# Review of Environmental Chemicals and Neurotoxicity

## Focus on Neurological Diseases

**S Bull**

## ABSTRACT

This review was conducted to support Health Protection Agency (HPA) strategic Goal 2 and 5 namely to protect against adverse effects of acute and chronic exposure to chemical poisons and other environmental hazards and to protect and improve the health of children. The possible neurotoxic effects of environmental chemicals, particularly those involved in progressive degenerative disease such as Parkinson's disease (PD) and Alzheimer's disease (AD) and also those adversely affecting neurological development in children following early life stage exposure, are of increasing public concern. It is important that HPA is aware of current scientific development in this area.

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Centre for Radiation, Chemical and Environmental Hazards
Chemical Hazards and Poisons Division
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## Abbreviations

| | |
|---|---|
| Aβ | Amyloid beta peptide |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ACP | Advisory Committee on Pesticides |
| AD | Alzheimer's disease |
| APP | Amyloid precursor protein |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BAEP | Brainstem auditory evoked potential |
| BBB | Blood brain barrier |
| β-CIT | 2-β-carbomethoxy-3-β-[4-iodophenyl] tropane. |
| BDE | Brominated phenyl ethers |
| CCI | Colour confusion index |
| CEI | Cumulative exposure index |
| CNS | Central nervous system |
| COT | Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment |
| CSF | Cerebrospinal fluid |
| DNB | 1,3-dinitrobenzene |
| DNT | Developmental neurotoxicity |
| DTZB | Dihydrotetrabenazine |
| DWI | Diffusion Weighted Imaging |
| ECD | Ethyl cysteinate dimer |
| EEG | Electroencephalography |
| EMG | Electromyography |
| EP | Evoked potential |
| EPA | Environment Protection Agency |
| FAS | Foetal Alcohol Syndrome |
| FDDNP | 2-(1-(6-[(2-fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile. |
| FDG | 2-fluoro-2-deoxyglucose. |
| FDRI | Field-dependent R2 increase |
| FOB | Functional observation battery |
| $H_2O_2$ | Hydrogen peroxide |
| HPA | Health Protection Agency |
| HMPAO | Hexamethyl propylamine oxime |
| ILSI | International Life Sciences Institution |
| IPCS | International Programme on Chemical Safety |
| JECFA | Joint Expert Committee on Food Additives |
| MA | Mandelic acid |
| MPP+ | 1-Methyl-4-phenylpyridine |
| MPTP | N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine |
| MRI | Magnetic resonance imaging |
| MRM | Magnetic resonance imaging microscopy |
| MRM | Magnetic resonance imaging microscopy. |
| MRS | Magnetic resonance spectroscopy. |
| MRV | Magnetic resonance volumetry. |
| MTI | Magnetisation transfer imaging. |
| NCTB | Neurobehavioural Core Test Battery |
| NMDA | N-methyl-D-aspartate |
| NSC-60 | Neurotoxicity Symptom Checklist-60 |
| OECD | Organisation for Economic Co-operation and Development |
| OEL | Occupational exposure limit |
| PBDE | Polybrominated diphenyl ethers |

| | |
|---|---|
| PCB | Polychlorinated biphenyls |
| PCE | Perchloroethylene |
| PD | Parkinson's Disease |
| PET | Positron emission tomography |
| PGA | Phenylglyoxylic acid |
| PIB | Pittsburgh-compound B |
| PTWI | Provisional Tolerable Weekly Intake |
| R2 | Relaxation rates |
| REACH | Registration, Evaluation and Authorisation of Chemicals |
| ROS | Reactive oxygen species |
| SB-13 | 4-N-methylamino-4'-hydroxystilbene |
| SEP | Sensory evoked potential |
| SPECT | Single photon emission computerised tomography |
| SSEP | Somatosensory evoked potential |
| T2 | Relaxation times |
| TaBro | 1-tribromomethyl-1,2,3,4-tetrahydro-beta-carboline |
| TaClo | 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline |
| TGD | Technical Guidance Document for Risk Assessment |
| THP | Tetrahydropapaveroline |
| TIQ | Tetrahydroisoquinoline |
| TWA | Time weighted average |
| VEP | Visual evoked potential |
| VMAT 2 | Vascular monoamine transporter type 2 |
| WAIS-R | Wechsler Adult Intelligence Scale Revised |
| WHO | World Health Organisation |

# Executive Summary

This review was conducted to support Health Protection Agency (HPA) strategic Goal 2 and 5 namely to protect against adverse effects of acute and chronic exposure to chemical poisons and other environmental hazards and to protect and improve the health of children. The possible neurotoxic effects of environmental chemicals, particularly those involved in progressive degenerative disease such as Parkinson's disease (PD) and Alzheimer's disease (AD) and also those adversely affecting neurological development in children following early life stage exposure, are of increasing public concern. It is important that HPA is aware of current scientific development in this area.

Neurotoxic chemicals are a diverse range of compounds that are capable of causing neuronal injury or neurodegeneration. Methods for the identification of compounds with neurotoxic potential have been reviewed. With many chemicals the only available information on which to base an assessment is experimental data in animals. A range of *in-vivo* tests have been established for the identification and characterisation of neurotoxic chemicals but there is currently an international effort to further refine or replace such methods by using *in-vitro* test systems. However, further assessment of such models in terms of validation, use, advantages and limitations is necessary. Human data on the neurotoxicity of chemicals are particularly relevant when available. Neurotoxicity tests have been routinely used in clinical practice and the increase in neuroimaging has aided the diagnosis of neurological disease, as well as providing a tool with which to following neuronal degeneration in a non-invasive manner.

Epidemiology studies have identified several chemicals as being potentially involved in chronic neurodegenerative disease or as having developmental toxicity. The available data are reviewed. In addition consideration is given to research on potential mechanisms.

Neurodegenerative diseases such as PD and AD are both recognised as being multifactorial diseases and epidemiological studies now focus on genetic and environmental analyses rather than simply carrying out descriptive studies. Research into several neurodegenerative disorders have tentatively identified a number of potential mechanisms that may be of relevance to acute or delayed neurological effects. However, there remains considerable scope for further investigation, especially with regard to elucidating the fundamental mechanisms involved with neurodegenerative disorders.

The purpose of this review was to summarise scientific developments in the field of neurotoxicity and evaluate putative environmental exposure to chemicals and the possible association with neurodegenerative diseases. With regard to consideration of health detriments and environmental exposure to chemicals, the report concentrates on chronic progressive diseases such as PD and AD.

# Introduction

Neurotoxicity is concerned with the adverse changes in the structure or function of the nervous system. Herein, a neurotoxin is considered to be a substance which elicits a pathological response primarily or specifically on the nervous system. The complexity of the nervous system results in a broad range of potential targets and adverse sequelae, since the activity of the nervous system maintains a balance between all the various organs in the body.

The importance of the potential impact of chemicals on human neurological function has been recognised by recent activity in the Organisation for Economic Co-operation and Development (OECD) test guidelines programme relating to specific methods and a general guidance document, and by the International Programme on Chemicals Safety (IPCS) in their recent (2001) Environmental Health Criteria (EHC) Document on 'Neurotoxicity; risk assessment for human health; principles and approaches'.

The deleterious effects of chemicals may not become clinically evident for some time following exposure, aptly called the silent period. Such latent toxicity has been defined as 'persistent morphological and/or biochemical injury, which remains clinically unapparent' (1). Several hypotheses have been proposed to explain the asymptomatic period prior to clinical expression of neurotoxic injury. Exposure to neurotoxic chemicals may cause cell death of a subpopulation of neuronal cells, but the total number of cells lost is insufficient to cause adverse effects because of the reserve capacity regarding this fraction. Such a deficit is observed following stress, disease, further chemical exposure or by the natural aging process in which the number of neurones progressively decreases (figure 1). Alternatively, toxic exposure may cause sublethal injury to neuronal cells in critical areas of the brain such as the substantia nigra, that leads to a progressive loss of function (2). Initially this can be compensated for, although over time loss of function arises due to the lack of plasticity of the brain (1). Such hypotheses also exist for developmental neurotoxicity; exposure to chemicals or infections during the appropriate gestational age can result in fewer dopaminergic neurones. Although no clinical signs may be evident in early years, the subjects may be predisposed to Parkinsonian traits later on in life (1, 2). This has lead to particular concern about exposure to low levels of environmental chemicals and effects on neurobehavioural/development in children and on the development of neurodegenerative diseases in the adult.

Neurodegenerative diseases are of great concern, bearing in mind the aging of the population. Alzheimer's disease and PD are the most common neurodegenerative diseases, with approximately 500,000 and 120,000 people being affected, respectively.

Alzheimer's disease is the most common type of dementia, accounting for 55 % of all cases. Other types of dementia include vascular dementia (20 % of dementia cases), dementia with Lewy bodies (15 %), fronto-temporal dementia including Pick's disease (5 %) and other dementias (5 %)(3).

Epidemiological studies have shown that AD below 65 years of age is quite rare, with only 1 in 1000 people having AD. However, 1 in 20 65 to 80 year olds and 1 in 5 people over the age of 80 have AD (3, 4). In fact, the prevalence of AD approximately doubles every 5 years after 65 years of age, reaching 20 % by 80 years of age. Overall, approximately 500,000 people in the UK have AD (4, 5). The median survival of someone in their 60s with AD is between 7 and 10 years (5).

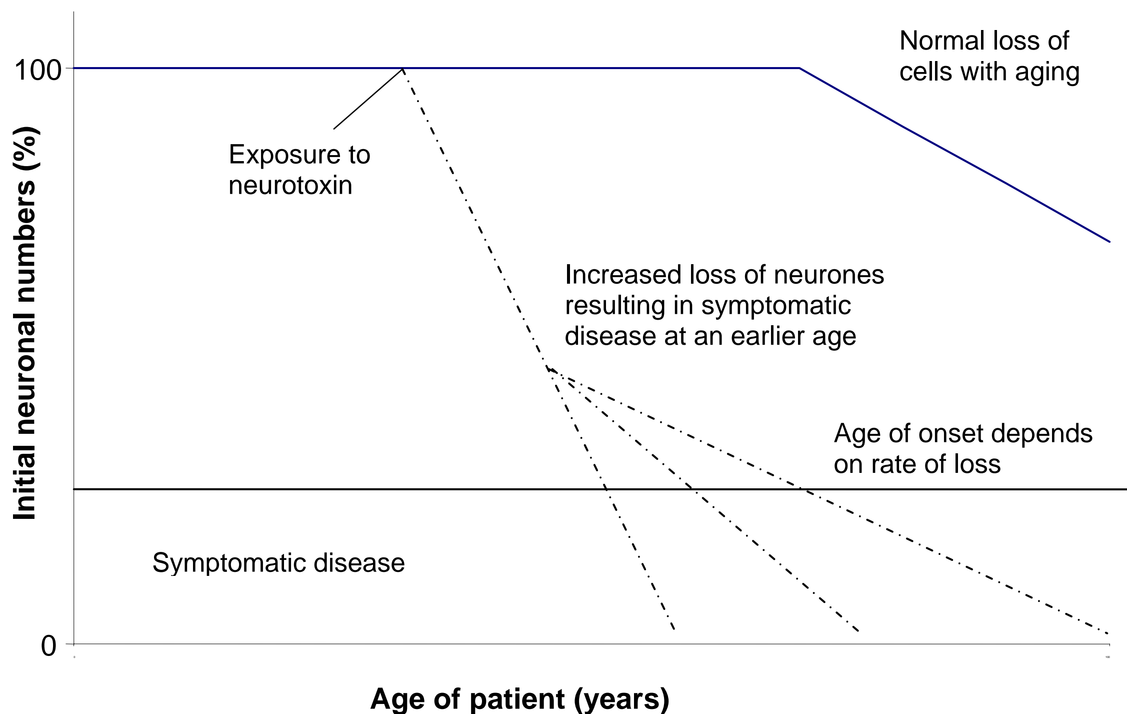


Figure 1. An increased loss of neurones following exposure to a neurotoxin producing symptomatic neurological disease at an earlier stage (reprinted with permission from Medical Neurotoxicology ed. P Blain and H Harris).

Parkinson's disease is the second most common neurodegenerative disease after AD. Symptoms often start around 55 years of age, although early-onset PD below the age of 40 is seen in approximately 5% of patients (6).

Overall, 1 in 500 people has PD, hence approximately 120,000 people are affected in the UK and approximately 10,000 are diagnosed each year (4, 6). There is a rising prevalence of PD with age. In people over 65 years old, 1 in 100 are affected and 1 in 10 over 80 year olds has PD (7, 8).

Certain literature has suggested a putative association between environmental exposure to chemicals and neurodegenerative diseases (9). To date, evidence about the long-term effects of chemicals in potentially vulnerable groups, such as children and the elderly is lacking. The aging individual represents a sensitive subpopulation due to the natural aging process, which results in a reduction of plasticity and diminished compensatory capacity of the nervous system. Developmental neurotoxicity is also a sensitive endpoint, as in addition to the *in utero* phase, the CNS has a relatively long post-natal development process, with critical time periods for different effects. Thus, children are also a sensitive population for chemical-induced neurotoxicity.

Several occupational and environmental chemicals that have been implicated in the aetiology of neurotoxicity, developmental neurotoxicity and neurodegenerative diseases (10, 11) (table 1). It has been suggested that many chemicals listed by the American Conference of Governmental Industrial Hygienists (ACGIH) affect the nervous system (12, 13). In addition, Anderson and colleagues stated that at least 138 industrial chemicals were thought to be neurotoxic to humans, causing clinical signs and symptoms such as addiction, ataxia, coma, delirium and hallucinations, muscle fasciculations, narcosis,

paralysis, paraesthesia, Parkinsonism, peripheral neuropathy and tremors (14). The group of chemicals included 56 pesticides and related compounds, 24 solvents and organic compounds and 18 metal and organometal compounds. Anger (13) estimated that a third of the 200 chemicals to which people are exposed may have some adverse effects on the nervous system if sufficient exposure occurs. In addition, neurotoxic effects were also reported as one of the ten most common disorders in the work place in US (12, 15) and stress, mental illness and hearing loss were within the 6 most commonly reported occupational disorders in the UK (16). However, in both cases, data obtained are largely dependent on the definition of neurotoxic effects and whether they are indeed chemical-induced. Therapeutic drugs (including some anticancer and antiviral agents) can cause neurotoxicity at therapeutic doses as well as drugs of abuse (12, 17).

***Table 1. Examples of neurotoxic agents (18, 19).***

| Class | Neurotoxic agents |
|---|---|
| Agrochemicals: | Dieldrin, Paraquat, Maneb, Rotenone |
| β-Carbolines: | Harman, Norharman, TaClo, TaBro |
| Alkaloids: | 1-benzyl-isoquinoline, tetrahydroisoquinoline (TIQ), tetrahydropapaveroline (THP) |
| Metals: | Aluminium, Arsenic, Barium, Bismuth, Copper, Lead, Iron, Manganese, Mercury, Methyl mercury, Thallium, Trimethyltin, Triethyltin, Zinc |
| Solvents and industrial compounds: | Acrylamide, Benzene, Carbon disulfide, Ethanol, Ethylene glycol, Methanol, Methyl chloride, Methylene chloride, n-Hexane, Methyl *n*-butyl ketone, Methyl chloride, Perchloroethylene, Phenol, Toluene, Tetrachloroethane, Trichloroethylene, Polychorinated biphenyl heptachlor, Tinuvin 123, Toluene |
| Endogenous dopamine-related compounds: | 6-HODA, Benzthiazoles + mercapturates, Dopaminachrome, Dopaldehyde, Melanin, Thiocatechol adducts of dopamine, 3,4-dihydroxyphenylacetic acid (DOPA) and 3,4-dihydroxyphenylacetic acid (DOPAC) |
| Other: | Cyanide, Carbon monoxide, β-Methylamino-L-alanine |

Overall, there is a need for an overview/position paper to ensure that the Health Protection Agency (HPA) is up to date with scientific developments regarding methodology for risk assessment in the area of neurotoxicology and of data on the role of environmental chemicals in the causation of such effects, particularly in susceptible groups. This should help to provide authoritative advice in this area, and enable research relevant to HPA's priorities to be identified.

# Current methods for assessing neurotoxicity and developmental toxicity

In most cases the assessment of the neurotoxicity of chemicals needs to be based on data from animal experiments. Data in humans from several epidemiological studies is only infrequently available, but is particularly valuable.

## Studies in animals and experimental systems

### *In-vivo models of neurotoxicity*

A multidisciplinary approach is required in order to adequately assess potential neurotoxic effects of compounds, due to the diverse function of the nervous system. Many effects may be measured using neurophysiological (e.g. electroencephalography, measurement of evoked potentials), neuropathological (e.g. microscopy, histochemistry, immunohistochemistry) or behavioural techniques (20).

Due to the variety of biochemical targets and toxic effects, test strategies used to evaluate the neurotoxic potential of chemicals should be determined on a case by case basis. A staged approach is commonly used consisting of three tiers aimed to identify (tier 1) and characterise (tiers 2-3) the neurotoxicity of the chemical (21). Tier one is usually a basic repeated dose toxicity study. Due to the numerous possible effects on the nervous system, batteries of tests may be necessary to ensure the detection and characterisation of possible neurotoxic effects. Guidelines from the U.S. Environment Protection Agency (12) and OECD (22, 23) outline a number of tests that should be used.

The OECD guideline 424 for the testing of chemicals was designed to either confirm or further characterise potential neurobehavioural and neuropathological effects observed in adult animals during the repeat dose toxicity study (23). It may be carried out in combination with the repeat dose toxicity studies or can be carried out as a separate study.

