is the reverse of that seen in sequential aminoglycoside and noise exposures in the chinchilla (Ryan and Bone, 1982) and Marques et al. (1975). Another difference between aminoglycoside and toluene (and other solvent) ototoxicity is that the hearing loss in the latter is mid-frequency rather than high frequency. As Johnson (1993) points out this implies that a special mechanism is involved.

The authors speculated that toluene causes structural damage which decreases hair cell membrane or stereocilial resistance to ensuing mechanical stress. However, they believed that their observation of sequential differences was essentially unexplained. Lataye and Campo (1997) studied the combined simultaneous effects on rats of toluene (2000ppm, 6h/d, 5d/w, 4w) and noise (8kHz OBN, 92dB SPL). Over the test range (2-32kHz) BAER thresholds were elevated in excess of the summated elevations caused by toluene alone and noise alone. Threshold elevations were maximal at 16kHz for toluene alone and 10 or 12kHz for noise alone and the summated frequency patterns corresponded roughly to the patterns obtained separately.

A cochleogram study after toluene exposure alone showed similarities to the bimodal distribution observed for trichloroethylene (Campo et al., 1997) with similar row effects also, damage decreasing from outer hair cell row 3 to row 1 and inner hair cell sparing. In the combined exposure the bimodal effect was less obvious in the context of greater combined damage.

There was good correlation between the histological and the electrophysiological results. Histologically, it was evident that the toluene exposure alone was associated with outer hair cell loss and phalangeal scarring. The noise alone exposure was associated with stereocilial fusion with row effects in the opposite direction to toluene alone.

The authors interpreted these data to mean that the damage caused by combined exposures to toluene and noise is produced by the same mechanisms (poisoning and mechanical , respectively) as in

separate exposures. In other words, there does not appear to be, from the histological evidence, any different mechanism in action with the combined exposures.

However, it should be noted this observation does not preclude the possibility that some parts of the metabolic pathways associated with noise and with toluene damage are common to each other.

STYRENE AND XYLENE

Besides toluene two other aromatic hydrocarbons have been investigated for ototoxicity. Xylene, a toluene analogue, carries a second methyl group in one of three possible positions giving three isomers. Any of these may be toxic (Zenz, 1994) and, industrially, a mixture of isomers is usual. Styrene is another toluene analogue with an extra methyl group double-bonded to the first.

Xylene is in widespread use in many industries. It is used as paint thinner, in mastics, in pharmaceutical preparations, in dyes and as an aviation fuel additive.

The double bond in the vinyl group of styrene is associated with reactive potential - useful for polymerisation but also related to toxicity. It is widely used in the production of polyester resins and transparent plastics. "Spray on" uses are encountered in the manufacture of boats, swimming pools and containers.

Both substances are well known neurotoxins (Zenz, 1994). Like trichloroethylene the biodegradation of styrene is inhibited by ethanol but also by toluene. This could be of occupational significance.

The range of human and animal studies of ototoxicity has, so far, been limited for these compounds.

Human Studies

Muijser et al. (1988) used Bekesy audiometry up to 16kHz to assess 59 workers who produced glass-fibre reinforced plastics products. Styrene and noise levels in the factory were measured. The noise levels varied with location within the factory and across exposure groups including controls. So although the frequencies tested were higher than those usually considered to be damaged by noise, there can not be complete confidence that different noise exposure patterns did not affect the results.

It was found on multivariate analysis that at 8kHz there was a significantly higher threshold elevation in the more heavily exposed workers. (Mean styrene exposure 32±1SD 18ppm, maximum 85ppm). The study is suggestive of a high frequency styrene effect in human workers but is not conclusive.

Möller et al. (1990) examined workers from a plastic boat plant with between six and fifteen years service. Although there were some higher peak exposures, generally exposures had been below the Swedish limit (25ppm). Conventional pure tone audiometry did not indicate any special differences which could have been due to styrene. However, it may be noted that in a volunteer study (Larsby et al., 1982) test subjects to styrene did show significant changes in saccade tests and visual suppression of nystagmus.

A larger study of nearly 300 workers was carried out in the fibre-reinforced plastics manufacturing industry (Sass-Kortsak et al., 1995). This was unable to confirm the suggestion of a specific styrene associated loss at 8kHz although the styrene exposures were somewhat lower than in the Muijser study. The Sass-Kortsak study was able also to be much more rigorous in controlling for noise exposure. They were able to measure noise exposure for each subject and to estimate lifetime noise exposure.

There are no reported epidemiological findings in humans of a specific xylene ototoxic effect whether in conjunction with noise exposure or not.

Animal Studies

Yano et al. (1992) reported on the electrophysiological and morphological effects on rats of styrene vapour (800ppm, 14 h/d, 5 d/w, 3w). Tone-pip generated waveforms of decreased amplitude and increasing latency were found upwards from 4kHz. The latter was minimally affected, 8kHz showed moderate effects with severe effects occurring at 16 and 30kHz. These results are similar to those

obtained with toluene (Pryor et al., 1984A; Sullivan et al., 1989). But they were in contrast with the data of Pryor et al. (1987) which (using younger rats) showed sensitivity decrements at all frequencies tested.

The morphological findings generally confirmed the electrophysiological results. There was significant outer hair cell loss with patchy inner hair cell loss in the lower middle regions of the cochlea and, especially in the lower basal turn corresponding to 8-16kHz. There was no information or speculation concerning the mechanism of the ototoxicity.

Fechter (1993) demonstrated the effects on guinea pigs of (a) a single injection of styrene, (b) a single seven-hour inhalation of 500ppm with or without noise exposure and (c) similar exposures of 500 or 1200ppm with or without noise but with a one week recovery time. Noise groups received 95dBA white noise for the seven hour period. Effects were determined by electrocochleographic measures of threshold, compound action potential and amplitude of the cochlear microphonic.

No ototoxic effect of styrene by injection or by inhalation alone was demonstrable. Styrene with noise and noise alone did raise CAP threshold. One week post exposure there was a small CAP threshold elevation in the 500ppm styrene plus noise group but not for the 1200ppm styrene plus noise group. The cochlear microphonic one week post exposure was affected in all treatment groups. But the effect with styrene plus noise was not greater than the noise alone effect. Overall these experiments on the guinea pig failed to demonstrate an acute effect of styrene alone or a significant potentiation of noise damage by styrene. However, the number of animals was small and the exposure periods were brief although the concentration was well over the TLV.

The effects of xylene on the auditory system have been studied electrophysiologically in experiments involving separate and combined (with n-hexane) organic solvent exposures. There have been differences in the results possibly attributable to the different strains of rats used. Pryor et al. (1987)

found a 20-25dB hearing loss at 12.5kHz while Nylén and Hagman (1994) found only a maximum 7dB loss at this frequency.

Taking the human and animal studies together several statements can be supported.

- Styrene can produce a variable mid-frequency hearing loss (12.5kHz) in rats.
- Styrene vapour is capable of causing cochleotoxic effects in rats under short-duration exposure conditions at concentrations much higher than accepted TLV's. Higher frequencies are affected than for xylene. The principal effect in the cochlea is on the outer hair cells with relative sparing of the inner hair cells.
- Guinea pigs seem relatively more resistant than rats to styrene induced ototoxicity. Styrene may not potentiate a noise-alone effect in guinea pigs.
- More research is required to see if there is an additive or synergistic effect of styrene in any experimental animal.
- The human studies are affected by the difficulty of knowing and controlling exposures both of noise and solvent both in the short and long term.
- For levels at or below the range of current TLV's no styrene ototoxicity has been convincingly demonstrated in humans although there is one flawed study suggesting ototoxicity at 8kHz.
- Xylene can probably cause a mid-frequency hearing loss in rats. The site of action has not been determined.

CARBON DISULPHIDE

Carbon disulphide is used as a raw product for organic synthesis, especially viscose rayon, and as a solvent in vulcanising rubber, degreasing, and oil extraction. It is toxic to the central nervous system and to cranial and peripheral nerves as well as to the heart, liver and kidneys. High dose acute exposures (200 to 500ppm) can be fatal. For exposure standards see the Appendix.

Investigation of auditory pathway neurotoxicity and ototoxicity have been carried out in viscose rayon plants. There have been BAER and behavioural audiometry investigations in rats but no cochlear histopathological studies.

Human Studies

In a study of current and former workers from a viscose rayon filament manufacturing facility in Japan (Hirata et al., 1992B) seventy-four male subjects aged between 40 and 55 years without renal or neurological disease, diabetes mellitus or a history of high tobacco or alcohol consumption were placed in one of three groups. The first was a long exposure currently working (>20 years) group (L), the second a short exposure currently working group (2-7 years) (S), and a group of former workers with exposure duration of more than 10 years which ceased at least 7 years before the study (R). There was also a non-exposed control group (N).

The average time-weighted carbon disulphide concentration in the factory at the time of the study was 4.8 ppm although it may have been higher in years prior to the commencement of the study. In the L group there were significant increases Wave V latency and in III-V interpeak latencies compared with controls. Other differences were not of statistical significance.

III - V interpeak latency increases are indicative of central brainstem effects. (Møller and Jannetta, 1986). (Hirata et al., 1992B) believed that the III-V IPL increases in the long-exposed (L) group of current spinning workers suggest that current exposure of less than 5 ppm and past exposure at

higher levels may have caused brainstem dysfunction. Further, since the short-exposure group showed no significant III-V latency differences they believed that the current exposure level to carbon disulphide did not detectably affect brainstem function. The role of exposure time as distinct from dose was not discussed as a factor capable of explaining these results.

However, the absence of significant effects in the previously employed group exposed for a long period to unknown higher concentrations suggested a recovery from the carbon disulphide effect after cessation of exposure.

The authors did not carry out pure tone audiometry or present any noise data in respect of the exposed workers or the controls. As sensory (i.e. cochlear) hearing loss whether or not due to noise can effect BAER, for example, by causing both Wave I and Wave V delay this omission may confound the authors suggestion of brainstem dysfunction.

Morata (1989) studied the effects of simultaneous exposure to noise and carbon disulphide on male workers in a viscose rayon factory in Brazil. Pure tone audiometry was carried out and the results classified by severity, age and exposure duration. Workers thought to have a hearing loss not related to their occupation were separately classified. No workers were included who had exposure to noise or carbon disulphide prior to their employment in the rayon plant.

Pure tone audiometry showed two thirds of the workers tested had a hearing loss. The prevalence of hearing loss was higher than expected for age in all groups. As prevalence of hearing loss even in the youngest group (18-29 years) was greater than 50% presbycusis was excluded as a principal cause. In addition, it was observed that a high proportion of losses involved the frequencies 500-2000 Hz in addition to a high frequency loss. This is not typical of uncomplicated noise induced hearing loss.

The association between hearing loss and exposure time to noise and carbon disulphide was found to be statistically significant and could be observed after a simultaneous exposure time as little as three years. As simultaneous exposure duration grew so did prevalence and severity of loss.

The level of noise exposure was given as 86-89dBA but no variations over time were reported. Working hours were 48 per week in weekly rotating shifts. Atmospheric carbon disulphide concentration was measured at 30ppm, well above the range of international recommendations of 7-20ppm.

No control group was used but some comparisons were able to be made with unpublished material. The demographic and occupational parameters were comparable except that there was no carbon disulphide exposure. However, noise exposure levels were not published. The proportion of hearing losses involving the range 500-2000 Hz was greater (12.7%) in the simultaneously exposed group than in the noise alone group (3.5%). The overall prevalence of hearing loss was greater (60.1%) in the simultaneous exposure group than in the noise alone group (53.1%).

Morata (1989) discussed earlier work in which 259 viscose industry workers had been exposed to as much as 15 times the now current OSHA standard. There was a significantly greater incidence of pure tone hearing losses in the exposed group compared with non-exposed worker controls. Each group had been subjected to the same level of noise.

These human epidemiological studies are limited in the following ways:

- No correlation between pure tone audiometric studies and brainstem evoked potentials is available. The studies examined one or the other.
- Because of study limitations no firm, epidemiologically reliable conclusions can be drawn as to the effects of carbon disulphide and noise in simultaneous exposures compared to solvent exposure only.

- No correlations between audiometric or electrophysiological findings and pathological changes are available.

Nevertheless the following useful information has been obtained.

- Brainstem effects may be reversible.
- Hearing loss associated with simultaneous exposure to carbon disulphide and noise may produce a more severe loss than noise alone at least in some people.

The data are sufficient to support Morata (1989) who pointed out the need to ensure that work environments were modified so as to reduce exposure to both agents at their source or to reduce the duration of exposures.

Animal Studies

Rebert and Becker (1986) studied the effects of carbon disulphide on female Long-Evans rats using concentrations of 400 and 800ppm (7h/d, 7d/w). Brainstem auditory evoked response testing was carried out firstly, before the carbon disulphide treatments, then at the end of the first, fourth, seventh and eleventh treatment weeks and finally, at the end of the first and third weeks of a recovery phase during which there was no carbon disulphide exposure.

The auditory stimuli used were conventional clicks as well as 16kHz tone pips at 10dB intensity level steps between 25dB and 95dB. Throughout the experiment there was an evident trend in the 800ppm group toward increasing latency in the acoustic nerve (Wave I) response but the difference observed was not statistically significant. Nearly half of this latency increase was reversed during the recovery phase.

Wave V latency here was dramatically different, increasing from 3.17 msec to 3.58 msec at the end of the treatment period. Because more of the latency increase attached to Wave V than to Wave I the

change represents, in large part, brainstem tract dysfunction. In I-V interpeak latency terms the high dose group was significantly different from controls and this effect was maximal after seven weeks exposure and was largely maintained thereafter. The low dose group had slightly increased latencies throughout the dosing period but these reverted to near normal in the recovery period.

Further examination showed that the increased I-V IPL was entirely due to an increased III-V IPL throughout the experiment - thus locating the retrocochlear effects to the brainstem nuclei and tracts. It was also observed that in the 800ppm group the amplitude of the BAER was reduced - at least during the treatment phase.

Tone pip stimuli at 16kHz were used to establish a rough estimate of hearing threshold. Mean hearing thresholds were 57dB for the 800ppm group. The 400ppm group and the controls were not significantly different from each other but were significantly lower, at 40dB, than the 800ppm group. The authors believe that, despite the absence of a statistically significant difference between the 400ppm group and the controls, there appeared to be a relationship between hearing threshold and carbon disulphide dose.

Latency-intensity functions for Wave I and Wave V were compared. The findings were that for Wave I there was significant difference between the three groups in the 55 to 75dB range with, overall, slightly different slopes. By contrast the Wave V latency-intensity functions were parallel with a substantial latency increase in the 800ppm group and a moderate effect in the 400ppm group. In another study Clerici and Fechter (1991) used acoustic startle reflex diminution as a marker of increased hearing thresholds in rats exposed to 500ppm of carbon disulphide six hours per day, five days per week for 5 or 12 weeks. The basis of the techniques involved are discussed by Young and Fechter (1983).

The dose (500ppm) and periods involved were roughly comparable to the 400ppm group in the experiments of Rebert and Becker (1986). They found auditory thresholds at 10 and 40kHz relatively unaffected by either the 5 or 12 week dosing schedule. However neuromuscular integrity was severely compromised and it is clear that hearing loss cannot be used as an early marker of carbon disulphide toxicity.

In another BAER study (Hirata et al., 1992A) rats were exposed (800ppm or 200ppm - the latter being a lower dose than used in earlier experiments) and allowed a six week recovery period rather than just three weeks. Results at 800pm were similar to those of Rebert and Becker (1986). However by using more rats they were able to detect significant delays of BAER latencies in the 200ppm group. They also observed complete recovery of IPL III-V (the central part of the brainstem) over the six weeks but not of IPL I-III (the peripheral part of the brainstem).

Rebert et al. (1986) also noted disruption of cortical evoked potentials and Ferrarro et al. (1979) found a possible pathological correlate in neuronal degeneration in the temporal cortex of cats exposed to carbon disulphide.

The results of animal experiments concerning the ototoxicity of carbon disulphide can now be summarised. Rats exposed to carbon disulphide develop dose-dependent disruptions of Wave I and Wave V components of the BAER. The most remarkable changes were increased III-V IPLs and decreased response amplitudes. This suggests that the primary effect is located from the superior olivary complex to the inferior colliculus. It is not known why this part of the brainstem is particularly sensitive to carbon disulphide but Rebert and Becker (1986) suggest that there may be regional metabolic differences within the brainstem which is thought to have a high metabolic rate.

Hirata et al. (1992A) drew the opposite conclusion as to the site of the greatest brainstem damage. They concluded that the recovery over six weeks of IPL III-V but not of IPL I-III meant that the

peripheral section of the brainstem ascending auditory pathway is more severely damaged than the central section. The vulnerability of the peripheral section they attribute to its greater length than the distance from the superior olivary complex to the inferior colliculus. Rebert and Becker (1986) did not observe the recovery of IPL III-V but their last observation was only three weeks after the cessation of exposure compared with six for Hirata et al. (1992A).

The experiments also point to peripheral auditory dysfunction. Factors suggesting this include the possible effect of carbon disulphide on Wave I latency and the overall decline in waveform amplitude. The location of the peripheral effect may be conductive as indicated by the Wave V dose-response curves being parallel. Thus, there is a need for a histological study of the entire auditory system in exposed rats as well as immittance audiometry including stapedial reflexes.

Further Discussion

A comparison of the human and animal studies is useful. In the rat studies the dose regimen was two orders of magnitude higher than that experienced by factory workers. However, Rebert and Becker (1986) say that rats appear to be less sensitive to carbon disulphide than people. In addition, the time course of the dosage was much longer in humans.

The stability of auditory thresholds in the investigations of Clerici and Fechter (1991) may reflect sparing of cochlear function on the one hand and increasing redundancy of the ascending central innervation on the other, which may render pure tone tests relatively insensitive to progressive central deterioration.

However if there is co-incident cochlear damage such as that caused by noise exposure there may be a potentiated effect on overall auditory function. This may explain why humans experience hearing loss at much lower doses of carbon disulphide and why a hearing loss can appear early in the course of a combined exposure. It may also be the reason for early middle frequency losses not typical of

noise exposure alone. If so, the apparent relative insensitivity of rats to carbon disulphide may be explained by their exposure only to a single noxious agent.

The questions which remain open are:

(a) do human exposures to noise and carbon disulphide need to be simultaneous to have a maximal potentiating effect on hearing loss, that is are they inter-dependent or completely independent effects?

(b) what are the relative effects of concurrent and sequential exposures to noise and carbon disulphide in rats?

It may be that carbon disulphide ototoxicity is only detectable as a pure tone audiometric decrement when associated with a cochlear loss due to noise or other factors.

MIXED SOLVENTS

The study of animal exposures to single solvents has been useful to the understanding of auditory function. To a lesser extent, because exposure levels are not controlled, the same is true of human exposures. In occupational settings exposures may occur to multiple solvents and noise either simultaneously or sequentially. The level of these exposures may fluctuate.