Essentially, the neurotoxicity study comprises:

- Detailed clinical observations.
- Changes in skin, fur, eyes, mucous membranes.
- Occurrence of secretions and excretions.
- Autonomic activity (e.g. lacrimation, piloerection, pupil size, respiratory pattern, unusual urination/defecation, discoloured urine).
- Body position, activity level, co-ordination of movement.
- Changes in gait, posture, reactivity to handling.
- Clonic or tonic movements.
- Convulsions or tremors.
- Stereotypes (e.g. excessive grooming, repetitive circling).
- Strange behaviour (e.g. biting, walking backwards).
- Functional tests.
- Assessment of sensory function (e.g. sensory irritation).
- Assessment of motor function (e.g. limb grip strength, foot splaying).
- Assessment of cognitive function (e.g. habituation, avoidance).

The US Environment Protection Agency (EPA) guidelines for neurotoxicity risk assessment describe concepts and procedures that the Agency follows in evaluating data on potential neurotoxicity associated with exposure to environmental toxins (12). The guideline describes five categories of neurotoxicity endpoints including structural or neuropathological, neurophysiological, neurochemical, behavioural and neurological and developmental (12).

The functional observational battery (FOB) is a standardised screening battery for assessing many aspects of behaviour and neurological functions in rodents and is designed to detect and quantify major overt behavioural, physiological and neurological signs (24). The tests have been validated with many known neurotoxic chemicals and are often used in conjunction with other measures of toxicity (21). The FOB comprises a number of tests to identify specific deficits in motor and sensory functions by measuring neuromuscular, sensory and autonomic function (24).

### *In-vitro models of neurotoxicity*

To address problems associated with increasing cost and time required for neurotoxicity testing, the large number of chemicals in commercial use that have not been investigated and animal welfare issues, considerable effort is being directed at the development of *in-vitro* alternatives. These may be considered as part of the tiered system with which to identify potential neurotoxic chemicals (21), although such approaches have not been validated as replacements for animal studies. The EPA has stated in the proposed guidelines for neurotoxicity risk assessment that "demonstrated neurotoxicity *in vitro* in the absence of *in-vivo* data is suggestive but inadequate evidence of a neurotoxic effect. On the other hand, *in-vivo* data supported by *in-vitro* data enhance the reliability of the *in-vivo* results" (12).

Several *in-vitro* models are commonly used in neurotoxicity evaluations, including synaptic fractions, primary cultures of rat astrocytes, rat cerebellar granule neurones, primary motor neurones of dissociated cultures of murine spinal cord, rat brain region organ cultures and hippocampal slices (25). Alternatively, many continuous cell lines of various origins (glioma neuroblastoma or pheochromocytome) such as C6, NIE115, PC12, neuro-2a, B65 or IMR32 cells have been used for screening of potential neurotoxic compounds (26). Recently chromaffin cells (27) and the AF5 CNS cell line (28, 29) have been used as *in-vitro* models for neurotoxicity studies.

Many endpoints may be used to detect basic cytotoxicity. However, more specific endpoints are required in developing *in-vitro* systems to evaluate neurotoxic potential of chemicals. Possible target sites of neurotoxicity include changes in:

- Morphological endpoints.
- Neurite outgrowth.
- Peripheral nerve or central nervous myelin.
- Axonal transport.
- Electrophysiological indices.
- Blood-brain barrier.
- Calcium homeostasis.
- Neurotransmitters and hormones (26).

Recent studies have described a three-dimensional *in-vitro* blood-brain barrier model (30) comprising an astrogliomal cell line, epithelial or endothelial cells and a neuronal cell line have been grown as a co-culture system and cytotoxic responses of the target cell layer and the barrier cell layer are measured (30).

Overall, further assessment of *in-vitro* neurotoxicity models in terms of validation, use, advantages and limitations is necessary.

### *Developmental neurotoxicity tests*

It is widely accepted that the degree of neurotoxicity varies with the developmental period and can be particularly high in the early stages of development, due to characteristics of the cellular and molecular processes involved in brain development including slow maturation, immature protective systems, cell specialisation and limited capacity for regeneration (31). However, there is currently no specific regulatory requirement that chemicals be tested specifically for developmental neurotoxicity prior to registration and use in the EU (31-33). In this regard, it is pertinent to note that much reassurance can be obtained from the absence of any effects on the development of offspring in a multigeneration study, since the ability to grow, mate and rear offspring is affected by subtle changes relating to developmental toxicity.

A guideline for developmental neurotoxicity studies was issued by U.S. EPA in 1991 and a revised version was proposed in 1995 (USEPA, 1995). Recently, an OECD test guideline has been developed based on the US guideline (34) and the revised EU Technical Guidance Document for Risk Assessment (EU, TGD) has now included the OECD draft TG 426 as a possible test in the testing strategy for new and existing chemicals (EU-TGD, 2001).

A tiered approach to determine when a chemical should undergo developmental neurotoxicity (DNT) testing, using a weight of evidence approach was proposed in the U.S. EPA and EU-TGD. Criteria used to recommend DNT testing include CNS/behavioural teratogens (and structural analogues), adult neuropathic agents, adult neuroactive agents, hormonally-active compounds and developmental toxins that do not necessarily produce CNS effects (31). According to this tiered scheme, chemicals not meeting any of the criteria would not be recommended for DNT testing. However, inter-laboratory validation are needed to improve interpretability of data from DNT studies (32). The International Life Science Institution (ILSI) neuropathology review describes the strengths and weaknesses of the various morphological approaches in DNT studies that provide a number of suggestions for the appropriate use of different techniques (35). However, clear recommendations for the use of techniques in DNT studies is lacking (32).

## Studies in humans

### *Clinical neurotoxicity tests*

The assessment of neurotoxicity begins with a clinical evaluation of the patient, including medical history and a standard neurological examination. The clinical evaluation of neurotoxic diseases assesses various functions: These include, amongst others:

- Mental status: level of consciousness, orientation, speech disorders (dysphasia, dysarthria, dysphonia), concentration, memory, mood and affect.
- Cranial nerve function: I-olfactory, II-optic, III-oculomotor, IV-trochlear, V-trigeminal, VI-abducens, VII-facial, VIII-acoustic, IX-glossopharyngeal, X-vagus, XI-accessory and XII-hypoglossal.
- Motor function: muscle tone, muscle strength, muscle atrophy, unusual movements, tremor, pronator drift.

- Coordination and gait: rapid alternating movements, resistance to passive stretch, point-to-point movements, Romberg, gait.
- Reflexes: deep tendon reflexes, clonus, plantar response (Babinski.
- Cutaneous sensory indices: vibration, position, subjective light touch, dermatomal testing (pain, temperature, light touch), discrimination (24).

Depending on the clinical signs observed or type of chemical exposure, standard tests may be supplemented by additional procedures such as neuropsychological evaluation, neurophysiological tests and neuroimaging techniques (24).

*Neuropsychological and neurobehavioural testing*

Neuropsychological testing is often carried out during a clinical examination. The tests used are aimed at assessing a wide range of neurological functions including different aspects of verbal function, visuospatial ability, memory, attention, cognitive tracking and flexibility, and psychomotor abilities (24).

Assessments are carried out on a one-to-one basis and often take five or more hours to complete. Due to time and expense of carrying out such tests, experimental and epidemiology studies commonly use a shortened battery of clinical neurobehavioural tests that can be administered in a shorter period of time but are considered to be equally sensitive. The shortened approach includes cognitive testing batteries, psychiatric and symptom questionnaires and behavioural neurophysiological tests.

An example of tests used in a clinical neuropsychological battery are shown in table 2.

<u>Cognitive testing batteries</u>

In order to standardise test methods in human neurotoxicology and epidemiology, the World Health Organisation (WHO) published a Neurobehavioural Core Test Battery (NCTB). This encompasses a shortened battery of tests that focus on effects commonly observed in CNS toxic disorders. The battery of tests include:

- Perceptual coding (Digit Symbol).
- Attention and short-term memory (Simple Reaction Time Digit Span (WAIS-R), Benton Visual Recognition).
- Psychomotor performance (Aiming Motor Santa Ana Forum Board).
- Mood and affect (Profile of Mood States questionnaire).

The battery of evaluations encompasses relatively inexpensive, easy to administer 'pen and paper' tests. However, a number of automated test batteries have been developed that either replicate the pen and paper tests with an automated system (Neurobehavioural Evaluation System) or were designed according to theoretical concepts in experimental psychology (Swedish Performance Evaluations System). Specifically, such automated tests include:

- Behavioural assessment and research system.
- Cognitive function scanner.
- Information processing and performance test battery.
- Milan automated neurobehavioural system.
- Microtox system.
- Neurobehavioural evaluation system.
- Swedish performance evaluations system.

**Table 2. Examples of tests used in a clinical neuropsychological battery (24)**

| Domain/tests | Description/comments |
|---|---|
| General intellect | |
| Wechsler Adult Intelligence Scale Revised (WAIS-R) | Full-scale, verbal and performance IQ constitute measures of general level of cognitive ability compared with population norms. |
| Peabody Picture Vocabulary Test | Measures verbal intelligence in adults. |
| Wide-range Achievement Test | Measures academic skills in arithmetic, spelling and reading. |
| Attention and executive function | |
| Digit Span Subtest (WAIS-R) | Immediate recall of digits forwards and backwards. Measured simple attention and tracking. |
| Trail Making Test | Connect-a-dot task. Measures attention, sequencing and scanning. |
| Continuous Performance Test | Automated test of attention. |
| Paced Auditory Serial Addition | Serial calculation tests. Sensitive measure of attention/tracking. |
| Wisconsin Card Sorting Test | Requires inference of decision-making rules. Assesses mental flexibility. |
| Verbal Ability And Language | |
| Information Subtest (WAIS-R) | Recall of information usually learned at school. Robust estimate of native ability in adults. |
| Vocabulary Subtest (WAIS-R) | Fairy robust estimate of verbal intelligence in adults. |
| Similarities Subtest (WAIS-R) | Calls for inference of similarities between nominative words. Assesses verbal reasoning. |
| Boston Naming Test | Naming objects from line drawings. Sensitive to aphasia. |
| Visuospatial And Visuomotor | |
| Digit Symbol Subtest | Requires matching symbols to digits. Assesses perceptual coding and motor speed. |
| Rey-Osterreith Complex Figure | 'Copy condition' assess visuospatial planning and construction. |
| Santa Ana Form Board | Measures motor speed and coordination. |
| Finger Tapping Test | Electronic or mechanical counting of finger tapping speed. |
| Memory | |
| Wechsler Memory Scale Revised | Assesses learning and retention of verbal and visual material. |
| California Verbal Learning Test | Provides multiple measures of new verbal learning, recall, recognition memory, use of strategies, inference. |
| Rey-Osterreith Complex Figure | Recall condition assesses memory for complex visual information. |
| Mood And Personality | |
| Profile Of Mood States | Multiple scales of severity ratings of affective symptoms. Sensitive to mood disturbance and affective changes. |
| Minnesota Multiphasic Personality Inventory-Revised | Personality inventory. Provides description of current personality function. Some scales sensitive to toxic exposures. |

Recently, a test battery encompassing both hand-administered and computerised tests has been proposed by the Agency for Toxic Substances and Disease Registry (ATSDR). This Adult Environmental Neurobehavioural Test Battery consists of some of the NCTB tests recommended by WHO, computerised tests from the Neurobehavioural Evaluation System and additional behavioural neurophysiological tests of sensory and motor function not included in the other tests batteries (24, 36, 37).

Psychiatric and symptom questionnaires

Affective disorders are commonly observed following severe neurotoxic exposures. Psychotic symptoms including delusions, hallucinations, paranoia and suicidal depression have been reported following exposure to neurotoxic chemicals (38, 39). Less severe effects have also been documented including mood swings and lethargy. In some cases, such effects may be the earliest indication of neurotoxic exposure (40).

The Minnesota Multiphasic Personality Inventory (revised) was developed to assess personality and psychological changes in the clinic (41). However, due to it comprising over 500 questions, it is poorly suited for large studies of exposed subjects. As an alternative, the Profile of Mood States was developed to assess mood state, and has been incorporated into the NCTB and Neurobehavioural Evaluation System test batteries. Other questionnaires and rating scales have also been developed in order to ascertain subjective symptoms following neurotoxic exposure. These include the Q16 questionnaire (42) and the Neurotoxicity Symptom Checklist-60 (NSC-60) (43) (24).

Behavioural neurophysiological tests

Exposure to neurotoxic chemicals often initiates sensory or motor changes hence neurological examination and symptom questionnaires include items to distinguish such changes. However, contemporaneous questionnaires have been designed to ascertain quantitative measures of visual, somatosensory and olfactory function (44). To date, most work has been carried out on colour vision disturbances, contrast sensitivity, vibration sensitivity and olfactory discrimination (24).

*Electrophysiological tests*

Electrophysiological tests are used to augment data obtained from neurological examination. Nerve conduction velocity testing and needle electromyography (EMG) are widely used to examine peripheral nerve and muscle function in the clinical setting (45). Other electrophysiological techniques for evaluating CNS damage include different types of evoked potentials (EPs) and electroencephalography (EEG). The availability of digitalised EEG data has led to the development of quantitative EEG, which includes signal analysis such as detection and frequency analysis or brain mapping which allows comparison of the subject with normative values or different groups of patients for the purpose of diagnostic discriminant analysis.

In contrast to EEG, EPs are cortical responses elicited by the specific stimulation of specific sensory pathways. Several EPs may be measured, including:

- Sensory evoked potentials (SEPs); peripheral sensory stimuli (auditory, visual, somatosensory).
- Visual evoked potentials (VEPs); retinal stimuli (flashing light).
- Brainstem auditory evoked potentials (BAEPs); auditory stimuli (click, pulse).
- Somatosensory evoked potentials (SSEPs); peripheral nerve stimuli (electric shock to nerve in wrist, knee or ankle).

Dose-response relationships have been reported between EPs and blood concentrations of neurotoxic compounds. For example, a curvilinear relationship was found between BAEP and blood lead concentrations and a biphasic relationship was observed between VEP and tetrachloroethylene exposure (24).

### *Use of neuroimaging techniques*

Neuroimaging techniques present a unique and invaluable tool with which brain function or dysfunction may be assessed in a non-invasive manner. Neuroimaging has traditionally been used as a diagnostic tool, usually to verify a diagnosis based on clinical symptoms. However, neuroimaging characteristics allow it to be used to predict the predisposition of a healthy individual to develop a neurological disorder, to identify the presence of the disease and to follow the progression of the disease.

Recently, imaging is being increasingly used in neurotherapeutics to assess the effectiveness of a treatment in neurodegenerative diseases over time. In diseases such as AD or PD, neurological changes occur prior to clinical symptoms, hence biomarkers are required that ideally detect changes in the presymptomatic stage. Once identified, therapies could possibly be used to slow down or reverse neuronal loss or damage that lead to the development of symptoms (46).

Several types of neuroimaging are currently in use. Magnetic resonance imaging (MRI) and various advanced MRI techniques produce images of the brain whereas positron emission tomography (PET) and single photon emission computerised tomography (SPECT) give functional or biochemical information in a non-invasive manner (24). The results obtained can be integrated with neurobehavioural data to get an overall picture of the disease state, of the effect of chemicals on the brain and behaviour (20). Different imaging techniques allow the study of gene and protein expression, the induction or inhibition of enzymes or, in the case of AD, the formation and density of amyloid plaques.

#### *Magnetic resonance imaging*

Over the last decade conventional MRI and advanced MRI techniques have offered the potential for the diagnosis of neurodegenerative Parkinsonian disorders. With the use of MRI it is possible to determine whether individuals showing behavioural changes also exhibit neuronal loss and/or regional cell volume. Structural MRI, proton magnetic resonance spectroscopy ($^1$H-MRS), diffusion weighted imaging (DWI), magnetisation transfer imaging (MTI), magnetic resonance volumetry (MRV), field-dependent-$R_2$ increase (FDRI) and magnetic resonance imaging microscopy (MRM) have all reported abnormalities in the substantia nigra of PD patients compared with healthy individuals (47-51). In the early stages of PD newer MRI techniques such as MRV, MTI, DWI and $^1$H-MRS have better sensitivity in detecting abnormal features compared with conventional MRI techniques (48).

Structural MRI uses proton density and so called proton relaxation to form a high resolution structural image of the brain. In contrast, $^1$H-MRS measures the resonance of proton-containing chemical markers, the frequency of which reflects the local environment and the amplitude indicates the concentration. Examples include changes in N-acetyl aspartate or myoinositol, markers of mature neuronal integrity or glial cell markers, respectively. Hence reduction of such markers detected by $^1$H-MRS may be indicative of neuronal loss or dysfunction (52). Functional MRI techniques measure changes in blood flow by measuring the paramagnetic differences between oxygenated and deoxygenated haemoglobin in response to altered demands due to neuronal activation (52).

Field-dependent-$R_2$ increase MRI has been used to quantify brain iron concentrations. The degree to which MRI magnetic field strength affects the measured transverse relaxation rates ($R_2$) defines the FDRI measurement and the relaxation times ($T_2$) are obtained with two instruments with different field strengths (53). The presence of ferritin increases $R_2$ with increasing magnetic field-strength hence this field dependent $R_2$ increase correlates with the iron contained in the ferritin molecules (49). $T_2$-weighted MRI is also able to indirectly measure tissue iron concentrations. The $T_2$ is shortened in proportion with regional iron content thereby reducing the signal intensity (49, 51).

Magnetic resonance imaging microscopy has several advantages over clinical MRI in that it gives higher resolution. Clearly, the three dimensional images produced by MRM are superior to conventional histological techniques as it allows visualisation of the brain in any plane or depth. It is also quicker to perform as the brain does not need to be sectioned and stained (20).

*Positron emission tomography*

Positron emission tomography and the newly developed technique of microPET may be used to assess neurotoxicity and cell loss such as apoptosis. Specific ligands have been developed to elucidate the function of dopaminergic and serotonergic synapses, as well as ligands to assess dopamine re-uptake and post-synaptic dopamine receptor binding (20). Several studies have recently reported the application of PET in various aspects of neurotoxicity research (52, 54-57).

Positron emission tomography is commonly used to diagnose and follow the progression of neurodegenerative diseases and can quantify the extent of neuronal loss and the development of compensatory changes. It has a spatial resolution of 4-6 mm hence is highly sensitive and many radioactive nuclides have been produced for use in PET scanning (52). The ability to label many compounds enables different mechanisms to be investigated, such as the presynaptic loss of dopamine terminals, adaptive postsynaptic changes in dopamine receptors and the synaptic release of dopamine. Furthermore, quantitative information on blood flow, glucose, oxygen, dopamine metabolism and brain receptor binding may be obtained, although biological and pharmacological factors that may lead to such changes should be taken into account (55). The disadvantage of PET is that the positron emitters used have short half lives of between 2 and 100 minutes hence are expensive to produce.

*Single photon emission computerised tomography*

Single photon emission computerised tomography is one of the most commonly available functional brain imaging techniques used in clinical practice to diagnose or assess the progression of neurodegenerative diseases or to investigate cognition or image neuroreceptor systems. It has been used in a number of studies as a clinical tool for the differential diagnosis of neurotoxicity (52, 54, 58, 59).

Initially SPECT was less sensitive than PET as it had a lower spatial (8-16 mm) and temporal resolution. However, much work has recently been carried out to improve sensitivity of SPECT by improving the resolution to approximately 4 mm (60). Although the number of radiopharmaceuticals available for SPECT is lower than for PET, those used in SPECT have longer half lives hence are easier and cheaper to produce, making SPECT more widely available than PET (52, 54). However, SPECT does not correct for scatter and common SPECT radionuclides have a more limited binding affinity with neurochemicals than those used in PET. In addition, safety aspects of administration of radioisotopes to the patient should be considered (52).

### *Neuroimaging in Parkinson's Disease*

Functional imaging techniques significantly aid in the understanding of pathophysiology of PD and help in its diagnosis. The development of PET and SPECT with different tracers, $^{1}$H-MRS and high-field MRI with 3-dimensional volumetric acquisitions and DWI have been used for structural imaging and as biological markers for diagnosing PD and can be used to follow the progression of the disease, to study disease mechanisms and the effect of therapy. Such techniques are able to detect brain abnormalities, changes in brain metabolism, cerebral blood flow and presynaptic and postsynaptic dopaminergic abnormalities in Parkinsonian syndrome (52, 54, 55).

Positron emission tomography and SPECT differentiate between clinically probable PD patients and healthy individuals (figure 2) or those with tremor by evaluating the severity of the dopamine loss using presynaptic dopamine tracers and other adaptive changes in mechanisms related to the dopamine system (55). Currently there are three main ways of monitoring pre-synaptic dopamine function with PET. $^{18}$F-6-fluoro-L-dopa ($^{18}$F-dopa) radiotracer is used to reflect the dopaminergic nerve density, storage of dopamine as well as the terminal dopa decarboxylase activity that converts dopa into dopamine (54, 61, 62). Other radionuclides used to monitor pre-synaptic dopamine function with PET include a variety of $^{18}$F and $^{11}$C labelled antagonists to determine dopamine transporter density, using SPECT, and $^{11}$C-dihydrotetrabenazine (DTZB) to determine vascular monoamine transporter type 2 (VMAT 2) density (62).

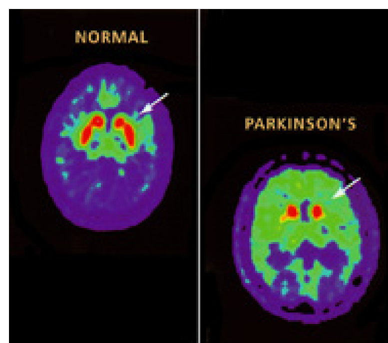


Figure 2. PET scans of a normal volunteer (left) and a Parkinsonian patient (right).

Studies using $^{18}$F-dopa PET showed that 25 % of asymptomatic adult relatives of PD patients had subclinical putamen loss of dopamine terminal function and reduced $^{18}$F-dopa uptake, some of which developed clinical symptoms during the subsequent 5 years. Such reduction in uptake correlated with neuronal degeneration as shown by pathological studies (52, 54, 61). There is also a relationship between $^{18}$F-dopa uptake and the degree of motor disability and disease progression. The presence of motor fluctuations slightly correlates with $^{18}$F-dopa uptake, suggestive of a role for altered storage capacity in dopaminergic terminals. Recent studies have also shown a correlation between $^{18}$F-dopa uptake in the frontal cortex and deficits seen whilst carrying out verbal tasks and memory tasks, and a significant decrease in $^{18}$F-dopa uptake in PD patients with dementia as compared with controls (61).

The disadvantage of using $^{18}$F-dopa PET is that false negatives may be obtained in early stage PD due to the compensatory upregulation of dopa decarboxylase activity, in order to increase dopamine turnover and preserve dopaminergic function in the remaining neurones. Such an increase in enzyme activity underestimates the degenerative process (52, 54, 61). The use of dopamine transporter ligands such as $^{76}$BR-FE-CBT are more sensitive than $^{18}$F-dopa to detect dopaminergic degeneration in early stage PD and

reduces the probability of obtaining a false negative result as dopamine transporters are regulated by different mechanisms to dopa decarboxylase (61).

Studies with SPECT ligands have used visual or semiquantitative methods to measure striatal dopamine transporter binding. A number of dopamine transporter ligands have been developed for use in SPECT and are mostly tropane derivatives. $^{123}$I-2-β-carbomethoxy-3-β-[4-iodophenyl] tropane ($^{123}$I-β-CIT) is commonly used in SPECT, although due to its slow kinetics is not suitable for PET. A fluoropropyl derivative of βCIT may be labelled with $^{18}$F and $^{11}$C and are commonly used (54). Dopamine transporters are found exclusively in dopamine axons and dendrites and levels correlate with striatal DA concentrations, making it a suitable marker of dopamine nerve terminal density. Studies have shown significant reductions in striatal binding in PD compared with healthy individuals, predominantly in the caudate and anterior putamen (54, 62). The reduction in uptake of $^{123}$I-β-CIT (2-β-carbomethoxy-3-b-[4-iodophenyl] tropane) radiotracer had a good correlation with the severity of symptoms and stage of PD. However, despite its high specificity and sensitivity for diagnosis of younger PD patients, due to the decrease in specificity in older patients, its non-selectivity for dopamine transporters, and it being affected by regulatory changes and age, it is not the most suitable radiotracer with which to follow the progression of PD (58, 59, 62).

The use of SPECT has also been used to monitor neuroprotective agents in PD. However, the uptake of $^{123}$I-β-CIT is not influenced by the use of dopaminergic medication indicating that dopamine transporters are not influenced by such therapies (61). Postsynaptic receptors have also been investigated using SPECT which showed that striatal binding was conserved or upregulated in PD, suggesting that only presynaptic nerve fibres are likely to be affected (52).

Vascular monoamine transporter type 2 is found exclusively in the brain and has been used as a marker of disease progression in PD. This transporter is responsible for the uptake of intra-cytoplasmic monoamines into synaptic vesicles. $^{11}$C-dihydrotetrabenazine binds to VMAT 2, blocking the uptake of monoamines into the vesicles therefore has been used to monitor the integrity of striatal monominergic nerve terminal density (62). The use of DTBZ has a number of advantages over using ligands for dopamine or dopamine transporters. It undergoes only limited metabolism and metabolites do not cross the BBB (63). Furthermore, VMAT is not regulated by conditions that alter synthesis, turnover or release of dopamine, and is not affected by medication (64, 65). Theoretically, DTBZ is not specific for dopamine, although more than 95 % of the monoaminergic nerve terminals in the striatum are dopaminergic. It is therefore considered that any binding observed largely reflects dopaminergic nerve terminal density (66). Despite this lack of specificity and it being less sensitive than measuring dopamine transporters DTBZ is still considered a useful biomarker of PD (62).

Postsynaptic receptors have also been investigated using PET. $^{11}$C-Raclopride, a dopaminergic receptor ligand, has been used in most studies. During early-onset PD an increase in binding in the putamen may indicate a compensatory mechanism in which receptors are upregulated. In more advanced PD $^{11}$C-raclopride binding normalises in the putamen and decreases in the caudate, reflecting either a decrease in dopaminergic receptors due to the progression of the disease or alternatively an effect of dopaminergic medication (61).

In addition to assessing pre- or post-synaptic dopamine function, other mechanisms involved in the pathogenesis of PD can be investigated. Increased uptake of $^{18}$F-2-fluoro-2-deoxyglucose (FDG) radiotracer demonstrated increased glucose metabolism prior to the appearance of clinical symptoms (52). $^{11}$C-PK11195 PET has been used to detect activated microglia as it binds selectively to benzodiazepine sites. In PD, loss of the

substantia nigra is associated with the activation of microglia, as indicated by an increased signal and therefore uptake of the $^{11}$C-PK11195 marker (54). Parallel changes in microglial activation and dopaminergic terminal loss in the nigrostriatal pathway in early PD suggests that the neuroinflammatory response of microglia contributes to the degenerative process occurring in PD (67).

High-field MRI can be used to identify abnormal substantia nigra pars compacta pathology in idiopathic PD patients, and in theory, measure the volume of this nucleus. In atypical PD, atrophy of the putamen, cortex and midbrain may be identified on T2 or DWI. Recent studies have investigated iron accumulation in the substantia nigra pars compacta using T2-weighted MRI and correlated data with clinical observations. Data showed a decrease in intensity, indicative of increased iron accumulation and oxidative stress in PD patients, that correlated with poor clinical score (68).

In summary, much research has been carried out on the use of biological markers to identify PD such as the measurement of dopamine or Vascular monoamine transporter type 2 (VMAT 2) by PET and SPECT imaging, or the identification of brain abnormalities or changes in brain metabolism or blood flow using high field MRI and DWI. Such neuroimaging techniques have aided the diagnosis of PD, as well as providing a tool with which to follow the progression of the disease and the effect of therapy in affected individuals.

### *Neuroimaging in Alzheimer's Disease*

Neuroimaging, such as the use of PET and SPECT, has been often used as a tool with which to diagnose AD or differentiate it from other dementias or normal aging (figure 3), to identify AD in asymptomatic or presymptomatic patients, as well as to monitor the progression of the disease or assess the effect of therapy (69). However, to date, it is unclear how PET and SPECT correlate with clinical diagnosis when used as a biomarker for AD (70). Many studies have found that the severity of functional lesions correlated with severity and type of cognitive impairment (71), and others reported a reduction in metabolism as the disease progressed (72).

The use of PET and SPECT imaging has been commonly used as biomarkers of disease progression. Regional cerebral metabolism, cerebral perfusion and cerebral activation can all be investigated using imaging techniques, as well as investigating the presence and density of amyloid plaques, neurofibrillary tangles and activated microglia. Moreover, MRS allows quantitative studies to be carried out on various compounds important for brain function (69).

Regional cerebral metabolism studies with PET have been carried out using FDG as a marker of glucose metabolism (figure 3). In early AD, regional metabolism decreases and during disease progression, other regions become affected. Such changes correspond to the neuropathologic changes in AD although the sensitivity and specificity are debatable (69).

Regional cerebral perfusion studies with SPECT are widely carried out in clinical practice. Several radiotracers have been used, the most common being Tc-99 m hexamethyl propylamine oxime (HMPAO) and Tc-99m ethyl cysteinate dimer (ECD) (69). Using such tracers, studies have demonstrated that regional perfusion was decreased in AD in several areas of the brain with a relatively high sensitivity and specificity (73). Other studies compared the accuracy of clinical diagnosis based on clinical findings, with and without the use of SPECT. Data showed that a 'positive' SPECT increased the probability of a clinical diagnosis of AD in cases of both probable and possible cases of AD (74).

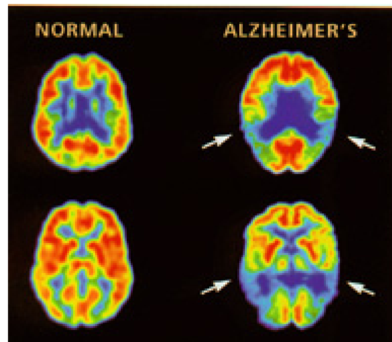

Figure 3. PET scans of a normal volunteer (left) and an Alzheimer's patient (right).

Cerebral perfusion has also been evaluated by using MRI. Regional cerebral blood volume can be measured by injecting a paramagnetic contrast that causes a signal decrease in the microvasculature in AD patients. Recently, arterial spin-labelled blood flow MRI has been used and was able to detect a decrease in perfusion in AD patients compared with healthy individuals. This latter technique does not necessitate the use of ionising radiation, provides similar information to that of SPECT and is easier to calculate blood flow. However, compared to SPECT, it has a poorer signal to noise ratio, is more sensitive to movement during imaging and can underestimate blood flow (69).

Positron emission tomography has been used to measure regional cerebral perfusion and activation studies whilst performing specific tasks. The radiotracer $H_2{}^{15}O$ is used to measure regional cerebral blood flow, which increases in parallel to the amount of regional oxygen required and is indicative of an increased synaptic activity in that specific area of the brain. For activation studies, PET scanning of either a specific region or the whole brain is carried out at rest and when the patient is undergoing a specific task, such as memorising words. Studies have demonstrated that to carry out the same task, some areas of the cortex are more extensively activated in early AD compared with controls (69).

Functional MRI has also been used in activation studies, as has MRS. Functional MRI has a greater temporal and spatial resolution compared to PET, and has the advantage of not exposing the patient to ionizing radiation. As with PET, an increase in activation appears to reflect functional compensation for neuronal loss. Magnetic resonance spectroscopy allows the relative measurement of a number of neurochemical important for brain function. It can measure N-acetyl aspartate and myoinositol which decrease and increase, respectively in AD patients. Similarly, phosphodiesters and glycerophosphoethanolamine may be evaluated, which are associated with the amount of amyloid plaques and the presence of psychotic symptoms, respectively. Such quantitative studies have a high sensitivity but low specificity (69, 75).

One of the major pathological features of AD is the presence of neuritic plaques composed of β-amyloid fibrils, neurofibrillary tangles of hyperphosphorylated tau and deficits in neurotransmitters. Several markers are available for use in PET imaging. The three main compounds used are 2-(1-(6-[(2-fluoroethyl)(methyl)amino]-2-naphthyl) ethylidene)malononitrile ($^{18}$F-FDDNP), $^{11}$C 6-hydroxy-benzothiazole (6-OH-BTA-1; $^{11}$C-PIB; Pittsburgh-Compound B), and 4-N-methylamino-4'-hydroxystilbene ($^{11}$C SB-13). In order to be useful for imaging amyloid plaques, the radiolabelled tracers must show high specific and selective binding to β-amyloid aggregates and have a high brain penetration (76). $^{18}$F-FCCNP, $^{11}$C-PIB and $^{11}$C SB-13 bind to neurofibrillary tangles and amyloid plaques. Initial studies using post-mortem samples have demonstrated that the compounds accumulated in certain areas of the brain in AD samples compared with

controls (76-78). Furthermore, $^{11}C$ PIB was tested in AD patients and controls and deposition of amyloid plaques were only observed in individuals with AD (69).

As with PD, activated microglia are present in the brains of AD patients. It is thought that they mediate neuronal damage or act in a protective manner following damage caused by other agents. Using PK11195-PET, an increase in expression of benzodiazepine receptors was demonstrated in AD individuals, indicative of microglia activation (69).
In summary, PET, SPECT and MRI may be used to assess cerebral metabolism, perfusion and activation, as well as identifying the presence of amyloid plaques, neurofibrillary tangles and activated microglia. Such techniques have aided the diagnosis of AD. Furthermore, they have been used to differentiate between AD and other dementias or normal aging, to identify AD in asymptomatic or presymptomatic patients, to follow the progression of the disease and to assess the effect of therapy.

# Neurodegenerative diseases: mechanisms and possible chemical aetiology

## Parkinson's Disease

### *Mechanisms of neurotoxicity in Parkinson's Disease*

Parkinson's disease is a common neurodegenerative disorder characterised by four cardinal signs, *viz.,* resting tremour, bradykinesia, rigidity and postural instability. The aetiology of PD is thought to be multifactorial, being derived from environmental factors acting on genetically predisposed, aging individuals (10, 79, 80).

Parkinson's disease is characterised by loss of dopaminergic neurones in the substantia nigra pars compacta that leads to the depletion of dopamine, leading to disruption of cerebral neuronal systems responsible for motor functions (80). Such neurodegeneration is accompanied by the presence of neuronal cell bodies (Lewy bodies) in the substantia nigra and Lewy neurites in the surviving dopaminergic neurones (81-83). Lewy bodies are found in many regions of the nervous system including the substantia nigra, locus ceruleus, limbic areas, hypothalamus, nucleus basalis, cranial nerve motor nuclei and central and peripheral divisions of the autonomic nervous system (79, 84).

Two hypotheses exist regarding the pathogenesis of the disease. Firstly, misfolding and aggregation of proteins such as $\alpha$-synuclein lead to death of dopaminergic neurones in the substantia nigra pars compacta. Secondly, degeneration of dopaminergic neurones may be due to apoptosis, oxidative stress or excitotoxicity. Defects in the mitochondrial complexes are thought to cause decreased ATP synthesis and oxidative stress leading to lipid peroxidation, DNA damage and inhibition of the mitochondrial respiratory chain. Alternatively, excitotoxicity occurs when excess glutamate changes calcium permeability via N-methyl-D-aspartate (NMDA) receptors, leading to excessive nitric oxide formation and uncoupling of the mitochondrial proton gradient (79, 82, 85).