Ototraumatic agents may interact when given simultaneously, having cumulative effects on hearing by affecting the same or different auditory system sites. A knowledge of individual effects of agents may not be sufficient to predict mixed exposure outcomes. A knowledge of the outcomes is necessary to assist understanding of auditory function generally and to contribute to workplace hazard assessment. A summary of human and animal mixed solvent interaction studies appears as Table I in this section.

Human Studies

Barregård and Axelsson (1984) reported cases where four shipyard workers who had been exposed to noise as well as organic solvents had a greater sensorineural hearing loss than would have been expected from exposure to noise alone. With low numbers and high expected variability little can be made of this by itself but the paper did provoke further research. They also reported that in a group of 32 painters with mixed solvent and noise exposure there was no clustering of inexplicably pronounced sensorineural hearing losses. Although there were no definite findings the authors speculated that a retrocochlear neurotoxic effect might be found in conjunction with cochlear damage caused by noise or solvents or both.

Bergström and Nyström (1986) found a greater degree of hearing loss in the solvent-exposed workers in a paper mill in its chemical department where noise levels were lower than elsewhere in the plant.

Ödkvist et al. (1982A) studied 11 workers in the painting, printing or petrol transport businesses who had exposures to aromatic or aliphatic hydrocarbons and found prolonged latencies in CERA and interrupted speech discrimination. However, BAERs were normal. A more detailed later study (Ödkvist at al., 1987) supported these findings. A dose-effect relationship was also found.

Similar findings were made in the majority of nine workers with psycho-organic syndrome (on the basis of case history, neurophysiological and psychological testing) due to 8-30 years of solvent exposure (Möller et al., 1989). However the workers in this study and those of Ödkvist et al. (1982A, 1989) did not show hearing losses to pure tone audiometry attributable to causes other than age or noise exposure.

However all these studies were of small numbers of workers and not necessarily sensitive to solvent-induced cochleotoxicity sought to be detected by means of pure tone audiometry against a background of unquantified noise exposure. The auditory cortex, however, does appear to be vulnerable to permanent solvent damage on the basis of the CERA and speech test results.

Another small numbers study (Taniuchi et al., 1992) had the advantages that the solvent mixture could be specified and that a control group and hearing norms were available. Although styrene was the only known toxic agent the combination with other agents may have been significant. One third of the workers in the noise and solvent-exposed group (n = 42 ears) had hearing losses above the 90^{th} percentile (noise only group 12% - n = 33 ears, control group 9% - n = 23 ears).

Johnson and Nylén (1995) discuss unpublished data showing no consistent relationship between cumulative noise or styrene exposure and audiometric thresholds. Thus the study results of Taniuchi et al. (1992) remain to be confirmed.

Morata et al. (1993) studied a group of printers where the nature and concentration of the solvents was known in some detail. The hearing in four groups of workers was classified as normal, conductive, unilateral or on a four-point severity scale of high frequency sensorineural loss. The groups were of exposure to noise only, noise and toluene, solvent mixture (including toluene) but not noise and a not exposed group. Adjusted relative risk estimates for hearing loss were 4 times for the noise group, 5 times for the solvent mixture group and 11 times for the noise and toluene group. This suggests an interaction between noise and toluene - discussed further in the section on toluene. The relationship was not evident in the study of Jacobsen et al. (1993) perhaps because the solvent profile was different or less precise (or indeed unknown) and because of the poorer content quality of the impairment data. An additional finding was a significantly higher prevalence of stapedial reflex decay in the noise plus toluene group, especially contralaterally, indicating possible brainstem toxicity. This was not seen in the mixed solvent group but this group appears to have been exposed to much lower doses of solvents than the noise and toluene group. Further, it has been pointed out (Cary et al., 1997) that this fact undermines any deduction that the risk factor difference (11 versus 5) is itself indicative of interaction between noise and toluene.

The Copenhagen male study of 3284 men used a questionnaire methodology (Jacobsen et al. 1993). Respondents self-identified their solvent exposure and the agent(s) was not identified across the group. Nor was hearing impairment measured but rather, self-assessed. Adjusted relative risks for hearing impairment after at least five years solvent exposure was 1.4 without noise-exposure, compared with 1.9 for noise alone or for noise and solvent exposure. Thus a solvent effect on self-assessed hearing was found but no additional solvent effect was evident in the noise-exposed.

Animal Studies

Animal studies may allow a more precise examination of possible additive or synergistic effects on hearing of controlled exposures to different solvents and noise. The non-auditory effects of combined noise and solvents have been studied (Kurnayeva et al., 1986) but animal hearing

experiments with combined solvents and noise have been restricted to single rather than mixed solvents.

Rats exposed (1000ppm each solvent, 22h/d, 28d) to toluene and n-hexane had decreased auditory sensitivity (2d, 3 months, and 1 year post-exposure) detected by BAER of 30-45dB at 2 days post-exposure with no improvement over time. This compared with a 25dB decrease in sensitivity at 2 days with slight improvement thereafter with toluene alone and 5dB at 2 days with reversion to normal thereafter with n-hexane alone. The combined exposure was interpreted as at least additive in its effect (Nylén et al., 1987).

Pryor and Rebert (1992), using higher doses (1200ppm toluene, 4000ppm hexane), also found the greatest decrease in sensitivity by BAER to be in the mixed exposure group. They raised the possibility of the final result arising from different components of the mixture having separate actions at different sites in the auditory system.

Rebert et al. (1993) looked for interactive effects in the rat between styrene and trichloroethylene by BAER. Additive effects only were found. Similarly Rebert et al. (1995) found additive effects only in rats for the following combinations - toluene and trichloroethylene, mixed xylenes and trichloroethylene, xylenes and chlorobenzene, and toluene and chlorobenzene. Finally, Nylén and Hagman (1994) found the interaction between xylene and n-hexane to have synergistic effects on auditory sensitivity as detected by BAER at the peripheral, presumably cochlear level, and antagonistic effects in the auditory pathway reducing IPL prolongation. Nylén (1996) suggested that the basis for the antagonism may be decreased concentration of the toxic n-hexane metabolite 2,5-hexanedione which has been implicated as the cause of neurofilament aggregation at the nodes of Ranvier - a known cause of reduced nerve conduction velocity. Toluene was suggested to have a similar effect on the concentration of this metabolite and that the basis of n-hexane-toluene synergism may be the same as n-hexane-xylene synergism.

Summary and Further Discussion

Various human occupational studies suggest that at levels prevailing in some workplaces over a sufficiently long period, a single solvent alone or mixed solvents can be associated with hearing loss. Although this is best documented for toluene the limitations of the studies do not allow the exculpation of other agents. In combination with noise, toluene exposure increases the probability of hearing loss although it is not yet sure whether this effect operates in occupational settings for other solvents or solvent mixtures. The (hopefully) increasing difficulty of finding and documenting combined solvent and/or noise exposures may prevent or delay more definitive findings.

Complex interactive effects (co-existing synergism and antagonism) have been found in rats for n-hexane in combination with xylene or toluene. The same mechanism has been hypothesised for each chemical.

On the other hand only additive mechanisms have been found for other chemical combinations tested. This implies that these agents act through the same or similar mechanisms (Rebert et al., 1995).

It should be noted that there are many difficulties in undertaking solvent interaction studies. These include experimental design generally, variation in dose-response relationships of agents and the need for assumptions to be made where data are scanty. There is variability of effect between solvents from one experiment to another the reasons for which are not always clear. In human studies the exact nature of the exposures(s) may not be known, nor the dose, duration, absorption, blood or tissue concentrations or histopathology.

The human and animal studies taken together have implications for occupational health and safety. The animal experiments on solvent mixtures have been conducted at higher doses than would be

expected to prevail in occupational settings. However, they usually show additive effects and may have synergistic effects. It can usually be anticipated that agents capable of cochleotoxicity will have additive effects at least. The difficulties, limitations and small numbers of animal experiments mean that synergistic effects have only been established in a few cases. However, they may not be uncommon. Three other factors should caution against complacency. Firstly, an agent which is not ototoxic in animals may be ototoxic in humans. Secondly, human exposures are long and not necessarily always at doses far below those applicable in animal experiments. Carelessness and accidents occur. Thirdly, human solvent exposures are often concurrent with long term noise exposure. (The combined effects of individual solvents and noise are discussed elsewhere it this study). Animal experiments with solvent mixtures and noise together are not yet reported although this will surely change. There are sufficient human data to show that mixed solvents and noise produce at least an additive effect. This implies a common or related metabolic pathway of damage (Rebert et al., 1995) although this is not supported by the experiments of Lataye and Campo (1997) considered in the section on toluene and noise.

There is a need for research which will determine whether and to what extent commonality of damage pathways exists at both structural and biochemical levels (Morata, 1998).

Table I

Reports of Combined Exposures to Ototoxic or Potentiating Agents

Animal/Human	Agents		Tests	Results	Reference
Human	Aliphatic and/or aromatic solvents		PTA SRT ART BAER CERA et al.	Central effects, brainstem spared	Ödkvist et al., 1982A
Human	Jet fuel and aliphatic and aromatic solvents		PTA BAER CERA ART SRT et al.	Possible solvent effects above brainstem level	Ödkvist et al., 1987
Human	Alcohol, aromatic and aliphatic industrial solvents		PTA PB-max CERA et al.	Possible auditory cortical pathology	Möller et al., 1989
Human	Mixture of toluene, xylene, methylethyl ketone and methyl and isobutyl ketone		PTA ART	Significant elevated risk ratios for hearing loss	Morata et al., 1993
Human	Self-reported, various and not specified		Self-Reports	No definite additive effect to noise damage Possible solvent ototoxicity	Jacobsen et al., 1993
Rat	Toluene	n-Hexane	BAER	Additive effect, at least	Nylén et al., 1987
Rat	Toluene	n-Hexane	BAER CAR	Possible potentiation by Hexane	Pryor and Rebert, 1992
Rat	Toluene	n-Hexane	BAER	Potentiation by hexane	Nylén et al., 1994
Rat	Toluene	Acetylsalicylic Acid	BAER	Aspirin potentiates toluene ototoxicity	Johnson, 1992

Animal/Human	Agents	Tests	Results	Reference
Rat	Styrene and Trichloroethylene	BAER	Additive not synergistic effects on hearing	Rebert et al., 1993
Rat	Trichloroethylene with toluene Mixed xylenes with trichloroethylene Xylenes with chlorobenzene Chlorobenzene with toluene	BAER	Additive, not synergistic effects on hearing	Rebert et al., 1995

LEAD

Introduction

Lead is a heavy metal which has been in use for over 5000 years. Toxic symptoms were first attributed to it by Hippocrates about 2500 years ago. In the early modern era the notable contributions to understanding were made by Ramazzini and Tanquerel des Planches (Zenz, 1994). Exposures can occur in mining, the user industries and in polluted environments. Industrial applications include the manufacture of car batteries, sheet metal, pipes and foil. There is a declining use in paints, enamels and glazes. Hazard arises from inhalation of dust or fumes or motor vehicle emissions. Organic lead compounds can be absorbed through the skin. The user industries are often noisy but no studies of combined effects with noise have been reported. Clinical features of lead toxicity are multifarious. Neurotoxicity is evident in the development of peripheral neuropathy and of encephalopathy. Clinical features become evident when blood levels exceed 0.08mg/100ml. This no longer common (Zenz, 1994). The elucidation of effects on hearing has been centered on attempts to detect neurotoxic effects by electrophysiological studies of the auditory brainstem. In animals, evidence of cochleotoxicity has been sought through electrocochleography.

Human Studies

Of the many studies of lead effects on workers and children only a few have been concerned with hearing.

A BAER study of thirty adults and children exposed to accidentally contaminated food for one to two years (Holdstein et al.,1986) found significant Wave 1 and Wave III latency increases in the adults and Wave III and Wave V increases in the children. Interpeak latency increases (I-III and I-V only) were significant only in the children. Stimulation was by 75dBHL clicks at 10/sec. When a rate of 55/sec was used there were significant interpeak latency increases (I-III and I-V) in adults only. It is not clear in this study that the results in separate ears were treated as non-independent observations as should be the case.

The result in which most confidence could be placed was the I-III interpeak latency delay. Thus the VIIIth nerve and/or the cochlear nucleus are suspected sites of damage. The authors speculated that the higher click rate may be uncovering synaptic damage with or without myelin impairment (in the adults) whereas the interpeak latency results in children may be more compatible with myelin impairment alone.

Workers subject to various occupational exposures to lead were shown (Discalzi et al., 1992) to have significant latency and interpeak latency increases (I, III, V, I-III, I-V and III-V) as compared with controls. Although there are well recognised problems with blood lead as an exposure measure the I-V interpeak latency was significantly increased in workers with a three year blood level average of greater than 0.05mg/100ml than in those below this level. Thus the results suggest brainstem and/or auditory nerve and/or cochlear effects. There was also a possible dose-response effect. Pure tone audiometry was normal in the group. However, this may only indicate the insensitivity of this technique to auditory neuropathology.

In the late 1970's a large scale health and nutrition survey was carried out in the United States (Schwartz and Otto, 1987). A small but significant relationship was found between blood lead concentration and audiometric threshold elevations at 0.5, 1, 2 and 4kHz. Although a causal association was not proved a need for periodic audiometric examination for children or workers exposed to lead was suggested, although neither sensitivity nor specificity issues were considered.

Animal Studies

There are three guinea pig studies of lead acetate effects on the cochlear microphonic, the endocochlear potential and the compound action potential (Yamamura et al., 1984, 1987 and 1989).

In the 1984 study thresholds of maximum voltage in the compound action potential were raised 15dB after a dose of 100mg of lead acetate spread over five weeks. No cochlear microphonic changes were observed leading to the conclusion that the known peripheral nerve effects of lead could be duplicated in the acoustic nerve and that no outer hair cell damage was apparent.

The implied resistance of outer hair cell function to toxic degradation by lead was confirmed in the 1987 experiments. However the pathological changes observed by Gozdik-Zolnierkiewicz and Moszynski (1969) of VIIIth nerve axonal degeneration and mild segmental demyelination were taken as consistent with the electrophysiological findings.

The 1989 study replicated earlier findings and observed no changes in endocochlear potential implying that no stria vascularis damage was detectable.

There is no histopathological study of the guinea pig cochlea after lead exposure to confirm the resistance to outer hair cell or other damage.

Summary

A large epidemiological study has suggested a relationship between blood lead levels and pure tone audiometric threshold elevation. Electrophysiological studies in animals and humans indicate that lead has neurotoxic effects on the VIIIth nerve and possibly on the auditory brainstem. The former has been confirmed histopathologically. Cochlear microphonic and endocochlear potential stability in acute lead intoxication in guinea pigs indicates resistance to toxic damage by the outer hair cells and the stria vascularis. There are no studies of the combined effects of lead and noise exposure.

MERCURY

Introduction

Mercury ore was used in prehistoric times (for wall painting) and the metallic form was known to early Egyptian cultures. Toxic effects were known in the ancient mining industry and in 1665 it was the first substance to be the subject of occupational disease control legislation. The toxicity of organic forms has been known since the nineteenth century. World production varies up to 150,000 tons per year (Schutte et al., 1994).

Mercury poisoning today is mainly by intoxication from alkylmercury salts, principally methylmercury. Inorganic mercury is methylated in the environment by microorganisms. Methylmercury is more lipid soluble, more readily absorbed from the gut (90% versus 10%) than the inorganic salts, and more readily crosses the blood-brain barrier (Gilman et al., 1980).

Recent advances in knowledge have arisen less from its occupational use than from disposal disasters caused when it enters the food chain and poisons humans who have eaten contaminated fish. The focus on environmental rather than occupational studies may also explain why there have been no investigations of the interaction of mercury and occupational noise on the auditory system.

Because of the dramatic non-auditory clinical features and mortality of severe mercury poisoning the associated hearing loss was not always regarded as an important feature. The careful studies after the mid-century disasters in Japan changed this perception.

The study of mercury as an ototoxic agent is still driven by environmental concerns but it is now recognised that the metal and its organic compounds may be useful in elucidating the physiology of the cochlea and auditory pathways.

Human Studies

Large toxic exposures to mercury are not characteristic of occupational settings in which mercury or its alkyl compounds are in use. Rather most such exposures have resulted from environmental releases due to accident or neglect.

Thus much of what is known of the effects on human hearing is derived from studies following environmental disasters. Chronic occupational exposure may be expected to have different, probably milder effects on hearing than acute poisoning. There may be no ototoxic effect at all (Miller, 1985).

In Japan, there have been two environmental methylmercury intoxication epidemics. The first was in Minamata on Kyushu between 1953 and 1960 and the second in the Aganogawa river region near Niigata on Honshu between 1965 and 1970. (Mizukoshi et al., 1989; Ryback, 1992 and Igata, 1993.) Neurotologic studies of these disasters have been carried out. (Mizukoshi et al., 1975 and Mizukoshi et al., 1989)

In Minamata, methylmercury intoxication usually presented with loss of feeling in the limbs followed by ataxia, dizziness, loss of hearing, speech disturbances and visual difficulties.

Mizukoshi et al. (1975) investigated 144 persons diagnosed with Minamata disease in the Aganogawa River region. A weighted average of pure tone hearing loss over 0.5, 1 and 2kHz was determined. Typically, the audiometric configuration was flat and affected all frequencies.

Fifty-three percent of ears were found to have a loss greater than 30dB and 17% had a loss greater than 90dB. Bekesy audiometry was carried out but only on 34 cases. The basis for selecting these was not stated. However 26 (76%) had type III or type IV patterns indicating retrocochlear loss. Abnormal recruitment as shown by the SISI or ABLB tests was absent in all but 32 of the 144 cases who were said to have inner ear disorders. The authors do not comment on any basis for cochlear disorder.

Two further observations suggest to the authors that the hearing impairment from methylmercury intoxication is mainly retrocochlear. Firstly, only a few cases with high SISI scores (4 out of 36) were suspected as having retrocochlear lesions from the results of speech discrimination or Bekesy tests and, secondly, all patients with low speech discrimination scores had Bekesy tests indicating retrocochlear loss.

Actually, the first observation only suggests that patients with cochlear disorders did not have detectable retrocochlear lesions which is not surprising as the latter would be better detected (if present) on the tests used (Bekesy and speech discrimination) if the cochlea was intact. The second observation only reflects an expected correlation between a retrocochlear speech discrimination pattern and a retrocochlear Bekesy pattern.

What does support a retrocochlear location for methylmercury intoxication is the high prevalence of Type III Bekesy tracings in the 34 cases so tested. However many of the ears with hearing loss were not subjected to tests which would help distinguish between cochlear and retrocochlear losses. Thus, although the study supports a retrocochlear site of pathology it enables no conclusion as to whether there can be intercurrent cochlear damage from methylmercury.

In 1989 Mizukoshi et al., reported on neurotological follow-up on 35 of the Aganogawa River patients. Sixteen of the fifty-eight ears (28%) tested showed a deterioration over the average of 13 years since the early post-exposure test. Four ears (7%) showed improvement. The possibility of ageing or other non-toxic factors in contributing to this deterioration was not discussed. Other neurotological tests found no significant further deteriorations except that the prevalence of spontaneous nystagmus increased. No further site of lesion data were presented in respect of the hearing losses.