The pathological process of PD involves serotinergic, norepinephrinergic (noradrenergic) and acetylcholinergic neurones. Loss of dopaminergic nerve terminals is responsible for most movement disturbances and imbalance of these neurotransmitters with acetylcholine are also involved in the psychiatric, cognitive and autonomic dysfunctions characteristic of PD (79).

Fibrils of $\alpha$-synuclein have commonly been reported in Lewy bodies as well as related proteins such as synphilin-1, parkin, ubiquitin and ubiquitin-pathway-related enzymes (10, 79, 85).

$\alpha$-Synuclein appears to play a key role in the pathogenesis of PD although the exact mechanism by which $\alpha$-synuclein causes dysfunction and death of dopaminergic neurones in the substantia nigra is unknown. Recent findings have shown that at low (nanomolar) concentrations, $\alpha$-synuclein protects neurones against oxidative stress and excitotoxicity through the PI3/Akt signalling pathway in primary neuronal cultures. In contrast, high (micromolar) concentrations of $\alpha$-synuclein exert neurotoxic effects, as the accumulation of $\alpha$-synuclein in dopaminergic neurones results in apoptosis, ultimately leading to neurone degradation (79, 81-83).

Synphilin-1 appears to represent a direct α-synuclein interaction partner. Therefore synphilin-1 associates with α-synuclein and promotes the formation of Lewy bodies (81).

It has been demonstrated that parkin (a 464 amino-acid protein) may be needed for the formation of Lewy bodies. Parkin appears to interact with glycosylated α-synuclein via interaction or ubiquitination of synphilin-1, rather than wild-type α-synuclein interacting with parkin directly (81). This subsequently allows ubiquitination and degradation by the ubiquitin system.

The protein parkin mediates the ubiquitination of cellular proteins destined for degradation. Mutations in the parkin gene showed that ubiquitin-mediated proteolysis may play an important role in the pathology of PD (79, 86, 87). When the ubiquitin system is inactivated due to mutations in the α-synuclein, parkin or ubiquitin C-terminal hydrolase L1 genes, or if degradation of the misfolded or mutated α-synuclein is insufficient, aggregation of proteins occurs leading to neurodegeneration (79, 85). Oxidative damage to α-synuclein can also enhance misfolding and aggregation (85). Neuronal expression of α-synuclein and parkin mRNAs in the brain are co-expressed, suggesting these proteins may both play a role in the pathophysiology of PD (79, 86).

To date, it is unknown why α-synuclein is transformed into insoluble Lewy bodies, how misfolding of α-synuclein occurs and what triggers the misfolding.

Neuronal damage due to excess glutamate, which alters the permeability of cells to calcium by acting through NMDA receptors, is thought to play a key role in neurodegeneration. The calcium-dependent NMDA neurotoxicity is based on two mechanisms. Firstly, increased calcium influx leads to activation of nitric oxide synthase, causing excessive nitric oxide formation, which reacts with superoxide to form peroxynitrite. Secondly, the influx of calcium affects mitochondrial membrane potential, ATP synthesis, glycolysis, and increasing production of reactive oxygen species (ROS), leading to mitochondrial dysfunction, lack of calcium homeostasis and cell death (88). Dopaminergic nigrostriatial neurones are rich in glutamate receptors, and since the substantia nigra receives rich glutamatergic inputs, it has been speculated that glutamate-induced neurotoxicity may be involved in PD (79).

Alternatively, it has been suggested that the selective loss of dopaminergic nigrostriatal neurones in Parkinsonism may be due to dopamine itself or a metabolite being neurotoxic, as it has been demonstrated that 3,4-dihydroxyphenylacetaldehyde, a metabolite of dopamine, is an endogenous neurotoxin that triggers dopaminergic neurone cell loss (79).

### *Chemicals involved in Parkinson's disease*

The concept that PD may be the result of chemical exposure arose following an epidemic of Parkinsonian syndrome in young intravenous drug addicts following N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) exposure. Observed effects closely mimicked idiopathic PD clinically, biochemically and pathologically. Other chemicals that are structurally similar to MPTP, such as rotenone and paraquat further increased interest in chemical exposure being possible risk factors for PD (89, 90).

*Alkaloids*

A group of naturally occurring alkaloids have been detected in the brains of patients with PD. Due to their structural similarity to MPTP, tetrahydroisoquinoline (TIQ) alkaloids are considered to be endogenous neurotoxic chemicals and may be responsible for some effects seen in PD. Besides endogenous formation, TIQ has been detected in certain

beverages and food stuffs, including soy sauce, chocolate, beer, port wine and dried bananas and readily crosses the blood-brain barrier (91-93). Tetrahydroisoquinoline derivatives have been identified in brains of other animal species and induce Parkinsonian symptoms in rodents and primates (93).

Increased levels of TIQ in the cerebrospinal fluid (CSF) of patients with PD may cause neurotoxicity via glutathione depletion and up-regulation of α-synuclein expression, leading to oxidative stress. As well as glutathione homeostasis being altered, dopamine levels may also be increased and undergoes spontaneous autooxidation to form ROS, free radicals and quinines. The alteration of dopamine and glutathione homeostasis may lead to damage of dopaminergic neuronal cells (91, 94).

Although mechanisms have been proposed, how TIQ causes degeneration of dopaminergic neurones, by which mechanisms it occurs and what initiates such mechanisms have not been fully elucidated (91).

*β-carbolines*

Several studies have reported significantly elevated levels of β-carbolines, specifically harman and norharman, in plasma and CSF of patients with PD (95). β-carbolines are ubiquitous and form during grilling or broiling of meat, fish, dairy products, food seasonings and are present in tobacco smoke. They are also produced by industrial pyrolysis processes and so arise in smoke, industrial waste and ground water (18). β-carbolines may act as a pro-toxin and need to be activated by one or two N-methylation steps in the CNS to form neurotoxic metabolites that may play a role in the pathogenesis of PD (95).

Other β-carbolines that may have a role in Parkinsonism include 3-carboxylated derivatives of typtophan and dopamine that are formed in brewing and fermentation processes and are found in smoked food and cheese. Few studies have been carried out on the neurotoxic properties of these compounds or metabolites (18).

*Pesticides*

Interest in the possible links between exposure to pesticides and the development of PD was stimulated by the close structural similarity of the MPTP metabolite 1-methyl-4-phenylpyridine (MPP+) to the herbicide paraquat. Furthermore the Medical and Scientific Panel of the Advisory Committee on Pesticides (ACP) noted apparent consistency of the epidemiology reports linking PD with exposure to pesticides. This led to the commissioning of a detailed review of the epidemiological and toxicological literature relevant to this area, in order to provide a critical evaluation of the possible role of pesticides in the development of PD. This was carried out by the Institute for Environment and Health (IEH) and their report has recently been published (96). The IEH review concluded that there does appear to be evidence for a potential role of pesticides in the development of PD. However the current body of evidence was insufficient to establish causation for any particular pesticide.

It was noted that only one epidemiology study had shown a dose-response relationship between a specific pesticide (paraquat) and an increased risk of PD. It was felt that this finding needed to be confirmed in further epidemiological studies on different populations before it will be considered an established association. Additional toxicological research was necessary to better understand the potential mechanisms by which paraquat might act. There was some toxicological evidence that rotenone and other insecticides, for example permethrin, could also potentially act by mechanisms relevant to the development of PD. Further research, both relating to epidemiology and to toxic

mechanisms of pesticides in relation to PD, and interaction of pesticides with heavy metals, was recommended.

The IEH report has been considered by the ACP. They agreed that further mechanistic research was necessary. In addition they considered that further epidemiology could be useful where exposure to specific pesticides could be ascertained with reasonable confidence (e.g. in cohort studies of pesticide production workers or long-term prospective studies users). It was noted that the review indicated a correlation between recalled pesticide exposure and PD, but did not point to a particular toxic mechanism or hazard from a specific compound or group of compounds.

In response to the ACP recommendation Defra is in the process of agreeing research proposals relating to both further epidemiology and mechanistic work.

*Metals*

The involvement of metals has been hypothesised in the pathophysiology of a number of neurodegenerative diseases such as PD and AD (97). Metals such as manganese, iron, lead, copper and combinations of lead/copper, lead/iron and iron/copper have been investigated as potential risk factors in PD due to their accumulation in the substantia nigra and their participation in oxidative reactions such as the production of hydrogen peroxide during the enzymatic oxidation of dopamine (with concommitant conversion of hydrogen peroxide to hydroxyl radicals). Recent reports have demonstrated that metals interact with $\alpha$-synuclein and may cause formation of fibrils *in vitro*, thereby suggesting a possible role in the development of PD (11, 92).

An important mechanism by which neurotoxic chemicals may selectively target dopaminergic cells is via uptake through the dopamine transporter. Moreover, environmental chemicals that inhibit mitochondrial complex I, leading to mitochondrial dysfunction, could be of particular relevance to Parkinsonism (11).

Recent research has reported that certain pesticides and metals could accelerate conformational changes of $\alpha$-synuclein and fibril formation, both individually and synergistically, by increasing the concentration of the partially-folded intermediate conformation that leads to aggregation (10).

### *Metal concentrations in Parkinson's disease*

This report gives an overview of the distribution of metals such as iron, zinc, copper, manganese, lead and aluminium in different regions of PD brains and presents possible hypotheses regarding the mechanisms of metal-induced neurodegeneration.

The distribution of several metals, including iron, zinc, copper, manganese, lead and aluminium in the brains of PD patients are outlined in annex I. Measurements have been carried out either on post-mortem samples (97-102) or on living patients (49, 51, 103, 104).

Iron has a diverse role ranging from facilitating cellular aerobic metabolism to synthesis and signal transduction of neurotransmitters. Iron may also be cytotoxic by facilitating the generation of hydroxyl radicals from hydrogen peroxide via Fenton reactions (105). Abnormalities in brain iron metabolism have been described for a number of neurological diseases such as PD and AD. Most studies reported an increase in iron concentrations in the post-mortem samples of the substantia nigra of PD patients compared with controls (51, 97, 99-104, 106-109). Most of the excess iron in PD is stored in the substantia nigra

pars compacta, the area that shows most neuronal loss. It has been hypothesised that the increase of iron is due to the contraction of the nigral neuropil due to degeneration in Parkinsonism, hence is a secondary event to degeneration. The iron is converted from $Fe^{2+}$ to $Fe^{3+}$ generating hydroxyl radicals, leading to lipid peroxidation and neuronal damage (99, 109, 110). In addition, an increase in iron content in the substantia nigra is observed in both astroglia and reactive microglia phagocytosing dopamine neurones in PD. An increase in reactive microglia may also lead to progressive neuronal loss (109). A reduction in free-radical scavenging and peroxide detoxification mechanisms also aid in the neuronal cell damage (99). The substantia nigra is specifically affected in PD and the increase in iron in this region reflects its involvement in dopamine receptor synthesis and dopaminergic neurotransmission (108).

Data for concentrations of iron in the basal ganglia were less consistent. Contrasting data were reported for the globus pallidus as Dexter and colleagues observed a decrease in iron in the medial and lateral globus pallidus (106, 107) whereas Griffiths and Crossman reported an increase in iron concentration in the lateral globus pallidus (99). Griffiths and Crossman hypothesised that iron increased in the globus pallidus as neurones become overactive and require additional iron to deal with the extrametabolic load (99), whereas the decrease could not be explained (106, 107)

Iron content was also measured in living patients using $T_2$-MRI. Studies reported an increase in iron content in the substantia nigra (51, 103, 104), and an increase of iron in the putamen and globus pallidus in PD patients (51). In addition, data obtained with FDRI showed an increase in iron concentrations in the substantia nigra reticulata, substantia nigra pars compacta, putamen and globus pallidus in early-onset PD patients but only in the nigra reticulata in later-onset PD patients compared with controls (49).

Ferritin concentrations in different regions of the brain were also altered in PD patients, although different studies reported conflicting data. Conflicting data have been presented regarding ferritin concentrations in different brain regions. An increase in ferritin concentrations wwas reported in the substantia nigra (108), putamen and globus pallidus of PD patients (98), whereas others described a decrease in the substantia nigra, caudate, putamen, globus pallidus, cerebral cortex and cerebellum (107, 111). In contrast, Mann reported that ferritin concentration in the substantia nigra of PD patients did not differ to that in controls (101). Cellular iron homeostasis is maintained due to the regulation of transferrin receptor and ferritin mRNA. When cells are replete of iron, the transferrin receptors decrease and ferritin concentrations increase; the reverse happens when cells are depleted of iron. In some cases, an increase in iron, as seen in PD patients, is paralleled by an increase in ferritin. Alternatively authors suggested that failure to do so supports the hypothesis of defective iron handling in PD brains, leading to oxidative stress and neuronal damage (101), whereas an increase in ferritin in the substantia nigra may be sufficient to be cytotoxic (108). The discrepancies may also have arisen merely due to the different specificities of the antibodies used for the individual ferritin subunits (101, 107).

Most of the non-haem iron in the brain is stored as ferritin, a ubiquitous protein that binds and inactivates iron (49). Iron entering cells via the receptor-mediated transport of transferrin may be utilised in several metabolic processes or is sequestered by ferritin. During periods of high concentrations of iron, the concentrations of the transferrin receptors fall and concentrations of ferritin increase in order to sequester and therefore inactivate iron (101). The potential toxicity of the increased iron load in the substantia nigra in Parkinsonism may be largely determined by the extent to which it is deactivated by binding to ferritin (107). The inconsistent data showing, in some cases, failure of ferritin concentrations to increase in parallel to concentrations of iron supports the conclusion that

there may be a defect in iron handing in the substantia nigra of PD brains and the excess free iron may lead to oxidative damage that contributes to the neuronal cell death (101).

Changes in zinc and copper concentrations in PD brain samples were also evident, although data were contrasting. Some studies reported that a significantly higher concentrations of zinc were observed in the caudate nucleus, lateral putamen, total substantia nigra and substantia nigra pars compacta in Parkinsonian post-mortem samples compared with control samples (106, 107) although several studies did not find such an increase (97, 101). Zinc was also higher in the raphe plus reticular formation in PD post-mortem samples (108). Lower concentrations of copper were observed in post-mortem samples of total substantia nigra and substantia nigra pars compacta of PD patients compared with controls (106, 107) and higher concentrations were reported in the raphe plus reticular formation (108). Dexter and colleagues suggested that the observed changes in concentration of both metals was unlikely to be directly linked with alterations in iron concentrations in PD brains, as the concentration of zinc was increased in the putamen and caudate nucleus where concentrations of iron were unchanged, and copper was normal in the globus pallidus where iron was reduced (106).

The concentration of manganese was decreased in the medial putamen in PD post-mortem samples compared with controls, but not in any other region of the brain (106, 107). Similarly, the concentration of lead did not differ between PD samples and controls in any of the brain regions studied (106, 107). Due to manganese and lead being unaltered in the substantia nigra of PD patients, authors suggested that they may not play a significant role in the pathogenesis of PD (106). However, more recent research has shown that manganese can induce oxidative stress and disturb neurotransmitter metabolism (112, 113).

Two studies measured the concentrations of aluminium in Parkinsonian samples and reported contrasting data. Yasui reported a significant increase in aluminium in CNS and basal ganglia in PD samples (114), but Hirsch failed to show any significant differences in the substantia nigra and central grey substance (97). Aluminium appears to inhibit choline acetyltransferase and acetylcholine esterase activity in the CNS, a reduction of which is associated with an increase in neurofibrillary tangles. It also interferes with synaptosome uptake of choline, serotonin, glutamate and gamma aminobutyric acid, as well as inhibits neurotransmitters (114).

## Alzheimer's Disease

### *Mechanisms of neurotoxicity in Alzheimer's Disease*

Alzheimer's disease is the most common form of adult onset dementia, the aetiology of which is multifactorial. Genetic defects, oxidative stress, β-amyloid toxicity and environmental factors have all been implicated to play a role (9).

Alzheimer's disease is associated with progressive neuronal cell death in the CNS; specific pathology includes plaques composed of β-amyloid and neurofibrillary tangles mainly composed of hyperphosphorylated tau protein (115, 116).

Two hypotheses were initially proposed regarding the cause of AD. In the 'amyloid cascade' hypothesis, dysregulation in amyloid precursor protein (APP) processing results in an imbalance in β-amyloid metabolism followed by the aggregation and deposition of plaques, causing neuronal death and dysfunction leading to dementia (117, 118). In the

'neuronal cytoskeletal degeneration' hypothesis, cytoskeletal changes lead to neurodegeneration, as hyper-phosphorylation and aggregation of tau into neurofibrillary tangles are related to cell death activation (116). Such neurofibrillary tangles are a pathological hallmark of AD (116, 118).

Mutations in the APP or presenilin genes predispose to β-amyloid deposition, either by increasing β-amyloid production or enhancing APP aggregation. However, the mechanism by which proteolytic cleavage of APP generates β-amyloid requires further investigation. Mature APP is proteolytically cleaved via the non-amyloidogenic pathway (mediated by protein kinase C) or the amyloidogenic pathway (which is not modulated by protein kinase C). How these two mechanisms are regulated is not fully understood, although it has been proposed that it may be determined by neurotransmitter and hormone status (116, 117).

Tau protein usually binds to microtubules and regulates their polymerisation. However, in AD, such stabilisation of microtubules within the neurones is impaired as tau protein is hyperphosphorylated and self-aggregates, leading to microtubule depolymerisation and the replacement of microtubules with tangles (aggregated tau). Tangles accumulate within the neurones, ultimately leading to cell death (117).