In 1971 in Iraq mercury-treated grain was accidentally used to make bread. Amin-Zaki et al. (1978) reported the effects. The range of estimated concentrations of mercury in the blood at a common post-exposure time in children at least mildly symptomatic was 7-35 micromol/litre. Partial or total hearing loss occurred in all but the mildly affected and asymptomatic children. No quantitative or site of lesion data were reported.

Two studies on workers (Discalzi et al., 1993 and Lille et al., 1988) have sought to detect effects of mercury on brainstem auditory evoked potentials. In each case exposure levels had produced average urinary mercury concentrations in the range of 170-195 micromol/mol creatinine. This level is high enough to impair psychomotor performance (Roels et al., 1982).

Lille et al. (1988) found normal brainstem auditory evoked potentials at these levels and Discalzi et al. (1993) showed no significant differences although the latter study was limited, the authors admitted, by the low numbers of subjects. No examination was made of other possible effects on hearing.

Human pathological data have been discussed by Mizukoshi et al. (1975). In a fatal heavily intoxicated case mercurial granules were found deposited in the transverse temporal gyri and other locations and it is argued that the retrocochlear site of lesion revealed by the Bekesy audiometry and other tests here finds its pathological correlation. As for the possibility of cochleotoxic lesions they point to their findings of recruitment in some patients but they were unable to establish any pathological correlation because of cochlear autolysis in their autopsy cases.

Autopsy studies of patients acutely poisoned with methylmercury reported by Gerstner and Huff (1977) showed no evidence of cochlear damage.

Animal Studies

The effects of mercury and mercury compounds on the cochlea and auditory pathways have been studied in rats, guinea pigs and seals. Techniques have included electrocochleography (Hotta et al., 1997), cochlear uptake studies (Konishi and Hamrick, 1979), modification of the acoustic startle reflex (Wu et al., 1985), the surface preparation technique (Ramprashad and Ronald, 1977 and Falk et al., 1973) and other morphological methods (Anniko and Sarkady, 1978).

Surface preparations (Falk et al., 1973) showed that methylmercury caused a low level of outer hair cell pathology in all four turns of the guinea pig cochlea at doses of 2mg/kg of body weight five times per week. Inner hair cells and supporting cells were normal. Maximum outer hair cell damage occurred in the outer row at 3½ turns from the base and decreased toward the base. Middle row damage was minimal at 1½ turns and greater both apically and basally. The inner row was the least damaged especially at the apex and base. Notably, about half the animals appeared to suffer little or no damage.

A study of six harp seals fed with 0.25 or 25mg/kg of body weight of methylmercury showed a general low level of outer hair cell damage throughout the cochlea similar to that seen in the guinea pig (Ramprashad and Ronald, 1977).

The nature of the hair cell changes seen were similar for the seal and the guinea pig. As in the guinea pig the changes in seals were most marked in the outer row of outer hair cells. They were also seen to be dose related. The surface preparation changes seen in both species included stereocilial disarray
cuticular plate bulging, cellular necrosis and scarring.
Sectional and surface preparation studies were carried out on the guinea pig (Anniko and Sarkady, 1978). Acute intoxication (7.5-25 mg/kg body weight) with early death caused little hair cell degeneration compared with chronic intoxication. Chronic intoxication (2.5 - 5.0 mg/kg body

weight) produced outer hair cell loss in all but the basal coil. The inner row of outer hair cells was relatively spared.

The first ultrastructural change seen was transformation of the internal structure of mitochondria in degenerating cells. In chronic intoxication the cristae lost their sharp contours and the whole mitochondrion appeared to shrink. Acute intoxication caused loss of cristae and early mitochondrial swelling with rupture and herniation. Later there was vesicular degeneration indicating a non-specific common path of cell disintegration.

Both afferent and efferent nerve terminals in the cochlea were affected. Degenerating myelinated nerve terminals were seen next to normal ones and in chronic intoxication the associated hair cell often appeared normal at least in the early stages. Again mitochondria were more greatly affected than other structures such as transmitter vesicles. Evidence of demyelination was also apparent. Spiral ganglion cells were subject to scattered losses in chronic intoxication and more generalised losses in acute intoxication.

Although the stria vascularis was usually morphologically intact, marginal cell vacuolisation sometimes occurred following either acute or chronic poisoning. Mitochondrial changes were similar to those seen in hair cells and intercellular oedema of the intermediate cell layer was sometimes observed.

Falk et al. (1974) referred to a slow continuous increase in blood mercury levels during chronic intoxication. If there is a delayed capacity of cochlear cells to eliminate toxic substances as suggested by Anniko and Plantin (1977) this factor may lead to prolonged increases in intracellular mercury concentrations within the cochlea and help to explain why hair cell losses in chronic intoxication are greater than in acute intoxication.

Mercury may cause a change in mitochondrial membrane permeability. Ion transport mechanisms and oxidative phosphorylation and DNA synthesis may be affected. Hearing may be affected because of metabolic changes before structural change is apparent (Anniko and Sarkady, 1978).

The changes caused by mercury intoxication vary with dose, dose timing and the subject animal. There is variation in damage within the cochlea, between animals and possibly between species.

Cochlear effects of mercury have also been examined electrophysiologically in guinea pig (Hotta et al., 1997, Konishi and Hamrick, 1979). The cochlear microphonic, the compound action potential and the endocochlear potential were recorded in comparison with blood levels of mercury. At a dose level lower than that required to produce clinical signs of mercury poisoning the cochlear microphonic was reduced in response to both high and low tone stimuli, most markedly for sound levels above 70dBSPL. The compound action potential voltage was also reduced and its latency increased. No significant reduction of endocochlear potential was found indicating that the ion pump of the stria vascularis remained intact. These results are consistent with what is known from pathological studies of the propensity for mercury to damage the cochlea.

Konishi and Hamrick (1979) also obtained pharmacokinetic data on the uptake and elimination of ^{203}Hg-labelled methylmercury in the cochlear fluids. Generally, the concentration of mercury peaks in the cochlear fluids within one to two days of dosage. There is then a rapid fall in concentration followed by further slower falls. Endolymph clearance is slower than perilymph clearance but the difference declines with dose. Methylmercury in extravascular spaces remains methylated. However intracellular mercury concentrations are higher than the extracellular. Thus the extracellular pharmacodynamics is not the whole picture. This may be why cochlear microphonic and compound action potential suppression continue after the cochlear fluid concentrations fall.

BAER testing has been used to study changes in physiologic thresholds in mice after methylmercury dosing at 4 and 8mg/kg body weight for 17 and 6 consecutive days respectively (Wassick and Yonovitz, 1985). Physiologic thresholds to tone burst were raised at all frequencies with the greater effect on the 4mg group. Interpeak latencies decreased for both groups for most frequencies. The authors interpreted this as an induced hypersensitivity in nerve tissue. The basis for this may be an impairment of slow conducting, small diameter fibres more sensitive to mercury toxicity. This would cause most of the transmission to be coming from fast conducting, large diameter fibers.

Summary and Comparison of Human and Animal Studies (Table II)

Human studies derive from environmental accidents where dose is high but uncertain, although blood levels may be known. Otherwise knowledge has been restricted to low dose studies of exposed industrial workers. Animal studies have used high doses. There are some common features.

Mercury poisoning causes a hearing loss in humans of a generally flat configuration but sometimes worse in the high tones. The same is true of animals. The extent of the loss is dose and time related but other unknown factors may cause some of the variability seen.

There have also been variations in site of lesion data not all necessarily attributable to dose differences. Generally mercury causes permanent cochlear loss in animals primarily by damaging outer hair cells and nerve terminals but direct evidence of cochleotoxicity in humans is limited. Mitochondrial damage is common to most affected sites indicating interference with energy production.

Mercury is also neurotoxic, affecting the auditory pathways, causing demyelination, but with some suggestion of relative sparing of larger diameter fibers. The damage may be partly reversible in the early stages. Electrophysiological and pathological studies indicate damage is possible at all levels in the auditory pathway.

Table II

Summary of Effects of Acute and Chronic Mercury Intoxication on the Inner Ear and Auditory Pathways

Structure	Early Effects	Later Effects
Inner Hair Cells	Usually spared	Usually spared-dose dependent
Outer Hair Cells		
• Severity • Row effects	Low Level of damage	Higher level of damage with chronic exposure
• Base to Apex Effects	All turns affected with some row variation and inter-animal differences	
• Surface Preparation	Stereocilial Disarray	Cuticular plate bulging cellular necrosis, scarring
• Ultrastructure	Mitochondrial damage	Various
• Variability of effect	Notable	Notable
Supporting cells	Unaffected	Unaffected
Nerve terminals at IHC		
• Severity	Variable	Variable
• Ultrastructure	Predominantly mitochondrial effects	
• Timing	Damage tends to be evident earlier than in outer hair cells	
Stria Vascularis	Effects usually mild and late Marginal cell vacuolisation Intercellullar oedema of intermediate cell layer Mitochondrial changes similar to those in hair cells and nerve terminals	
Spiral ganglion	Scattered cell loss more generalised in acute than chronic poisoning	
Myelinated nerves	Degeneration	

TRIMETHYLTIN

Introduction

Trimethyltin, one of the alkyltins, is toxic to the visual and auditory systems. It is associated with central nervous system pathology and has effects on restricted areas of the brainstem (Chang et al., 1983). Therefore it has been much studied as a model for clarifying the basic mechanisms of neurotoxicity. There is evidence for specific effects on neuronal cell bodies (Chang and Dyer, 1983; Fechter and Liu, 1995). Chemically, it acts as a potent uncoupler of oxidative metabolism (Fechter and Carlisle, 1990).

Alkyltins are widely used in the manufacture of plastics, polyurethane foam and biocidal compounds (Young and Fechter, 1986). Trimethyltin may be encountered as a potential contaminant in marine anti-fouling paints and in polyvinylchloride products. There is potential for occupational exposure to coincide with noise exposure. However there has been very little investigation of its ototoxic potential in humans. This contrasts with an interesting series of animal experiments which have now revealed more about its ototoxicity than is known for other metals or organometals.

Human Studies

Trimethyltin has been studied in humans after multiple severe intoxications of chemical workers (Rey et al., 1984). Exposures of allegedly protected workers for as much as 10 minutes per day for three days to a mixture of di- and trimethyltin produced (after a one to three day latency period) generalised neurotoxic symptoms. Tinnitus and loss of hearing were also noted.

There have not been studies of lower dose, longer duration exposures either with or without concurrent noise exposure. Epidemiological studies should be a future research priority.

Animal Studies

In the first morphological study of cochlear effects Chang and Dyer (1986) found widespread

sensorineural damage in rats which were exposed for periods of up to thirty days to doses of 6mg/kg body weight.

Organ of Corti damage was evident after 24 hours as swelling of the outer hair cells followed by segments of total hair cell loss after 15-30 days. Early (24 hours) spiral ganglion damage was evident as cell body vacuolisation subsiding by day three when increased nuclear chromatin condensation became patchily visible. The position(s) of the damaged segments in the cochlea was not reported.

These findings were extended by Young and Fechter (1986) showing frequency-specific dose dependent auditory impairment after only a single injection of trimethyltin at 2 or 4 or 6mg/kg body weight. The effects were partly reversible and significantly greater for higher doses and for a high frequency tone (40kHz) compared with a middle frequency tone (10kHz). No morphological data were presented concerning the pattern or the position of the loss.

The authors doubted whether the disruption of oxidative phosphorylation could be the basis of the ototoxic effect. Firstly, this was because other organometals such as methylmercury also disrupt oxidative phosphorylation and yet produce different damage patterns. Secondly, trimethyltin is 30 to 40 times more effective in uncoupling oxidative phosphorylation but it is not a correspondingly more potent ototoxic agent. However, they did speculate that differences in bioavailability attributable to differential penetration of the perilymph could be a confounding factor.

The evidence for a cochlear site of injury was reviewed by Fechter et al. (1986). They noted the similarity with the time course of noise-induced hearing loss - that is, the very persistent but partially reversible elevation of thresholds at higher frequencies (40kHz) and possibly complete reversibility in the low to middle frequencies. However the time course of such recovery as occurred (3-5 weeks

post exposure) was later than that from noise yet quite different from the very transient (e.g. loop diuretic) or permanent (e.g. aminoglycoside) effects of cochleotoxic drugs.

They went on to conduct an electrocochleography study in rats exposed to 5mg/kg body weight which showed all-frequency elevation of CAP thresholds especially apparent at higher frequencies. This was consistent with the significant decrements in cochlear microphonic found above 24kHz. Thus this study confirmed evidence for a cochlear basis of ototoxicity without excluding the possibility of more central auditory pathway damage. The cochlear microphonic decrement at higher frequencies suggested predominant outer hair cell damage to the basal cochlea and the CAP data are consistent with hair cell or spiral ganglion damage or both. Partial recovery of function may reflect restoration of mitochondrial metabolism and if so may suggest that the base-to-apex loss gradient reflects a base-to-apex metabolic gradient in the cochlea.

Crofton et al. (1990) were able to correlate electrophysiological changes with changes seen in cochlear surface preparations in rats exposed to 3, 5 or 7 mg/kg body weight. They confirmed a dose-related preferentially high tone (basal cochlea) effect electrophysiologically and morphologically. Cytocochleograms showed a much more prominent effect on outer hair cells than inner hair cells but no differential row susceptibility was evident for the outer hair cells.

Fechter and Carlisle (1990) broadened knowledge of the cochlear effects by studying, inter alia, effects on the stria vascularis in the guinea pig. Earlier, Eastman et al. (1987) had shown that factors related to pigmentation, including the presence of cochlear melanin do not have a quantitatively important effect on the magnitude or frequency dependence of trimethyltin induced hearing loss.

If trimethyltin toxicity is mediated by uncoupling mitochondrial oxidative phosphorylation, then effects may be sought in the stria vascularis which has an extremely high level of metabolic activity (Thalmann et al., 1975). Fechter and Carlisle (1990) were the first to report an effect there. They

found increases in blood vessel diameter more marked in the middle and apical regions, decreases in strial width, radiating arteriole density and collecting venule density. There was a progression of pathological change (worst at the midpoint of the cochlear duct). In the least affected areas there was basement membrane thickening around capillaries. More severely affected areas showed degeneration of the marginal cells and detachment from the spiral ligament.

Strial pathology was not directly related to corresponding hair cell loss. The worst affected areas for each were different. It was noted that vasodilatation was generally greatest in the areas where hair cells were least affected. This may mean that strial vasodilatation was protective. Further study of the relationship between strial and hair cell injury may be valuable in understanding the chemical and metabolic basis of trimethyltin ototoxicity.

Further studies in guinea pigs were carried out by Clerici et al. (1991), Fechter et al. (1992), and Liu and Fechter (1995). In the first mentioned study very short term effects (less than 30 minutes and 30-60 minutes) followed injection of 2mg/kg body weight. Parallel studies of triethyltin were also carried out. In this time frame results for both showed a marked rapid effect on CAP but with sparing of the cochlear microphonic. This indicates that the most sensitive cells to early toxic effects are not the outer hair cells. The early effect must be on the spiral ganglion cells or the inner hair cells. However, as histopathological studies above have noted, the inner hair cells are relatively spared in the longer term and the spiral ganglion cells are not.

Fechter et al. (1992) examined the 6-48 hour period after injection of 2mg/kg body weight to find both CAP and the cochlear microphonic affected. The effect was broad in the frequency spectrum at first (6hrs) but later was restricted to the higher frequencies suggesting recovery apically. Endocochlear potential was not observed to be disrupted in the 6-24 hour period.

The first morphological changes, detectable at 12 hours, were supranuclear vacuolisation, disruption of polyribosome clusters into free ribosomes, swelling of the rough endoplasmic reticulum and dark, punctate mitochondrial inclusions in the outer hair cells. The mitochondria themselves were distributed throughout the cytoplasm rather than being sequestered peripherally.

At 24 hours there was swelling of the basal region Type I spiral ganglion cells, with myelin sheath separation from the cell bodies. Type II cells appeared normal. Polyribosomal and rough endoplasmic reticulum disruption was evident in the Type I cells and outer hair cells. Thus disruption of neuronal and hair cell protein synthesis may be relevant in the causation of ototoxicity.

The lateness of spiral ganglion damage in the rat (4 weeks) (Hoeffding and Fechter, 1991) is contrasted with the early effects in the guinea pig. This difference is similar to that reported by Spoendlin (1975) for noise-induced injury for these animals.

Trimethyltin has been shown to produce 15% outer hair cell shortening in length *in vitro* (Clerici et al, 1993). Shortening was unrelated to concentration. Cell membrane disruption was also noted and as it was unrelated to volume or length changes the basis of membrane rupture was thought to be a weakening of the plasma membrane or the cortical lattice.

Recently, attention has turned to the effects of trimethyltin on intracellular calcium concentrations in both spiral ganglion cells and outer hair cells (Fechter and Liu, 1995; Liu and Fechter, 1996). *In vitro* experiments varied the level of extracellular Ca^{2+} or applied the Ca^{2+} channel blocker, nifedipine. Trimethyltin caused a sustained, and rapid but eventually saturating rise in $[Ca^{2+}]$ in spiral ganglion cells which was initially blockable by nifedipine. An attenuated rise was found in a calcium-free medium.

A smaller rise in intracellular [Ca^{2+}] was found for outer hair cells and its time course was much less rapid. However for outer hair cells a reduction of [Ca^{2+}]in the artificial perilymph medium or the blocking of Ca^{2+} channels with nifedipine did not affect the rise in intracellular [Ca^{2+}].

These results suggest that the trimethyltin induced increase in intracellular [Ca^{2+}] is mediated by disruption and release of intracellular stores in each of these cells and, additionally in the case of spiral ganglion cells, by enhancing influx of extracellular calcium. They also show direct spiral ganglion cell effects independent of the presynaptic inner hair cell. The nifedipine effects suggest Ca^{2+} channels are involved in trimethyltin toxicity.

The precise biochemical mechanism of the elevation of intracellular calcium remains uncertain. However, possibilities include:

- mitochondrial energy production inhibition impairing Ca^{2+} sequestration
- inhibition of Ca^{2+}-ATPase resulting in depressed function of plasma membrane Ca^{2+} pumps; and/or
- direct trimethyltin action to increase production of free radicals which may increase [Ca^{2+}] via membrane or other effects.

Other experiments (Fechter and Liu, 1995) showed glutamate application having similar effects to those of trimethyltin. Thus the biochemistry of glutamate as the excitatory neurotransmitter between the inner hair cell and the spiral ganglion cell is likely to be relevant to the mechanism of trimethyltin neurotoxicity.

Summary

Trimethyltin is cochleotoxic at far lower dose levels than those which produce central nervous system effects. (Fechter and Liu, 1995; Clerici et al., 1993). The dose-response and locational effects have been elucidated by an excellent series of experiments over fifteen years.