Phosphorylation of tau protein is a key factor in its aggregation, although the exact mechanism has not been fully elucidated. One hypothesis is that phosphorylation of tau protein reduces its binding capacity to microtubules, as aggregated tau protein is highly phosphorylated. Alternatively, tau phosphorylation may occur after aggregation and structural change in tau protein are association with aggregation (117).

At present, it is unclear whether β-amyloid or tau protein is central to the development of AD (117). Recent studies have shown that β-amyloid formation precedes the appearance of phosphorylated tau aggregates, suggesting that (over)expression of APP or elevation of β-amyloid concentration may induce tau protein assembly (118).

A new hypothesis suggests that AD arises due to a synaptic failure, as synapse loss correlates to the pattern and severity of cognitive impairment rather than amyloid plaques or neurofibrillary tangles. Patients with mild cognitive impairment present less synaptophysin (a synaptic marker) without senile plaque formation and animal models show synaptotoxicity without amyloid plaque formation. This supports the hypothesis that β-amyloid deposition into oligomers may be synaptotoxic *in vitro* and *in vivo* (116).

Acetylcholinesterase may play a key role in β-amyloid-induced neurotoxicity in subpopulations that are more susceptible to neurodegeneration, as a dysfunction of acetylcholine-containing neurones in the brain substantially contributes to the cognitive decline observed in AD patients. Acetylcholinesterase is predominantly associated with the amyloid core of mature senile plaques, pre-amyloid deposits and cerebral blood vessels. In transgenic mice, acetylcholinesterase interacts with β-amyloid accelerating amyloid plaque formation and promoting the size of the plaque formed (116).

Alzheimer's disease may also be associated with reactive oxygen and nitrogen species (resulting oxidative stress). Potential neurotoxins such as peptides, excitatory amino acids, cytokines or drugs induce neuronal cell death via oxidative downstream pathways which can cause acute oxidative destruction or activate secondary events leading to apoptosis (119).

Glutamate-mediated neurotoxicity is frequently cited as being involved in the pathogenesis of AD. Glutamate is a key neurotransmitter in primary perception and cognition. Glutamatergic neurones form the main excitory system in the brain and play a

predominant role in many neurophysiological functions. Excessive activation of glutamate receptors can result in excitotoxicity, namely neuronal dysfunction and neuronal death. In AD, excessive glutamate and glutamatergic activity occurs, although glutamate does not exert its normal physiological role as the NMDA glutamate receptors are overstimulated in a tonic rather than phasic manner. Such continuous activation of the NMDA receptor leads to excessive influx of calcium and an increase in synaptic 'noise', which impair long-term potentiation and neuronal plasticity (learning) and, in some cases, cell death (120).

***Chemicals involved in Alzheimer's disease***

*Acrolein*

Acrolein is a ubiquitous environmental pollutant that is generated as a combustion by-product of hydrocarbons (121) and is strongly nucleophilic. Acrolein-protein adducts have been detected in the amygdala and hippocampus of AD patients which show marked pathological changes in AD (121, 122). It has also been hypothesised that acrolein adduct formation with lysine residues on tau proteins may play a role in the development of neurofibrillary tangles (122) as acrolein was found in more than 50 % of AD neurofibrillary tangles and in dystrophic neurites surrounding senile plaques (121). Hence, acrolein appears to be a neurotoxic agent that may be involved in the pathophysiology of AD (121, 122).

*Aluminium*

Aluminium has been suggested to be associated with β-amyloid in AD, although this still remains controversial (123). It precipitates β-amyloid *in vitro* as distinct fibrillar structures and *in-vivo*, aluminium increases the burden of β-amyloid in the brain, although the aetiology of such an association is unknown (123, 124). *In-vivo*, aluminium also promotes the formation and accumulation of β-amyloid and hyperphosphorylated tau protein. In addition, aluminium mimics the deficit of cortical cholinergic neurotransmission seen in AD and also increases iron-induced oxidative stress (123, 125).

In the recent Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) subgroup consultation document on the Lowermoor water pollution incident it was stated that considering information derived from mechanistic studies, together with information from epidemiology, suggests that aluminium exposure does not cause AD (although it has not been established whether aluminium may contribute indirectly in some cases). It seems increasingly unlikely that there is a link between aluminium exposure and AD (126).

*Metals*

Redox reactive transition metals such as copper and iron may be involved in free radical-mediated oxidative stress, observed during AD progression. This hypothesis is supported by the increased concentration of Cu and Fe levels in AD brains and changes to expression of metal-binding proteins in Alzheimer's patients (127).

Although transition metals are tightly regulated to prevent excessive generation of free radicals, additional factors such as homocysteine can exacerbate their toxic potential by inducing excitotoxicity or through reduction of $copper^{2+}$ to $copper^{+}$ resulting in the production of free radicals. Increased plasma levels of homocysteine (hyperhomocysteinaemia) have been suggested as a risk factor in AD. *In-vitro*, homocysteine potentiates neurotoxicity via pathways involving Cu reduction, hydrogen peroxide ($H_2O_2$) production and the interaction between $copper^{+}$ and $H_2O_2$ to produce the

highly reactive hydroxide radical (127, 128). Homocysteine also induces neuronal damage through over activation of ionotropic glutamate receptors (excitotoxicity) in susceptible populations. Further work should be carried out to elucidate the relationship between homocysteine/copper-generated oxidative stress and neuronal damage *in vivo* (127, 128).

Many studies have shown that β-amyloid is neurotoxic *in vitro*, and that metal ions, such as copper$^{2+}$, iron$^{2+}$ and zinc$^{2+}$ can potentiate this neurotoxicity (115-117). Copper promotes the aggregation of β-amyloid, as well as the interaction between β-amyloid and copper$^{2+}$ and iron$^{2+}$ generating ROS and $H_2O_2$ causing lipid peroxidation, protein oxidation and oxidative stress (9). In addition, metal ions may also play a role in the oligomerisation of β-amyloid, which could lead to the formation of the β-amyloid plaques (116, 117).

Recent research has shown that neurodegeneration associated with AD appears to involve protein oxidation, increased expression of antioxidant enzymes and lipid peroxidation, which produce several α,β-unsaturated aldehydes such as acrolein and malondialdehyde.

***Metal concentrations in Alzheimer's disease***

The aluminium hypothesis has not been discussed in detail in this review since it has been dismissed following recent COT consideration of the issue (126).

The distribution of several metals, including iron, zinc, copper, manganese, lead, aluminium in the brains of PD and AD patients are outlined in annex I.

Contrasting data have been reported regarding the iron content in different brain regions in patients with AD. A significantly increased concentration of iron was reported in the post-mortem samples of the frontal pole, temporal pole, anterior parietal, cerebellum, hippocampus, globus pallidus and amygdala in AD patients compared with controls (105, 129-131), although others failed to measure a significant change in iron content (99, 132-134). It has been hypothesised that elevated iron, probably derived from ferritin, may be involved in the induction of free radicals leading to the formation of amyloid deposits commonly found in AD. This, together with increased peroxidation, indicate that the production of ROS may play an important role in the propagation of AD and neuronal degeneration (129, 131).

It has been suggested that zinc may play a role in AD. Studies with control patients revealed that the concentration of zinc in the brain remained fairly constant throughout adult life (130). Several studies measured the concentration of zinc in different regions of the brains from AD patients. Significantly increased zinc concentrations were measured in the frontal pole, temporal pole, anterior parietal, hippocampus, amygdala and cerebellum in AD samples compared with controls (129, 130, 132). The elevation of zinc may play a role in senile plaque formation in AD, especially in areas such as the amygdala, hippocampus and inferior parietal lobule. Zinc has also been shown to increase the aggregation of the amyloid beta-peptide (Aβ), possibly leading to the increased deposition of the peptide and formation of senile plaques (129, 130). Experiments carried out *in vitro* have suggested that zinc could play a role in Aβ aggregation or direct neurone degeneration. Therefore, it is possible that the disruption of zinc homeostasis is involved in the pathogenesis of AD (129, 130).

Copper was significantly decreased in the amygdala and hippocampus in AD patients compared with controls (130), whereas no change was observed in neuritic plaques or neuropil in the amygdala (132). Copper is bound to enzymes such as superoxide dismutase, cytochrome c and ceruloplasmin. Most, if not all physiological copper is bound,

the decreases observed in some brain regions affected in AD may reflect a decrease in some of the enzymes/proteins (130).

No significant changes in the concentrations of mercury (129) or manganese (99, 133, 135) were reported in any of the brain regions of AD patients.

## Developmental neurotoxicity

### *Mechanisms of developmental neurotoxicity*

Developmental neurotoxicity is the effect of chemicals on the developing nervous system during the pre- or postnatal period principally as a result of chemical exposure to the mother during pregnancy or lactation. It may cause changes in behaviour, neurohistology, neurochemistry, neurophysiology and/or gross morphology of the CNS (14). Limited cell regeneration occurs following damage hence chemicals have the potential to cause long-term or irreversible delays or deficits in neurological and neurobehavioural development (136, 137). A number of environmental chemicals such as lead and methyl mercury have demonstrated the potential to cause neurological disturbances following exposure during the perinatal period in animals (1, 138). Therefore, it has been hypothesised that pre- or post-natal exposure to chemicals may play a role in the aetiology of neurological developmental changes in humans. Pre- or postnatal exposure to chemicals has been reported to lead to reduced cognitive development and verbal communication, decreased auditory sensitivity and visuomotor performance, blindness, deafness, delayed walking and behavioural problems amongst others.

The developing nervous system is especially susceptible. Many of the vulnerable developmental processes are completed before birth hence the prenatal CNS is particularly sensitive to chemical insult (139). Furthermore, the rapid development during embryonic and fetal stages makes them more vulnerable to acute chemical exposures, whereas the later developmental processes are more likely to be affected by chronic exposure. As well as prenatal developmental process occurring, the development of some areas of the brain continues into the postnatal period (14). Completion of the blood brain barrier (BBB) that protects infants from some chemical exposures occurs in humans at approximately 6 months of age (1), hence chemical insult prior to this may result in neurological damage (140).

The maturation of the CNS consists of a series of processes that occur in sequence and are dependent on each other. The sequence of events may be categorised under several headings namely gross morphology, proliferation and cell death, migration, synaptogenesis, myelination, transmitters and receptors, and trimming, which is thought to eliminate unimportant synapses whilst preserving more important one (141).

Studies with rodents or with autopsy material have demonstrated that at the end of the embryonal stage (12$^{th}$ week of gestation) organogenesis of the brain is relatively well progressed (136, 137). Chemical insult during these early stages of CNS development can cause gross structural abnormalities (139).

Programmed cell death plays a fundamental role in the control of the final number of neurones hence exposure to chemical agents that interfere with cell proliferation have the potential to affect development (140). Chemicals may either prevent cell division or cause cell death to proliferating or migrating cells, but regardless of the mechanism, the number of actively proliferating cells decreases (140)

Developmental processes occur at specific times in specific regions, hence the effects of chemicals that interfere with cell proliferation are extremely time-dependent, resulting in different patterns of morphological anomalies and different behavioural effects (140, 141). Chemicals such as valproic acid can interfere with cell proliferation causing neural tube defects and mental retardation in humans (1, 142). In rats, loss of the motor neurones of the abducens nucleus occurs following exposure to valproic acid on day 12 of gestation (142). However, if rats are exposed on day 13, no loss of motor neurones is observed as all neurones have been formed. Effects may then be observed in other regions in which developmental processes occur slightly later in gestation (137). Similarly, exposure of rats to methylazoxymethanol during the mid-foetal period causes hyperactivity, due to damage to the cerebral cortex and hippocampus, whereas exposure on day 19 causes hypoactivity due to damage to the cerebellum.

An important feature of such early neurone loss is that expression of the deficit may not become evident for some time after injury. Several therapeutic agents and trophic factors have been identified that influence cell death of neurones, but to date, no environmental chemicals have been identified (140, 143).

During the developmental period most neurones do not stay in their place of origin but migrate to another region of the brain. A number of chemicals are known to lead to ectopic neurones, neurones observed in areas different to their intended region. However, it is unclear whether chemicals interfere directly with mechanisms of migration, whether they disturb the area through which cells need to migrate or injure the cells making them unable to migrate (137, 140).

As with cell proliferation and cell death, the timing of migration is well established in different regions of the brain. Migration of the cerebral cortex is complete at approximately five months after birth and cerebellar migration continues for about 12 months (144). In addition, although neuronal differentiation occurs prenatally, further neuronal maturation continues into the first postnatal years, including the arborisation of dendrites, synapse formation, and myelination of fibres (136, 140). Therefore, both the prenatal and early postnatal phases of CNS development are vulnerable to chemical insult.

Genetic control of migration is more fully understood. Research carried out in mice demonstrated that the gene *Reelin* acts as a signal to migrating neurones and is critical for normal migration in the layered cortical structures of the cerebral cortex, hippocampus and the cerebellum amongst others. An increased or decreased expression of *Reelin* results in polymicrogyria (too many folds on the surface of the brain) or lissencephaly (too few folds on the brain surface which appears smooth) with cerebellar hypoplasia, respectively (140). In addition, the gene encoding for cyclin dependent kinase 5 (*cdk5*) is expressed in migrating neurones and is thought to play a role in the cytoskeletal processes needed for cell movement (140).

It is widely accepted that mechanisms by which neurones form synapses, release transmitters and form receptors for transmitters are potential targets for chemical agents. The formation of synapses is a critical part of neuronal differentiation and the establishment of the basic pathways is essential to later development (140, 145). Synaptogenesis occurs early in development and continues throughout life. Synapses are produced at a high rate between mid-gestation until the end of the first post-natal year, during which time synaptic density reaches a maximum. Subsequently, synapse production slows down therefore elimination exceeds synapse production causing a decline in synapse density (145). Malnutrition or exposure to lead has been shown to decrease post-natal synaptogenesis, leading to a lower number of synapses per neurone (137, 140).

The formation of myelin occurs later in development than proliferation and migration of neuronal populations. Myelination begins in the second trimester of pregnancy in the peripheral nervous system in order to increase the signal conduction velocity. Shortly thereafter, oligodendrocytes in the CNS begin to produce myelin. Myelination peaks during the first postnatal year but takes approximately 40 years until the process is completed (145). Malnutrition, as well as perinatal exposure to ethanol or postnatal exposure to lead may cause significant decreases in myelination (141).

Research in both animals and humans has demonstrated that neurotransmitters can be detected prenatally and their distribution is usually broader in the fetus than in the adult. In addition the distribution of receptors can change as development proceeds. Hence both receptors and transmitters may undergo interference by chemical agents (137, 140). Recent studies of glutamate and acetylcholine transmitters showed different time courses of maturation in different brain areas as well as changing transmitter receptor modulation during development (137, 146). Muscarinic cholinergic receptor binding, NMDA receptor binding, and acetylcholinesterase were lower in prenatal or postnatal cerebellum compared with young adults, whereas kainate binding and nicotinic acetylcholine receptors were higher in the prenatal stage. Exposure to chemicals such as organophosphates and carbamate insecticides, which inhibit acetylcholinesterase, may affect the development of the cholinergic system in the brain (137, 146).

Interference with the development of receptors has been demonstrated in animal models. The number of dopaminergic receptors in rats was decreased following prenatal exposure to haloperidol, whereas exposure during suckling initiated an increase. Therefore exposure during the brief critical period when receptors are formed may result in impaired formation (137).

Other processes that may be vulnerable to environmental interference include trimming or pruning. However, to date no environmental chemicals have been identified that either increases or decreases trimming (137, 140).

***Chemicals involved in developmental neurotoxicity***

Several neuroactive drugs have been identified as human developmental toxins as they are able to pass the placental barrier and produce adverse effects on the developing fetus. However, few environmental chemicals have been specifically investigated for their neurodevelopmental toxic potential (14).

Many chemicals have been reported to be neurotoxic in experimental animals, some of which could also affect the developing nervous system causing malformation and growth abnormalities in the offspring (14). Andersen and colleagues identified nine pesticides, 14 solvents, ten metals and organometal compounds and six other industrial chemicals as having an adverse effect in the offspring after prenatal exposure in experimental animals. The authors stated that there was convincing evidence that low level exposure of neurotoxic chemicals during vulnerable developmental periods could induce permanent functional changes in the CNS. Due to neurogenesis and maturation of the nervous system being similar in animals and humans, they suggested neurological changes could occur if exposure to neurotoxic chemicals occurred at relevant concentrations at critical time points of development (14).

Only a small number of chemicals have been investigated for adverse effects on the development of the nervous system following prenatal exposure. These include lead, mercury, manganese, PCBs and alcohol, all of which can lead to irreversible changes in brain function during the period of rapid development of the neonatal brain (table 3). In

some cases, the dose at which nerve membrane sodium channels are affected in neonates is below that which gives no permanent effects in adults (137).

**Table 3. Neurotoxicological effects following chemical *exposure (141).***

| Chemical | Neurotoxicological effects | Exposure |
|---|---|---|
| Lead | Impaired psychological development, decreased auditory and visual sensitivity, decreased IQ | Postnatal exposure through hand-mouth activities, old lead-containing paint etc |
| Methylmercury | Ataxia, visual abnormalities, hearing loss, muscle weakness, mental retardation | *In-utero* exposure via maternal consumption of contaminated fish |
| PCBs | Poor cognitive development, developmental delays over time | *In-utero* exposure via maternal consumption of contaminated fish |
| Organophosphate pesticides | Lack of coordination, balance, stamina, short-term memory and reaction time, decreased IQ, gross and fine hand-eye coordination | Inhalation or ingestion of organophosphate pesticides. Postnatal exposure via breast milk. *In-utero* exposure. |
| Alcohol | Foetal Alcohol Syndrome (FAS), lower verbal communication, deficits in motor development | *In-utero* exposure, postnatal exposure via breast feeding |

*Lead*

Lead, a widely distributed environmental chemical, can cause peripheral neuropathy (myelinopathy) or encephalopathy after chronic high dose exposure. It is also capable of crossing the blood-brain barrier more readily in children than adults and can therefore affect cognitive ability and behaviour in children, as well as causing developmental neurotoxicity (113).