Trimethyltin produces a mainly high tone hearing loss because of damage in the basal coil to hair cells and spiral ganglion cells. Inner hair cells, more apical outer hair cells and the stria vascularis are generally less affected and are more likely to be able to recover.

The focus is now on the biochemical basis for the toxicity and a number of mechanisms have been proposed. However the precise cause and effect relationships at the neurotransmitter, cell membrane and biochemical levels are still the subject of investigation.

There is a need for epidemiological studies to determine whether there are low dose effects on human hearing.

MISCELLANEOUS AGENTS

There have been investigations of the ototoxicity or neurotoxicity of some substances which do not satisfy the criteria for inclusion in this study. However, for completeness and because some substances have potential for further research some information on them is presented.

Hexane and Heptane

Evidence accumulating from the 1960's shows that hexanes and other hexacarbons can cause a sensorineural peripheral neuropathy with giant axonal changes secondary to aggregation of neurofilamentous masses (Zenz, 1994). Brainstem neurotoxicity in humans is evidenced by BAER studies of long term exposed workers (Chang, 1987; Huang and Chu, 1989) showing increased I-V IPL and Wave V latency prolongation.

In rats a similar pattern of brainstem neurotoxicity was found (Rebert et al., 1982; Pryor et al., 1983A) with only slight effects on Wave I latency at doses of 1000ppm (24h/d, 5d/w, 11 w). Much higher doses were required to actually reduce Wave I amplitude. There are no cochlear histopathological studies available but there is little electrophysiological evidence of cochleotoxicity at lower doses.

Heptane has been shown (Simonsen and Lund, 1995) to reduce the amplitude of Wave I and Wave V in BAER experiments in rats at 4000ppm (6h/d, 28d) but not at 800ppm. However there were no IPL or wave latency prolongations in either dose group. There were no cochlear histopathological examinations.

These studies show that aliphatic hydrocarbons can cause auditory brainstem neurotoxicity. Previously, Rebert et al. (1991) had related the ototoxicity of organic solvents to their unsaturated structure. The relationship now seems to be more complicated. Cochleotoxicity seems not to occur or at least occurs only with high doses of these saturated hydrocarbons. This is in contrast to the

cochleotoxicity of unsaturated substances such as trichloroethylene or toluene. How or whether saturation is important in protection of cochlear function may be a useful line of research in the future.

n-Butanol

Franks and Morata (1996) discussed hearing loss in eleven workers exposed to this subtonic at levels of 80ppm. After excluding age and dose duration effects this group who had worked in noise levels of 75dBA, were found to have greater hearing loss than workers not exposed to n-butanol who worked in noise at 90-110dBA. More research is needed on this substance.

1,3-Dinitrobenzene

This widely used industrial chemical has been used in experiments to link the level of brainstem metabolic activity with its vulnerability to toxic damage (Ray et al., 1992). Glucose utilisation is reduced in the ipsilateral cochlear nucleus and the contralateral inferior colliculus after the tympanic membrane is ruptured. This effect can be prevented by exposure to white noise. The rupture substantially reduces the severity of the vasculo-necrotic lesions usually seen in these centres after dosing with 1,3-dinitrobenzene unless it is accompanied by additional white noise. The experiment implies that the toxin's effectiveness depends on the level of neural activity at the time of exposure. The sidedness of the effects is consistent with the anatomy of the auditory pathways.

No human studies of the ototoxicity of this agent are available.

Butyl nitrite

This substance is used in room de-odorisers. Ryback (1992) discussed loss of auditory sensitivity observed at 10 and 40kHz. There was recovery at the latter frequency but at the former dysfunction persisted over a six day period. No human studies or cochlear histopathological studies are available.

Carbon monoxide

Baker and Lilly (1977) reported a moderate hearing loss maximal at 2kHz following acute poisoning in a sixteen year old without known previous hearing loss. The loss was partly reversed especially over the first month and particularly in the lower tones. Recovery above 2kHz was much less.

Ryback (1992) discussed a report of prolongation of Wave I latency without IPL prolongation in 6 of 32 cases of acute poisoning indicating peripheral pathology. However two cases showed a central pattern of wave latency and IPL prolongation.

The toxic effects of carbon monoxide flow from the induced tissue hypoxia. It thus provides a simple model to study the effects of hypoxia on cochlear function. However secondary effects on blood flow have to be considered. Young et al. (1987) found that in rats exposed to carbon monoxide (1200ppm, 90 minutes preceding and then 120 minutes concurrent with exposure to noise at 110dBA) experienced greater high frequency threshold shifts than those produced by exposure to noise only. An equivalent exposure to carbon monoxide without noise exposure produced no threshold shift. Maximal effects were at 40kHz. Confirmatory experiments (Fechter et al., 1988) showed outer hair cell damage.

The propensity for carbon monoxide to potentiate noise-induced threshold shift could indicate that carbon monoxide acts to

(1) Reduce cochlear blood flow and/or

(2) Cause cochlear hypoxia directly and/or

(3) Otherwise lead to metabolic exhaustion.

The assumption that outer hair cells used more energy would explain the greater effects on these cells. The fact that carbon monoxide alone did not produce a threshold shift implies that it acts only to potentiate a series of metabolic events associated with the noise-induced damage.

Carbon monoxide alone can produce a permanent hearing loss (Baker and Lilly, 1977) but this did not occur in rats (Young et al., 1987). The reason for this is probably the dose difference.

Another interesting point is that the potentiation is frequency specific. The basis for this could lie in failure of cochlear blood flow compensation or a reshunting of blood toward that part of the cochlea most stimulated by noise although noise stimulation generally reduces cochlear blood flow (Axelsson and Dengerink, 1987). However, the explanation may be found elsewhere - for example in possible differences in the level of the metabolic rate in outer hair cells along the cochlea.

No recent research using the carbon monoxide model is available and it is not clear why. It would seem to be a promising avenue for investigation of cochlear metabolism.

Manganese

Manganese is used in steel and metal alloy production and has been known as a neurotoxin since 1837. Manganism is characterised by Parkinsonian - type symptoms and signs and lesions are variably present in the basal ganglia (Zenz, 1994). Auditory brainstem pathology has not been reported but any finding of dopamine receptors in the auditory pathway would point to that possibility because of manganese enhanced oxidation of that neurotransmitter. Franks and Morata (1996) and Ryback (1992) discussed reported ototoxicity in workers exacerbated by noise exposure but the nature of the interaction is not clear. However there are no recent human studies and there is a need for animal studies to decide the possibility of cochlear or brainstem lesions.

Arsenic and arsenic compounds

Arsenic, known as a toxin from ancient times, acts through the formation of covalent bonds with the sulphur atom of sulphydryl groups in tissues (Zenz, 1994). One of a series of papers published after an epidemiological and environmental survey of the effects of coal burning emissions with a high

arsenic content concerned hearing loss in children (Bencko, 1977). Compared to age-matched controls sensorineural losses were noted mainly at low frequencies.

Anniko and Wersäll (1977 and 1975, respectively) showed sodium arsanilicum (atoxyl) caused damage in the guinea pig vestibular sensory epithelia and in the cochlea. There was disintegration of the chalices surrounding the type I hair cells and dark cell damage of the cristae ampullari and utricular macula. In the stria vascularis there was early progressive vacuolisation, nuclear ectopia, loss of mitochondrial and Golgi membranes followed by chromatin fragmentation and nuclear membrane rupture. The marginal and intermediate cells were most affected particularly in the apical turns with relative sparing otherwise. Damaged cells tended to be extruded into the endolymph. There was also vesiculation and mitochondrial swelling and degeneration in endothelial cells of the labyrinthine blood vessels. The importance of the roles of toxic leakage from blood vessels, vascular occlusion and other effects is not clear. However there have been no recent studies.

No correlation of human with animal studies is possible and no noise interactions are known.

Interactions and Location of Effects

The concepts and nomenclature of interactions derive from their use in pharmacology. Their application to the study of the auditory system is not straightforward. There is no one satisfactory and comprehensive measure of dysfunction in the auditory system. For example, normal pure tone audiometry does not exclude the possibility of retrocochlear effects on hearing. Nor is there necessarily a direct relationship between pure tone sensitivity deficits and the damage inflicted on hair cells. Thus although the concepts of interaction and synergy are useful they do not precisely capture the totality of auditory system effects.

It may be most useful to consider the combined effects of noise and chemicals in terms of sites of auditory system damage and mechanisms of action and interaction. This approach has been used by Fechter (1995). Solvents and heavy metals may be cochleotoxic or neurotoxic - that is they may produce effects almost anywhere in the auditory system. Noise damage is restricted to the cochlea. For example, the effects of carbon disulphide and noise on hearing may involve retrocochlear neurotoxicity, chemical cochleotoxicity and noise damage to the cochlea - effects not easily summarised by one measure. It is better to try to understand the several effects.

A useful element for understanding is the simultaneity of exposure. Different mechanisms can be expected to be operating if the damage pattern differs between otherwise equivalent simultaneous or serial exposures to noise and chemicals. For example noise may result in higher levels of chemical penetration of the cochlea by changes in blood flow. However it should be remembered that noise is known to produce vasoconstriction in the cochlea. Also, there could be effects depending on noise-induced increased hair cell activity leading to injury to barrier mechanisms which normally act to prevent chemicals entering the cochlea or the hair cells. Another possibility is a common pathway of damage. Compromise of intracellular energy production is a principal candidate for this. Carbon monoxide ototoxicity may be an example. It may also be that a common feature of both noise and

industrial ototoxic exposure is increased release of the neurotransmitter glutamate. This may provide the basis of a link between cochleotoxicity and some form of neurotoxicity.