Many studies have reported the neurotoxic effects of lead in children. The main routes of exposure to lead are via hand-to-mouth activities and the diet (136). Early exposure may affect apoptosis, excitotoxicity, neurotransmitter storage and release, synaptogenesis transmission and affect mitochondrial metabolism, all playing a role in the pathogenesis of neurological effects (147, 148). At environmentally relevant concentrations, many of the toxic actions of lead arise from its ability to mimic the actions of calcium and/or disrupt calcium homeostasis thereby affecting several signal transduction pathways, such as protein kinase C (148). Such activation of protein kinase C has been reported to be involved in the inhibition of astroglia-induced microvessel formation, to contribute to the release of neurotransmitters from chromaffin and PC12 cells *in vitro* and to mediate a lead-induced rise in intracellular free calcium in bone cells. In addition, a lead-induced decrease in protein kinase C activity, due to down-regulation following sustained activation, may be related to learning and memory problems caused by exposure to lead (149). Lead also alters second messenger systems, acetylcholine, dopamine and amino acid neurotransmitter release, neurotransmitter receptor density and impairs myelin formation (137, 148).

Lead has been extensively studied as a cause of impaired psychological development in children. Children are considered to be a more sensitive risk group for lead than adults due to ingesting environmental lead though hand-mouth activities, lower body weight and higher absorption and total retention from the gastrointestinal tract than in adults (150). In addition, the fact that the BBB is not yet fully developed makes children more vulnerable to toxicity (136, 148).

Neurodevelopmental effects are the most sensitive toxicological endpoints of lead, the most significant health effect being the association with reduced cognitive development and IQ in children (151). There is some controversy as to whether there is a dose threshold; however, extensive studies relating the effect of lead with IQ are consistent with the absence of any threshold.

Electrophysiological studies have shown that children exposed to lead have a decreased auditory sensitivity and visuomotor performance as well as increased latency in brain auditory-evoked potentials (152). The meta-analyses for both cross-sectional and prospective studies concluded that lead has a small but significant effect on children's IQ (136), that did not exhibit a threshold. Lead exposure has also been reported to be associated with antisocial and delinquent behaviour but it is unclear if this is a causal effect. Dietrich and colleagues reported that prenatal lead exposure was associated with parent-reported deviant behaviour, and prenatal and postnatal exposure was associated with higher self-reported antisocial behaviour (147). However, such a hypothesis is contentious as other important factors should be considered such as parenting and social factors. Such factors are likely to be more important than environmental factors with regard to the effects of lead on cognitive ability and social behaviour (150).

Blood lead concentrations can be routinely measured and there is evidence of a detrimental effect on behaviour and cognitive development. However, lead has a short half life in the blood, and the period during which lead can be measured in the blood is shorter than the duration of toxic effects in the brain, as once deposited in the brain, it is eliminated very slowly, and cannot be removed by chelating agents. Hence, even when blood concentrations are insignificant, lead that has accumulated in the brain can continue to cause neurotoxic effects (148). Lead concentrations have been measured in blood or shed deciduous teeth. Blood lead levels showed a high correlation with childhood IQ, even after adjusting data for potential confounders (137).

*Methylmercury*

Many studies have reported the health risks from organic mercury, particularly methylmercury. Exposure of the general population to mercury can occur through the diet, largely due to the consumption of contaminated fish (methylmercury and inorganic mercury). Methylmercury readily crosses the placenta hence foetal exposure following the consumption of contaminated fish is commonly observed, especially in populations where fish is a substantial part of the diet. In addition, small amounts are present in the breast milk contributing to post-natal exposure (136, 153, 154).

The developing brain is especially sensitive to methylmercury due to its rapid development and different neuropathology has been reported following prenatal, postnatal or adult exposure (140, 154). Prenatal exposure results in generalised damage but no neuronal loss, resulting in blindness, deafness, mental retardation and delays in walking and speech development whereas brain damage following adult exposure is primarily localised to specific regions, such as the granule layer (cerebellum) and visual cortex (cerebrum) as well as axon degeneration, leading to symptoms such as visual abnormalities, ataxia, hearing loss, muscle weakness, tremor and mental retardation (1, 154, 155). Symptomatic postnatal exposure appears to cause intermediate effects (154). Prenatal exposure causes neurotoxicity at lower concentrations than postnatal exposure and at concentrations that may not cause adverse effects to the mother (140, 153, 155). Thus the critical effect of methylmercury is on the developing CNS as pregnant women are the most susceptible population because of risks to the fetus.

A number of molecular targets and mechanisms have been suggested to play a role in the toxicity of methylmercury. It is known to cause neuronal degeneration either by apoptosis

or necrosis, disrupts the integrity of microtubules, impairs mitosis, disrupts neuronal migration, affects calcium homeostasis and causes oxidative stress. In addition, methylmercury has been reported to be associated with an imbalance in neurotransmission and inhibits protein synthesis (113, 155). Such effects are especially damaging during CNS development, leading to alterations of structure and function of the CNS.

Maternal hair has been predominantly used as a biological marker of exposure; mercury has also been measured in cord blood. However the association between the latter and brain mercury concentrations has not been fully elucidated (154). Several studies have shown a correlation between methylmercury in maternal hair and language development, IQ, motor speed, hand-eye coordination, memory and neurobehavioural deficits (136).

Two long-term prospective epidemiological studies have been carried out using cohorts from the Faroe Islands and from the Seychelles. These studies attempted to identify the lowest dietary mercury exposure associated with subtle effects on the developing nervous system. However, conflicting data were reported by each study. The Faroe Islands study showed an association between methylmercury exposure and performance in neuropsychological tests. In contrast, data from the Seychelles showed no adverse effects, despite mean mercury exposure measured by hair levels being similar (154, 156). Discrepancies between these studies may be ascribed to potential differences in genetic susceptibility between the two cohorts, disparity in diagnostic tests used to detect neurological changes and biomarkers used to assess mercury exposure.

In 2000 the Joint Expert Committee on Food Additives (JECFA) proposed a provisional tolerable weekly intake (PTWI) of 3.3 $\mu$g kg $bw^{-1}$ $week^{-1}$ that was considered sufficiently protective of the general population, but was not protective against developmental effects. In 2003, JECFA revised the PTWI to 1.6 $\mu$g kg $bw^{-1}$ $week^{-1}$. The COT concluded that such a PTWI was sufficient to protect against neurodevelopmental effects in the fetus, and that a guideline of 3.3 $\mu$g kg $bw^{-1}$ $week^{-1}$ was appropriate in considering intakes by breastfeeding mothers as the intake of the breast-fed infant would be within the new PTWI of 1.6 $\mu$g kg $bw^{-1}$ $week^{-1}$ (156).

*Polychlorinated biphenyls*

Polychlorinated biphenyls are a large group of chemicals previously used in many industrial processes. Although banned for some time, their persistence in the environment has resulted in ubiquitous human exposure (139, 143). Increasing evidence has suggested that exposure to PCBs may be related to neurotoxicity and developmental neurotoxicity, although mechanisms are still unclear. PCBs consist of many congeners that have different structures and mechanisms by which they exert their neurotoxic effects, and are usually divided into ‘dioxin-like’ or ‘non dioxin-like’ PCBs. Such different mechanisms of action may result in various neurological or behavioural effects (157).

The main route of exposure to PCBs is through dietary intake, mainly through the ingestion of animal fat and milk products. Childhood exposure is predominantly related to prenatal rather than postnatal exposure despite concentrations in breast milk being greater than those *in utero* (140).

Several studies have demonstrated that signal transduction pathways can be disrupted by PCBs. Calcium homeostasis is affected, protein kinase C translocation is increased and hydrolysis of membrane phosphoinositides is inhibited. All these may contribute to neuronal cell death and prolonged increase in synaptic responses that are thought to be involved in learning and memory (149).

Two main poisoning events involving the consumption of contaminated rice oil revealed persistent growth retardation, movement disorders, IQ deficits and behavioural disorders, although in one study there did not appear to be a relationship between available indices of PCB exposure and behavioural disorders (158). Exposure predominantly occurred prenatally, as mothers were advised not to breast feed (136).

Several other epidemiological studies demonstrated that PCBs may cause a variety of developmental effects including reduced birth rate, deformed nails, hyperpigmentation, motor immaturity, decreased reflexes, IQ impairment, decreased cognitive development and verbal communication, mental retardation and behavioural problems. In older children, reduced attention and memory, decreased reading capabilities and decreased IQ were evident (139).

Overall, the COT concluded that PCBs have the potential to cause a wide range of adverse health effects, and that the most critical effects i.e. those seen at the lowest level of exposure, are those on reproduction and postnatal behavioural development, as well as on skin and the immune system (159).

*Polybrominated diphenyl ethers*

Polybrominated diphenyl ethers (PBDEs), commonly known as brominated flame retardants are a large group of environmental contaminants, used in the manufacture of electrical appliances, building materials and textiles (160-163). The diet is probably the predominant route of exposure, including consumption of contaminated fish and dairy products (162, 164). In addition, due to PBDEs being used in indoor environments, inhalation of contaminated indoor dusts is possible (164).

The commercial PBDE products predominantly consisted of penta-, octa- and deca-brominated diphenyl ethers (BDE) products. However, the EU banned the use of penta- and octa-BDE as flame retardants in 2004 because of their persistence/bioaccumulation and because levels in human breast milk appeared to be increasing. Deca-BDE are generally considered to have the lowest toxicity compared to penta- and octa-BDEs (165).

The PBDE congeners that dominate in the environment are mainly tetra-BDE (BDE-47) and penta-BDE (BDE-99) and have both been detected in plasma samples and breast milk (160). In a recent study carried out in the UK, PBDE congeners were measured in human breast milk. Data showed the presence of tetra-BDE (BDE-47) (mean 3 ng/g lipid; 1-37 ng/g lipid) in all samples tested, followed by hexa-BDE (BDE-153) and penta-BDE (BDE-99) in 89 and 92 %, respectively (164). Other studies also reported the occurrence of both congeners in human blood plasma and adipose tissue (160, 163-165).

The developing CNS is a potential target for toxicity of PBDEs and neurobehavioural toxicity is widely observed in experimental animals exposed to PBDEs. Neonatal mice exposed to a single oral dose of tetra-BDE showed permanent impairment of spontaneous motor behaviour in adulthood, and exposure to penta-BDE also affected learning and memory functions and was associated with permanent behavioural effects (161). In addition, both pre- and postnatal exposure to penta-BDE has been shown to cause permanent neurobehavioural disturbances at doses below that causing maternal toxicity (163). The neurodevelopmental toxicity of PBDEs appears to involve changes in the cholinergic system as cholinergic nicotinic receptors appear to be targets for PBDEs. In addition, PBDEs have been suggested to alter thyroid hormone homeostasis thereby disrupting brain development causing permanent neurological damage. However, the mechanisms involved in PBDE-induced neurotoxicity are not fully understood (161-163).

Few human epidemiological studies have been carried out to investigate the effect of PBDEs on human development and behaviour but animal data indicate that pregnant women should be considered a sensitive subpopulation due to effects on the developing fetus (162).

## Chemical-induced dyschromatopsia

Chemical-induced deficits in colour perception (dyschromatopsia) is a relatively new area of research. Impairment of colour discrimination is considered an early sign of neurotoxicity or optic neuropathy (166-168). Such effects can generally be detected at low exposure, if the method used to test colour vision is adequately sensitive and conducted under correct conditions (169).

In the human eye, there are two main types of visual receptor cells, namely rods and cones, responsible for the perception of light and colour, respectively. Colour perception involves retinal receptors (cones) and adequate conduction of optic nerve fibres (170). Short wavelength light is perceived by S cones, medium wavelengths by M cones and long wavelengths by L cones, hence colour vision arises from stimulation of all three types of cones (171).

Retinal changes and damage to the optic nerve have both been implicated to play a role in dyschromatopsia (172, 173), as well as acute depression of the CNS, effects on the electrical membrane potentials in the retina and depression of dopaminergic function in the neuro-optic pathways (174).

Colour vision deficiencies may be either congenital or acquired and are characterised by regions of the spectrum (axis of colour confusion) where there is poor discrimination and by the degree of impairment. Congenital loss of colour vision is usually in the L- or M-cone photoreceptors leading to red-green deficiencies (protan and deutan deficiencies, respectively), affecting approximately 8 % of men and 0.4 % of women. Congenital blue-yellow (tritan) deficiencies caused by abnormalities of the S-cone photoreceptors are extremely rare, having a prevalence in the general population of approximately 0.005 % (174).

Loss of colour discrimination related to a direct effect of chemicals on the cones usually are in the form of blue-yellow deficiencies (166). S cones, which represent less than 10 % of the total cone population, are thought to be more sensitive to chemical-induced damage (171), leading to deficits in the blue-yellow range of colour vision (175). Colour vision loss may also arise due to chemical-induced defects in any of the three types of cones (171). According to Kollner's rule, blue-green defects commonly observed following chemical exposure are caused by damage to the external retinal layers, whereas red-green defects reflect damage to the internal retinal layers or to the optic nerve (172, 173, 176).

Loss of sensitivity of cone photoreceptors may also occur as well as neuronal alterations in the peripheral system (177). In some cases, i.e. following exposure to carbon disulfide, colour vision may be affected due to impaired receptiveness of the ganglion cells for polarising and depolarising signals generated by the cones.

Damage to the optic nerve following chemical exposure may also lead to dyschromatopsia and is thought to occur due to demyelination of the nerve filaments (173) or by alterations in visually evoked potentials (166). Solvents and styrene are thought to cause alterations in the myelin sheath of the axon and the cell membrane by binding to membrane proteins thereby causing neuronal damage. Electrophysiological data suggest peripheral neuropathy may occur following occupational (chronic) exposure to solvents or styrene, as a decrease in motor and sensory nerve conduction velocities occurs, including impairment of the faster myelinated fibres peripheral sensory nerves (178, 179). Loss of colour discrimination may also be related to neurotransmitters in the retina or effects on the optic nerve or the brain (166, 170).

A number of studies have investigated colour vision loss following occupational exposure to chemicals such as styrene, toluene or n-hexane (annex II) and the number of chemicals known to or suspected to affect visual function appears to be increasing (180). For several chemicals, such as styrene and toluene, effects on chromatic perception have been reported at lower concentrations than the current environmental or occupational limits (167) although the evidence is not consistent. In addition, solvents such as Perchloroethylene (PCE), carbon disulfide, toluene and n-hexane have also been reported to cause colour vision impairment at low concentrations (169).

Other chemicals have also been reported to cause dyschromatopsia. For example, PCE-induced colour vision loss may be the result of distal axonopathy of the optic nerve (168).

Several tests have been developed to assess colour discrimination, such as the Farnsworth-Munsell 100 hue test, Ishihara colour vision test or the Lanthony D15 hue desaturated panel (Lanthony D15 test), the latter being the most commonly used. Most tests are based on the ability to recombine a set of 15 desaturated colours according to a specific chromatic sequence, and should be undertaken under specific conditions i.e. under a daylight fluorescent lamp (175).

The Lanthony D15 test was most commonly used in all the studies considered in this review (166, 167, 172, 175, 181-192). It is commonly used as it is sensitive enough to detect mild dyschromatopsia, is quick and simple to carry out in the workplace and is inexpensive. However, the test must be carried out under the correct light conditions, using a 1200-lux daylight fluorescent lamp. In addition, the test should ideally be carried out on multiple occasions, and each eye should be tested separately to assess for monocular or asymmetrical colour vision loss (185). One group carried out the Lanthony D15 test under natural sunlight (190-192) rather than standardised conditions, hence data obtained are less reliable.

Another main advantage of the Lanthony D15 test is the possibility to quantitatively express data, by calculating the colour confusion index (CCI), enabling the possibility to assess dose-response relationships and comparisons between exposed workers and control subjects (185).

The Farnsworth-Munsell 100 hue test has been used in a small number of studies (170, 193-195). This test does not identify slight colour vision deficits but can provide more detailed information on more severe colour vision loss. Repeated testing can significantly affect the data obtained and error scores have been reported to improve upon re-testing (171).

The Ishihara test, which is widely used to test red-green colour deficiency, was used in two studies (169, 196), although details about the test were not fully given. Due to it not being sensitive to detect tritan (blue-yellow) defects, it is less suitable to detect acquired colour vision deficiency (171).

The Lanthony new colour test was used in one study and those who had difficulty in completing it were subsequently tested with the Ishihara test (196). The Lanthony new colour test is relatively insensitive to differences in colour discrimination and the Ishihara test is unsuitable for detecting acquired dyschromatopsia, hence no conclusions can be drawn from the negative data reported in this study.

Muttray and colleagues used a battery of tests to assess colour discrimination in printshop workers, including the Farnsworth D15 test, the Lanthony D15 test, three Velhagen plates designed to detect impaired tritan discrimination and the standard pseudoisochromatic plate part 2 plate test. All were carried out under appropriate conditions (197, 198).

## Styrene

Many studies have investigated the effects of styrene on lack of colour discrimination. Most authors reported a significant increase in CCI in subjects exposed to styrene compared with controls, indicative of a loss of colour discrimination (167, 175, 178, 184, 186, 187, 189). In contrast, Fallas and colleagues failed to show a significant difference between exposed and non-exposed subjects (193).

Styrene vapour is well absorbed by inhalation, but dermal absorption of both the vapour and liquid is limited (174). The majority of styrene is metabolised to mandelic acid (MA) and phenylglyoxylic acid (PGA), which are excreted in urine. Many studies use the level of metabolites in the urine as a surrogate measure of styrene exposure, although some studies measured styrene in personal air samples.

In the UK the current approved occupational long-term exposure limit (8-hour time-weighted average (TWA)) for styrene is 100 ppm (430 mg $m^{-3}$) and the short-term exposure limit (15 minute reference period) is 250 ppm (1080 mg $m^{-3}$).

The effect of styrene on colour vision appears to be dose-related. Gobba and colleagues showed that in a cohort of workers exposed to styrene, those not exceeding the occupational levels showed similar CCI values as did controls. In contrast, those exceeding the long-term exposure limits had significantly higher CCI values (175).

Most of the studies that reported similar concentrations of exposure (mean concentration 6 – 21 ppm or 69.02 – 205.78 mg $m^{-3}$) stated a loss of colour vision, with the exception of Fallas, who failed to show a loss of colour vision in ship constructors exposed to mean concentration of styrene of 24.3 ppm (193). However, Fallas and co-workers measured colour discrimination by using the Farnsworth-Munsell 100 hue test compared to the other studies that used the Lanthony D15 test, which may account for the differences observed.

In a number of studies, the CCI significantly correlated with the urinary metabolite MA (178, 181, 187) or styrene concentration (167, 175). In contrast, Chia and co-workers could not show a significant linear correlation between colour discrimination and urinary concentration of MA or PGA (184).

It has been suggested that styrene causes demyelination of the optic fibres (173). Raitta proposed an impaired receptiveness of ganglion cells for polarising and depolarising signals generated by cones or a direct effect on receptor lipids (194, 195).

In some studies, the loss of colour discrimination was not considered to be an acute effect as measurements were carried out after 2 days of non-exposure (178), and others reported a dose-dependent effect (175, 178, 181, 184). In addition, the reversibility of such effects require further investigation as Gobba and colleagues reported that workers exposed to styrene did not recover after one month of no exposure (175).

## Toluene

Eight studies were identified that measured colour discrepancy following toluene exposure. Several reported a significant increase in CCI in workers compared to controls (166, 182, 190, 191) although some failed to show a significant difference in CCI between workers and controls (192, 196-198).

Toluene is well absorbed following inhalation exposure and is rapidly metabolised and eliminated by the body (174). Toluene was measured in the air by personal air monitors or

in ambient air (182, 190). Alternatively, blood toluene concentration was measured, which is the recommended biological index of toluene exposure. Urinary concentrations were measured (166), although since approximately 0.06 % of inhaled toluene is excreted unchanged in the urine the latter is not such a good biological index of toluene exposure (174).

Many of the studies identified used the Lanthony D15 test to identify discrepancies in colour vision (166, 182, 190-192). Muttray and colleagues used a battery of tests including the Farnsworth panel D15 test, the Velhagen test and a standard Pseudoisochromatic plates part 2 (197, 198) and Nakatuka used the Ishihara's test and the Lanthony new colour test (196). Some of the additional tests are less sensitive than the Lanthony D15 test, hence the power of such studies may not have been sufficient to detect any effect of solvent exposure.

In the UK, the current long-term occupational exposure limit (OEL; 8 hour TWA) for toluene is 50 ppm (191 mg $m^{-3}$) and the short-term exposure limit (15 minute TWA) is 150 ppm (574 mg $m^{-3}$). Overall, there is no evidence that toluene causes acute effects on colour discrimination in the range of 300-350 ppm for 30 minutes, or 50-150 ppm 8hr TWA. There are limited data to suggest that chronic exposure to toluene causes dyschromatopsia. However, due to the limitations of some of the studies, whether exposure to toluene can cause such effects remains inconclusive (174, 180).

## Perchloroethylene

Two studies investigated the effect of PCE on colour discrimination of dry cleaners and ironers. In both studies the dry cleaners had a significantly increased CCI, as measured by the Lanthony D15 test, therefore exhibited decreased colour discrimination compared to controls (168, 199). Exposure ranged from 0.4 – 31 ppm PCE in the breathing zone, measured by personal samplers. Authors concluded that PCE exposure even at low concentrations can induce a dose-related impairment of colour vision (168). Furthermore, in a two-year follow up study carried out on dry cleaners exposed to PCE, workers with an increased exposure showed deterioration in colour vision, whereas the colour vision of those that had a decreased exposure to PCE remained unchanged (199). Authors concluded that a small increase in exposure to PCE may cause a loss of colour vision, whereas a reduction of exposure did not result in improvement of colour vision.

Perchloroethylene vapour is well absorbed following inhalation exposure but dermal exposure is markedly lower than for other solvents. Little of the absorbed PCE is metabolised or excreted in urine, the majority being exhaled unchanged in the breath (174). The visual system is the most affected organ following acute inhalation exposure to PCE, resulting in a decrease in visually evoked potentials and visual contrast sensitivity. However, visual effects following chronic exposure remain uncertain (168).

In the UK, the current occupational long-term exposure limit (8 hour TWA) for PCE is 50 ppm (335 mg $m^{-3}$) and the short-term exposure limit (15 minute TWA) is 150 ppm. The two studies noted above suggest an impairment in colour vision following exposure to PCE below the current occupational standards (168, 199).

Till and colleagues described a case report of loss of colour vision in one child whose mother was exposed to PCE during pregnancy when working in the dry-cleaning business. No further data are available on such effects following *in utero* exposure. Visual evoked potential measurements and a minimalist test was carried out and showed that the child had reduced contrast sensitivity and colour discrimination compared to controls

(200). However, no definite conclusion regarding the effects of PCE can be drawn from this single case report.

## n-Hexane

Two studies were carried out on adhesive bandage factory workers and vegetable oil extraction workers, both groups exposed to n-hexane (194, 201). Using the Farnsworth panel D15 and Farnsworth-Munsell 100 hue test 12 out of 15 subjects had decreased colour vision according to one or both of the tests used. However, authors did not present results in terms of occupation hence did not correlate data to n-hexane exposure, and did not include a control group. One worker had a congenital colour vision defect, but the other workers all exhibited signs of acquired dyschromatopsia, mainly in the blue-yellow axis (194). In a follow-up study of the same cohort, workers had significantly lower visual evoked potentials and electroretinograph amplitudes (201). Again, authors did not distinguish between workers exposed to low or high concentrations of n-hexane.

In the UK, the current occupational long-term exposure limit (8 hour TWA) for n-hexane is 20 ppm (72 mg $m^{-3}$). In the two studies examined, some workers were exposed to concentrations of n-hexane above the recommended exposure limit when equipment being used was faulty. Authors stated that although concentrations up to 3000 ppm were measured in ambient air (near the floor at a tape machine or following disturbances in processes or machinery) the overall exposure in workrooms usually stayed below 500 ppm, the permissible exposure levels set in Finland, where the factories were situated. Concentrations in the breathing zone of workers was reportedly lower, although data were not given (194, 201).

## Carbon disulfide

Conflicting data have been reported on workers exposed to carbon disulfide when working in a viscose rayon plant. Two studies reported impaired colour discrimination in workers, predominantly in the blue and green range (170, 195) whereas another study failed to show a difference in CCI between workers and controls (188). In the populations studied, there were no other effects on measurements of vision, including visual acuity, visual field, eye motility, depth of perception or papillary reaction. The colour vision loss did not correlate with duration of exposure (195). Workers that did experience colour vision loss were tested by using the Farnsworth-Munsell 100 hue test, whereas those that did not show any dyschromatopsia were measured by using the Lanthony D15 test. The exposure concentrations in all studies, measured by personal air sampling, were similar, and were below the exposure limit of 10 ppm (32 mg $m^{-3}$).

## Mixtures

Several studies have been carried out to investigate colour vision loss following occupational exposure to mixtures of chemicals, mainly organic solvents. Most authors reported which chemicals the workers were possibly exposed to, although few reported exposure data and no conclusions can be drawn regarding effects of specific chemicals. Subjects worked in a variety of areas including welding, fibre-glass reinforced plastic manufacture, furniture manufacture, microassembly, paint production and plastic product manufacture.

Most studies reported a increase in CCI, therefore a decrease in colour discrimination in exposed workers compared with controls (169, 172, 176, 177, 180, 202, 203), with the exception of two studies that failed to see such a correlation (173, 196).

Two studies focussed on colour discrimination of children following maternal exposure to solvents during pregnancy, although exposure was only ascertained via a questionnaire and no quantitative measurements were carried out. Data show that that children whose mothers had worked with solvents had a decreased colour discrimination, visual acuity and contrast sensitivity compared to unexposed controls (204, 205). The authors concluded that the developing visual system is vulnerable to organic solvent exposure and suggested that although current occupational exposure concentrations are deemed safe for adult workers they are not adequate to protect the unborn child (204).

### Recent review by Health and Safety Executive

The Health and Safety Executive (HSE) have recently reviewed in some detail whether occupational exposure to organic solvents causes loss of colour discrimination. In addition, the HSE's Advisory Committee for Toxic Databases is considering whether the data for styrene warrant a reduction of the current 8hr TWA of 100 ppm. The committee recognises that styrene had been most extensively studied, and the evidence supported the view that styrene affected colour discrimination, of which the tritan type was mainly affected, and that effects would not be expected at an 8hr TWA of less than 20 ppm. The significance of these subtle effects was not known. For example, it was not known whether such effects were reversible as conflicting data were presented.

There is limited information on the other solvents (mixed solvents, PCE etc) and no reliable conclusions can be drawn.

### Summary

To conclude, a brief literature overview of chemical exposure and colour vision indicates that there are reports of visual function being affected by a number of chemical agents such as styrene, toluene, PCE and carbon disulfide, as well as mixtures of organic solvents. The most convincing data relate to styrene as it was doubtful whether any conclusions could be drawn regarding causative agents in the studies involving mixtures of solvents.

Significant effects were observed on colour discrimination that was often dose-dependent. The Lanthony D15 test was most commonly used in studies identified and proved to be the most sensitive in detecting acquired deficiencies in colour vision. A battery of tests may be more appropriate in fully investigating colour vision loss. It is not known whether the effects seen in some of the studies identified are reversible or not and their true health significance has yet to be elucidated. Nor is it known whether these effects might be early indicators of more significant neurotoxicity, hence this area warrants further research.

# Conclusions and recommendations

The review has concentrated on progressive neurodegenerative diseases and adverse effects on nervous system following early life stage exposure. Consideration had been given to methods of investigation of neurotoxic potential both in animals and humans, mechanisms involved and evaluation of chemical involvement in the aetiology of these diseases.

Progressive neurodegenerative diseases are a major cause of health detriment and this is likely to be of increasing importance as the average age of the general population increases. The causative factors are not known but recent literature suggests that there may be a chemical aetiology. Evidence from epidemiological studies with regard to any associations with environmental chemicals has been sparse. Most data relate to a possible association with exposure to pesticides and PD, although only one study has shown a dose-response relationship with a specific pesticide (paraquat); this herbicide has structural similarities to MPP+, a metabolite of MPTP known to produce Parkinsonian syndrome. The ACP has recommended further epidemiological and mechanistic research relating to pesticides and PD. Defra are taking this forward. Progressive neurodegenerative diseases have been recognised by the HPA as a priority area for research in the HPA's research programme at the Medical Toxicology Research Centre at the University of Newcastle.

There is also increasing concern that exposure to neurotoxic chemicals in early life stages may adversely affect development of the nervous system (developmental neurotoxins). This has led to the development of a specific method for investigating this endpoint in animals. However, it is still unclear whether this approach is necessary when data are available from a well conducted multigeneration study. There are data on a few chemicals from epidemiology studies indicating such effects in humans, but these are essentially limited to established neurotoxic chemicals such as lead, mercury, PCBs etc, which are strictly controlled because of concerns regarding their toxicity.

A number of potential research areas have been noted in the review relating to the identification of compounds which may be implicated in neurodegenerative disease or relating to mechanistic studies.

The data on the role of metals in the aetiology of PD and AD have been reviewed in some depth and this may warrant further investigation. Some research has revealed that zinc concentrations were higher in certain regions of the brain in AD patients and *in-vitro* studies suggest that zinc may play a role in β-amyloid aggregation or neurone degeneration. The environmental pollutant acrolein has been shown to produce protein adducts in areas of the brain that show marked pathological changes in AD patients, and was found in neurofibrillary tangles. Although this association may be a chance finding, it is recommended that the possible role of zinc and acrolein in neurodegenerative diseases warrant further consideration.

Regarding mechanisms, chemically-induced dyschromatopsia (loss of colour vision) has been claimed to be an early sign of more serious neurotoxicity, as it may occurs at levels lower than those producing other neurotoxic effects. Few data are available to support this and currently the significance of this effect is not known. Further work is this area is recommended.

There are very limited data to suggest that exposure to environmental chemicals is associated with progressive neurological conditions. However, in view of the difficulties

relating to epidemiology studies in this area, it may be better to concentrate on obtaining an adequate toxicological database on chemicals to which the population is widely exposed, to ensure that any potential neurotoxic properties are recognised, and that if there are such concerns, they are only used in areas that are adequately controlled. Such data should be generated, in due course, by the EC Regulation REACH based on registration, evaluation and authorisation of chemicals of most concern. It is recommended that HPA needs is aware of the outcome of this Regulation.

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# Annex I: Metal concentrations in different brain regions of PD or AD patients

| Author | Metal | Subjects | Sample | Method | Results |
|---|---|---|---|---|---|
| **Parkinson's disease** | | | | | |
| Bartzokis *et al.* 1999 | Iron | Living PD patients (n=14) and controls (n=14) | Substantia nigra pars reticulate, substantia nigra pars compacta, caudate, globus pallidus, putamen, white matter | Field-dependent $R_2$ increase, MRI | Significantly increased iron in the substantia nigra reticulata, substantia nigra pars compacta, putamen and globus pallidus in early-onset PD patients compared with controls. Significantly increased iron in the substantia nigra reticulata in later-onset PD patients. |
| Chen *et al.* 1993 | Iron, ferritin, | PD (n= 10) and HD (n=6) patients and controls (n=6) | Post-mortem samples: Putamen and globus pallidus | Iron: Flame atomic absorption spectrophotometer Ferritin: Radioimmunoassay | Significantly increased iron and ferritin in the putamen and globus pallidus samples in HD and to a lesser extent in PD samples compared with controls. |
| Dexter *et al.* 1989 | Iron, Copper, Zinc, Manganese, Lead | PD patients (n=27) and age-matched controls (n=34) | Post-mortem samples: Substantia nigra, cerebellum, cortex, globus pallidus, caudate nucleus and lateral putamen | Inductively coupled plasma spectroscopy | Significantly decreased iron concentrations in the medial and lateral globus pallidus in PD samples compared with controls. Significantly increased iron concentrations in the total substantia nigra and substantial nigra compacta in PD samples. Significantly decreased concentrations of copper in the total substantia nigra and substantia nigra pars compacta in PD samples. Significantly increased concentrations of zinc in the caudate nucleus, lateral putamen, total substantia nigra and substantia nigra pars compacta in PD samples. Significant decrease of manganese concentrations in the medial putamen |

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| | | | | | in PD samples.<br>No significant difference in lead concentrations between PD samples and controls, in any of the regions. |
| Dexter *et al.* 1990 | Ferritin, Iron | PD patients (n=27) and age-matched controls (n=34) | Post-mortem samples: substantia nigra, caudate, putamen, globus pallidus, cerebral cortex and cerebellum.<br>Serum and CSF. | Ferritin: Radioimmunoassay<br>Iron: spectrophotometry | Significantly decreased ferritin concentrations in the substantia nigra, caudate, putamen, globus pallidus, cerebral cortex and cerebellum in PD samples compared with controls.<br>No significant difference in ferritin concentrations between PD samples and controls, in serum or CSF. |
| Dexter *et al.* 1991 | Iron, Copper, Zinc, Manganese, Ferritin | PD patients (n=27) and age-matched controls (n=34) | Post-mortem samples: cerebellum, substantia nigra (total or zona compacta only), caudate nucleus, putamen, globus pallidus and cerebral cortex. | Inductively coupled plasma spectroscopy | Significantly increased total iron in the substantia nigra but decrease in globus pallidus in PD samples compared with controls.<br>Significantly decreased copper concentrations in the substantia nigra in PD samples.<br>Significantly increased zinc concentrations in the substantia nigra, caudate nucleus and lateral putamen in PD samples.<br>Significantly decreased manganese concentrations in the medial putamen in PD samples.<br>Significantly decreased ferritin in all areas but marked differences in the substantia nigra and medial putamen of samples. |
| Gorell *et al.* 1995 | Iron | Living PD patients (n=13) and controls (n=10) | Substantia nigra | T2-weighted MRI | Significantly increased iron concentrations in substantia nigra in PD subjects compared with controls. |
| Graham *et al.* 2000 | Iron | Living PD patients (n=21) and controls | Caudate nucleus, putamen, globus pallidus | T2-weighted MRI | Significantly increased iron concentrations in the substantia nigra in |

| | | (n=13) | and frontal white matter | | PD patients compared with controls. Significantly decreased iron in the putamen and globus pallidus in PD patients. |
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| Griffiths and Crossman 1993 | Iron | PD (n=6) and AD (n=6) patients and age-matched controls (n=6) | Post-mortem samples: Basal ganglia and neocortex | Atomic absorption spectrophotometry | Significantly increased iron concentrations in the substantia nigra and lateral globus pallidus in PD samples compared with controls. Decreased iron concentrations in the medial globus pallidus in PD samples. No significant difference in iron concentrations in any of the AD samples compared with controls. |
| Hirsch *et al.* 1991 | Iron, Potassium, Silicum, Sodium, Sulphur, Zinc | PD patients (n=5) and matched controls (n=7) | Post-mortem samples: Substantia nigra, central grey substance. | x-ray microprobe analysis | Significantly increased iron concentrations in the substantia nigra without neuromelanin in PD patients compared with controls. Significantly decreased iron concentrations in the substantia nigra with neuromelanin in PD patients. No significant difference in potassium, silicum, sodium, sulphur or zinc concentrations in PD patients. Significantly increased concentrations of iron and aluminium in the Lewy bodies in PD patients. |
| Jellinger *et al.* 1990 | Iron, Ferritin | PD (n=8), PD+AD (n=5) and AD (n= 15) patients and controls (n=8) | Post-mortem samples: Frontal cortex, hippocampus, basal ganglia, brain stem | Iron: histological staining Ferritin: Immunohistochemistry | Significantly increased iron$^{3+}$ in basal in the substantia nigra zona compacta in PD compared with controls. Ferritin in and around senile plaques and neurofibrillary tangles was observed in PD+AD and AD patients compared with controls. |