Trichloroethylene, toluene and styrene can cause a mid frequency hearing loss in rats due to cochlear damage. This may be true too for carbon disulphide and xylene but there is less certainty. This is immediately suggestive of a common mode of damage at the cellular or biochemical level. However, what this may be is unknown.

These substances have neurotoxic effects on the auditory pathways but there seems no clear relationship between auditory neurotoxic and cochleotoxic potential. It may be that the basis of neurotoxicity varies. It is possibly more likely, though, that variations in the penetration of the cochlea and the blood-brain barrier rather than in the direct effects on hair cells or neurons will better explain their toxicity. Differences in pathways and speed of detoxication are also possible.

Other location effects are also unexplained. For trichloroethylene (Campo et al., 1997) and toluene (Lataye and Campo, 1997) there is the interesting bimodal distribution of outer hair cell loss. Some solvents show differential row effects with damage decreasing from outer hair cell row 3 through to the inner hair cells. Perhaps there is a concentration effect due to the solvent entering the endolymph through the spiral ligament and the stria vascularis with lower concentrations reaching the inner hair cells. Research which was able to establish that perilymph solvent concentrations were higher than endolymph concentrations could dispel this speculation. Of course, a converse finding would support it.

Mechanical studies able to show that the outer part of the basilar membrane is displaced further might support speculation that the row effects result from a higher metabolic rate in the outer rows - thus linking propensity for damage to interference with intracellular energy metabolism.

If it were possible to assess energy demands of the cochlea under conditions of high and low sound stimulation then it might be useful to see if row effects changed under conditions of variable energy demand. The study of Ray et al. (1992) although concerning the auditory brainstem rather than the cochlea illustrates the concept. (See1,3-dinitrobenzene in the section on miscellaneous agents).

Two ways forward are suggested. Firstly, in the ageing ear there is a row effect similar to that produced by solvents although in different coils. So, is it possible that there is some commonality in the biochemistry of these phenomena? Secondly, there is the consideration of the effects of noise.

It is known that outer hair cell row effects due to noise damage are the opposite of solvent effects (Rosenhall and Pedersen, 1995). This may counter speculation that solvent row effects might be due to endolymph concentration gradients and basilar membrane mechanical effects in combination. This is because if noise effects are mediated through metabolic energy exhaustion the maximum Row 1 effect is not compatible with greater mechanical movement at Row 3 as a component of higher solvent toxicity there. However, limited knowledge of cochlear mechanisms does not enable a preferred conclusion to these speculations. In any case when considering the row effects of noise a distinction must be made between temporary threshold shifts, which are associated only with Row 3 (stereocilial) changes, and permanent threshold shifts where Row 1 damage may be greater than Row 3 damage (Gao et al., 1992). Perhaps it is best to be sceptical about the various speculations whilst not abandoning them.

Of the solvents noise interactions are best known for toluene. Toluene followed by noise exposure produces greater effects than the reverse order of exposures (Johnson et al., 1988). Toluene does something which sensitises the hair cells to noise damage. It may be a membrane effect, a stereocilial effect or a general metabolic effect although these are not mutually exclusive. However it is clear that the effects of noise and toluene must be different from each other in some way - although this does not exclude some commonality of action.

The relative frequency effects of noise and toluene in the cochlea are similar but not identical. Johnson et al. (1988) showed maximum toluene and noise effects at the same frequencies. Lataye and Campo (1997) showed maximal effects at slightly different frequencies. However, neither the noise nor the toluene exposure protocols were strictly comparable. At this stage frequency distribution differences can be said to be small and do not elucidate the basic damage mechanisms. In any case noise damage locations can be expected to be somewhat stimulus dependent. Enough is known however to say that for toluene (and perhaps other solvents) a special mechanism is involved (Johnson, 1993). Noise is different and so is aminoglycoside toxicity which varies in a reverse way to toluene in relation to sequential exposure to noise (Ryan and Bone, 1982).

Heavy metals seem different again. They generally produce high frequency rather than mid-frequency losses. Their relative neurotoxicity is high compared with their cochleotoxicity with the exception of trimethyltin. Whether this is due to absorption or penetration differences is not known. Methylmercury and trimethyltin are readily soluble in lipid and can be expected to penetrate cell membranes easily. Lead (at least as lead acetate) is associated with preservation of outer cell function. This may be because of a lower solubility in lipids or to other barrier effects. The effects of methylmercury on mitochondria and thus on cellular energy production have been made quite clear.

Generally, there seems to have been more study of the histopathology of heavy metal damage to the cochlea than is the case with solvents. It is not clear why this is so but this situation is changing as shown by the recent experiments of Campo and Lataye.

Pharmacokinetics and Biochemistry

Very little information is available concerning the concentrations which industrial ototoxic agents maintain in the cochlear or the rate at which they are cleared. However, some speculations have

been discussed concerning the possibility that cochlear clearance is relevant to heavy metal damage, principally methylmercury.

This is presumably because of the difficulty of reliably collecting the tiny amounts of perilymphatic and endolymphatic fluids. There is some evidence (Pryor et al., 1991) that toluene induced hearing loss is not due to a metabolite. Although this was indirect evidence, it implies that toluene itself does penetrate the cochlea.

Some progress has been made in associating hair cell and Type I afferent neurone damage with effects on calcium metabolism. These have concerned trimethyltin and need no further summary in this section. However, these experiments demonstrate a promising way of elucidating the cellular effects of other industrial ototoxic agents.

Implications for Safety Practice

Do recent advances in the knowledge of industrial agent ototoxicity imply any inadequacy in occupational exposure standards? Standard setting is a compromise between economics, safety and uncertainty (risk). Knowledge has increased and uncertainty is somewhat reduced. However, the advances in knowledge are in respect of substances already known to have non-auditory toxic effects. So the question becomes whether the additional knowledge on auditory effects should have an impact on regulation or control measures. In part this depends on whether auditory effects predominate over other effects. The answer to this is complicated. In many instances the evidence for auditory effects being outstanding over other effects is lacking. Examples are styrene, xylenes, trichloroethylene, lead and mercury. However the dose pattern and period of exposure are important here. After long term exposure auditory effects may become more prominent than they are with higher dose lower duration exposures.

Although the animal experiments reviewed in this study typically used high dose exposures and cannot in any case be directly applied to humans they do give cause for concern. Human studies far less frequently clearly demonstrated either ototoxicity or combined ototoxicity and noise damage. Such studies always have limitations so little comfort can be derived from their restricted conclusions. Further, the best studies reviewed here are certainly cause for concern. It is pointed out that the preferred response to the new knowledge may be more stringent applications of existing controls and regulations rather than more restrictive exposure standards especially if the latter are routinely circumvented as is the case in some countries.

Future research

This has been discussed in the reviews of individual chemicals. However, there are a number of themes which emerge. In animal studies there should be more emphasis on correlation of structural and functional deficits. This is already happening with toluene and trimethyltin. There is a need for more *in vitro* studies of the effects of chemicals on hair cells and for advancement of the biochemical knowledge of these effects. For example knowledge of calcium metabolism in the cochlea (Wangemann and Shacht, 1990) has been increased by *in vitro* studies of TMT effects.

There remain many substances, some mentioned here, which have been little studied and yet promise to be revealing. Two criteria for selecting such substances are the likelihood of elucidating fundamental mechanisms and the determination of possible risks to human populations. Examples are carbon monoxide, manganese, and 1,3-dinitrobenzene. Laboratory research on trimethyltin is promising but there seems to only be limited current interest in methylmercury which seems just as promising. Perhaps the neglect of general environmental studies of hearing as compared to a purely occupational health focus explains the lack of interest in methylmercury. However, broad environmental studies of chemicals and noise are needed but they are difficult, requiring large numbers, a long period and careful exclusion of compounding factors.

In respect of human populations generally, the opportunity for good quality studies of hearing effects of industrial ototoxic agents and noise at the level of individual industrial plants should be decreasing but there is scope for well designed long term surveillance programmes. Further, with humans there needs to be a greater number of temporal bone studies. Finally, there is a great need for further research with animals and humans in combined effects of ototoxic agents and noise. The work of Morata and colleagues in humans and Johnson and colleagues in animals as well as that of Campo and Lataye is showing the way and there is increasing journalistic interest (Lang 1994).

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Appendix

Table of Exposure Limits

SUBSTANCE		ACGIH	NIOSH	OSHA	Non-USA
Trichloroethylene	TWA			100ppm	
	TWA ceil			150ppm/15min	
	TLV-Japan, Australia, USA	50ppm			50ppm
	STEL	150ppm			10ppm
	TLV (Sweden)				100ppm
	TLV (U.K.)				
Toluene	TWA		100ppm	200ppm	
	TWA ceil			300ppm	
	TWA peak			500ppm	
	10min ceil		200ppm		
	TLV (from1993)	50ppm			
Xylene	TWA		200ppm/10min	100ppm	
	STEL	150ppm	ceil		
Styrene	TWA			100ppm	
	TWA ceil			200ppm	
	TLV	50ppm			
	STEL	100ppm			
Carbon disulphide	TWA			20ppm	
	TWA ceil			30ppm	
	30min peak			100ppm	
	TLV		1ppm		
	TLV ceil		10ppm		
	TLV	10ppm			
Mercury	TWA ceil			0.1mg/m^3	
	TWA		0.05mg/m^3		
	STEL	0.15mg/m^3			
Lead	TWA			0.05mg/m^3	
	TLV				
	STEL	0.15mg/m^3			
Arsenic	TLV	0.45mg/m^3			
Organic Tin Compounds	TWA	0.2mg/m^3			
		0.1mg/m^3			

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