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| Jimenez-Jimenez *et al.* 1995 | Manganese, Cobalt and Chromium | PD patients (n=29) and matched controls (n=27) | Serum and urine | Atomic absorption spectrophotometry. | No significant difference in concentrations of manganese, cobalt or chromium between the patients and controls. |
| Loeffler *et al.* 1995 | Iron | PD (n=14), AD (n=14) patients, young controls (n=8) and elderly controls (n=8) | Post-mortem samples: Caudate, putamen, globus pallidus, substantia nigra and frontal cortex | Ferrochem II serum iron/TIBC analyser | Significantly increased iron concentrations in the frontal cortex in AD compared with young and elderly controls.<br>Significantly increased iron concentrations in the globus pallidus in AD patients compared with elderly controls. |
| Logroscino *et al.* 1997 | Iron, Total iron binding capacity, Ferritin, Transferrin | PD patients (n=104) and controls (n=352) | Serum | Iron and total iron binding capacity: atomic absorption.<br>Ferritin: radioimmunoassay.<br>Transferrin: radial immunodiffusion | Significantly decreased iron, ferritin and transferrin in serum in PD patients compared with controls. |
| Mann *et al.* 1994 | Iron, Zinc, Ferritin | PD patients (n=18) and controls (n=22) | Post-mortem samples: Substantia nigra | Iron: Inductively coupled plasma spectroscopy<br>Ferritin: Double antibody sandwich ELISA | Significantly increased total iron concentrations in the substantia nigra in PD samples compared with controls.<br>No significant difference in zinc or ferritin concentrations. |
| Ordidge *et al.* 1994 | Iron | Living PD patients (n=7) and age-matched controls (n=7) | Substantia nigra | T2-weighted MRI | Significantly increased iron concentrations in the substantia nigra in 3 out of 7 PD patients, compared with controls. |
| Riederer *et al.* 1989 | Iron, Iron$^{2+}$, Iron$^{3+}$, Copper, Zinc, Magnesium, Calcium, | PD patients (n=13) and age-matched controls (n=4)(expt 1); or PD patients (n=8) and age-matched controls (n=8)(expt 2) | Post-mortem samples: Substantia nigra, globus pallidus, putamen, nucleus basalis of Meynert, amygdaloid nucleus and frontal | Metals: Atomic absorption spectrophotometry.<br>Iron: spectrometry.<br>Glutathione/ascorbate: HPLC with | Significantly increased concentrations of total iron and iron$^{3+}$ in the substantia nigra in PD samples compared with controls.<br>No significant differences in magnesium or calcium in PD samples. |

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| | Glutathione, Ascorbate, Ferritin | | cortex. | electrochemical detection.<br>Ferritin: Dissociation-enhanced lanthanide solid phase fluoroimmunoassay | Significantly increased concentrations of copper and zinc in the raphe plus reticular formation in PD samples.<br>Significantly decreased glutathione content in the putamen, globus pallidus, substantia nigra, nucleus basalis of Meynert, amygdaloid nucleus and frontal cortex.<br>Significantly decreased total ascorbic acid concentrations in PD samples.<br>Significantly increased concentrations of ferritin in the substantia nigra in PD samples. |
| Sofic *et al.* 1988 | Total Iron, iron$^{2+}$, iron$^{3+}$ | PD patients (n=8) and age-matched controls (n=8) | Post-mortem samples: Substantia nigra, cortex, hippocampus, putamen and globus pallidus | Spectrophotometry | Significantly increased total iron concentrations, iron$^{3+}$ and iron$^{2+}$/iron$^{3+}$ ratio in the substantia nigra in PD samples compared with controls.<br>No significant change in concentrations in other regions of the brain. |
| Sofic *et al.* 1991 | Iron, Iron2+, Iron3+ | PD (n=7), PD+AD (n=6) and AD (n=16) patients | Post-mortem samples: Substantia nigra zona compacta and zona reticulate. | Spectrophotometry | Significantly increased level of iron$^{3+}$ in the substantia nigra zona compacta in PD patients and PD+AD patients compared with controls.<br>Significantly increased total iron content in the substantia nigra zona compacta in PD patients. |
| Yasui *et al.* 1992 | Calcium, Aluminium, Magnesium | PD patients (n=4) and controls (n=5) | Post-mortem samples: cortical grey matter (frontal, parietal, temporal and occipital cortex), white matter (crus cerebri, frontal parietal, temporal, occipital and cerebellar white matter), basal ganglia (thalamus, caudate nucleus, globus | Calcium/Aluminium: neutron activation analysis<br>Mg: inductively coupled plasma emission spectrometry | Significantly decreased magnesium concentrations in the CNS, grey matter, white matter, basal ganglia and brain stem in PD samples compared with controls.<br>Significantly increased aluminium concentrations in the CNS and basal ganglia in PD samples.<br>No significant difference in calcium concentrations in PD samples. |

|  |  |  | pallidus, putamen, substantia nigra and red nucleus), brain stem (pons, olicary nucleus and medulla) |  |  |
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| **Alzheimer's Disease** |  |  |  |  |  |
| Cornett *et al.* 1998 | Iron, Zinc, Mercury, Selenium | AD patients (n=58) and age-matched controls (n=21) | Post-mortem samples: Frontal pole, temporal pole, inferior parietal lobule, amygdale, hippocampus, cerebellum, olfactory region. | Instrumental neutron activation analysis | Significantly increased iron concentrations in the frontal pole, temporal pole, anterior parietal, hippocampus and amygdala in AD samples compared with controls.<br>Significantly increased zinc concentrations in the frontal pole, temporal pole, anterior parietal, hippocampus, amygdala and cerebellum in AD samples.<br>No significant changes in mercury concentrations in AD samples.<br>Significantly increased concentrations of selenium in the amygdale in AD samples. |
| Deibel *et al.* 1996 | Copper, Iron, Zinc | AD patients (n=10) and age-matched controls (n=11) | Post-mortem samples: Amygdale, hippocampus, inferior parietal lobule, superior and middle temporal gyri, cerebellum | Instrumental neutron activation analysis | Significantly increased iron concentrations in the hippocampus in AD patients.<br>Significantly decreased copper concentrations in the amygdale and hippocampus in AD patients compared with controls.<br>Significantly increased zinc in the amygdala, hippocampus and inferior parietal lobule in AD patients. |
| Griffiths and Crossman. 1993 | Iron | PD (n=6) and AD (n=6) patients and age-matched controls (n=6) | Post-mortem samples: Basal ganglia and neocortex | Atomic absorption spectrophotometry | No significant difference in iron concentrations in any of the AD samples compared with controls. |
| Hock *et al.* 1998 | Mercury | AD patients (n=33), age-matched controls | Blood | Cold vapour atomic absorption | Significantly increased blood mercury concentrations in AD patients |

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| | | with depression (n=45) and age-matched controls (n=65) with non-psychiatric disorders. | | spectrometry | compared with controls with depression, and controls without psychiatric disorders. |
| Jellinger *et al.* 1990 | Iron, Ferritin | PD (n=8), PD+AD (n=5) and AD (n= 15) patients and controls (n=8) | Post-mortem samples: Frontal cortex, hippocampus, basal ganglia, brain stem | Iron: histological staining<br>Ferritin: Immunohistochemistry | Ferritin in and around senile plaques and neurofibrillary tangles was observed in PD+AD and AD patients compared with controls. |
| Kala *et al.* 1996 | Total Iron, $iron^{2+}$, $iron^{3+}$ | AD patients (n=4) and age-matched controls (n=5) | Post-mortem samples: Frontal cortex and cerebellum | Spectrophotometry | Significantly increased iron and $iron^{3+}$ concentrations in the frontal cortex and cerebellum in AD samples compared with controls. |
| Loeffler *et al.* 1995 | Iron | PD (n=14), AD (n=14) patients, young controls (n=8) and elderly controls (n=8) | Post-mortem samples: Caudate, putamen, globus pallidus, substantia nigra and frontal cortex | Ferrochem II serum iron/TIBC analyser | Significantly increased iron concentrations in the frontal cortex in AD compared with young and elderly controls.<br>Significantly increased iron concentrations in the globus pallidus in AD patients compared with elderly controls. |
| Lovell *et al.* 1998 | Copper, Iron, Zinc | AD patients (n=9) and controls (n=5) | Post-mortem samples: Neuritic plaques and neuropil in amygdala | Micro-particle-induced X-ray emission | Significantly increased concentrations of zinc in neuropil in AD samples compared with controls.<br>No significant difference in iron and copper in AD samples. |
| Markesbery *et al.* 1984 | Manganese | AD patients (n=) and controls (n=) | Post-mortem samples: anterior and middle frontal lobes, anterior and middle temporal lobes, hippocampus, amygdala, superior parietal lobule, occipital pole, globus pallidus, putamen, | Instrumental neutron activation analysis | No significant difference in manganese concentrations in AD patients compared with controls. |

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| | | | caudate nuclei, thalamus, substantia nigra, cerebellar vermis, cerebellar hemispheres | | |
| Rulon *et al.* 2000 | Zinc | AD patients (n=9) and controls (n=8) | Post-mortem samples: Amygdala, hippocampus, cerebellum, superior and middle temporal gryi and serum. | Flame atomic absorption spectrometry and instrumental neutron activation analysis | Significantly increased serum zinc level in AD patients compared with controls. No significant difference in zinc concentrations in the amygdala, hippocampus, cerebellum, superior and middle temporal gryi. |
| Sofic *et al.* 1991 | Iron, Iron2+, Iron3+ | PD (n=7), PD+AD (n=6) and AD (n=16) patients | Post-mortem samples: Substantia nigra zona compacta and zona reticulate. | Spectrophotometry | Significantly increased level of $iron^{3+}$ in the substantia nigra zona compacta in PD+AD patients compared with controls. |
| Squitti *et al.* 2002 | Copper, Iron, Transferrin | AD patients (n=79) and age-matched controls (n=76) | Serum | Copper: atomic absorption spectrophotometry Iron: colourimetry Transferrin: immunoturbidimetry | No significant difference in iron or transferrin concentrations. Significantly increased copper concentrations in AD patients compared with controls. |

## Annex II: Chemical-induced dyschromatopsia following occupational exposure to chemicals

| Author | Chemical | Subjects | Exposure data | Method to detect colour vision | Results |
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| **Styrene** | | | | | |
| Campagna *et al.* 1995[1] | Styrene | Fibre-reinforced plastic boat manufacturing plant workers (n=79). | 4-hr personal air sampling carried out. Styrene (205.78 mg $m^{-3}$ (19.75-378.49 mg $m^{-3}$)); Urinary metabolite MA measured. MA (0.36 mmol-mmol $creatinine^{-1}$ (0.04-0.60 mmol-mmol $creatinine^{-1}$)). | Lanthony D15 hue desaturated panel carried out 1x (type of lamp used not stated) and CCI calculated. | Significant difference between the best and worse eyes for both CCI and colour sensitivity. Urinary MA positively correlated with age and CCI. Colour sensitivity decreased with age and urinary MA. |
| Campagna *et al.* 1996[1] | Styrene | Reinforced plastics plant workers in Canada (n=67) and Italy (n=51). | 8-hr personal air sampling carried out. Styrene- Canada (17.6 ppm (3.5-101.9 ppm)); Italy (14.0 ppm (2.6-69.3 ppm)). | Lanthony D15 hue desaturated panel carried out 1x (type of lamp used not stated) and CCI calculated. | Significant increase in CCI with 4ppm styrene. Positive relationship between CCI and environmental styrene exposure. |
| Castillo *et al.* 2001[2] | Styrene | Various reinforced plastics plant workers (n=18) and low exposure controls. | 8-hr personal air sampling in breathing zone carried out in previous studies. Styrene Painters + laminators (209 - 1035 mg $m^{-3}$); Finishers (10 – 84 mg $m^{-3}$). Urinary metabolite MA measured. Cumulative exposure index (CEI) calculated for each worker. | Lanthony D15 hue desaturated panel and total colour confusion score (TCDS) calculated. | Improvement in colour vision since previous studies, whereas near visual contrast sensitivity decreased over time. |
| Chia *et al.* 1994 | Styrene | Fibre-reinforced plastic(167) boat manufacturing plant workers (n=21) and sex-matched | Estimated styrene in air calculated. Estimated styrene (6.0 ppm). Urinary metabolites MA and PGA measured. MA (84.0 mg/g | Lanthony D15 hue desaturated panel carried out 1x with a 1000-lux lamp, and TCDS calculated. | Significantly poorer colour discrimination in the TCDS in workers compared with controls. No linear correlations between colour |

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| | | controls. | creatinine (1.3-504.1 mg/g creatinine)); PGA (0.7 mg/g creatinine (0.3-1.9 mg/g creatinine)). | | difference score and urinary conc. of MA or PGA. |
| Eguchi *et al.* 1995 [3] | Styrene | Fibreglass and reinforced plastic factory workers (n=64) and controls (n=84). | Mean atmospheric styrene concentrations measured. Styrene (18.5 ppm (6.6-36.4 ppm)). Urinary metabolite MA measured. Urinary MA (0.22 g/l). | Lanthony D15 hue desaturated panel carried out 1x with a 1000-lux lamp, and CCI calculated. | Significantly higher mean CCI in exposed workers compared with controls. Positive correlation between urinary MN and CCI. |
| Fallas *et al.* 1992 | Styrene | Ship constructors (n=60) and age/sex matched controls (n=60). | Ambient air measured. Styrene (24.3 ppm). Urinary metabolite MA and PGA measured. MA (230 mg/g creatinine (2-1460 mg/g creatinine)); PGA (57.4 mg/g creatinine (0.4-421.2 mg/g creatinine)). | Farnsworth-Munsell 100 hue test carried out 1x and error scores calculated. | No significant difference in error scores between workers and controls. |
| Gobba *et al.* 1991 [1] | Styrene | Fibreglass-reinforced plastic factory workers (n=75) and controls (n=60). | 8-hr environmental monitoring by personal passive sampling and urinary MA and styrene measured. Airborne styrene (69.02 mg $m^{-3}$ (3.2-549.2 mg $m^{-3}$); urinary MA (342 mg/l (15.1-3002 mg/l)); urinary styrene (49.5 μg/l (1.6-202 μg/l)). | Lanthony D15 hue desaturated panel carried out 1x with a 1200-lux lamp, and CCI calculated. | Significant increase in CCI in older exposed workers compared with controls. Higher CCI in workers exposed to higher conc. of styrene. |
| Kishi *et al.* 2000 [3] | Styrene | Workers exposed to styrene (n=87) and controls (n=87). | Urinary metabolite MA measured. MA (<0.1->0.2 g/l) equivalent to atmospheric styrene conc. of 8 - >16ppm. | Lanthony D15 hue desaturated panel carried out 1x (type of lamp not stated) and CCI calculated. | Significant increase in CCI in exposed workers compared with controls, which significantly correlated with urinary mandelic acid. |
| Kishi *et al.* 2001 [3] | Styrene | Fibreglass and reinforced plastics workers (n=105) and non-exposed controls | Urinary metabolite MA and area atmospheric styrene conc. measured. Styrene (21.0 ppm (6.6-36.4ppm)) | Lanthony D15 hue desaturated panel carried out and CCI calculated. | Significant increase in CCI in exposed workers compared with controls. Significant difference in CCI between |

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| | | (n=117) from same factory | | | high and medium exposure groups but not low exposure group and controls. |
| Triebig *et al.* 2001 | Styrene | Laminators (n=50) and controls (n=27). | Urinary MA and PGA measured. Ambient styrene estimated.<br>MA+PGA (11-2399 mg/g creatinine). Styrene (approximately 20 ppm) | Lanthony D15 hue desaturated panel carried out 1x with a 1000-lux lamp, and CCI calculated. | Increased in CCI at end of working week. Mean CCI decreased during short period of non-exposure. Tritan and red-green errors made. |
| **Toluene** | | | | | |
| Campagna *et al.* 2001[1] | Toluene | Photogravure plant workers (n=92), controls with ambient exposure (n=43) and controls from a bookbinding plant (n=31) | 8-hr personal air sampling in breathing zone carried out. Toluene (136 mg $m^{-3}$ (50-296 mg $m^{-3}$)) above the OES. | Lanthony D15 hue desaturated panel carried out and CCI calculated. | Increased CCI in exposed workers and workers with ambient exposure compared with controls. Higher number of workers and workers with ambient exposure with dyschromatopsia (type I, II and III) than controls. |
| Cavalleri *et al.* 2000[1] | Toluene | Rubber plant workers (smearers (n=24 and solutioners(n=9)) and controls (16). | Urinary excretion of unmodified toluene (TolU) measured. Urinary Tol – smearers (59.9 µg/l); solutioners (34.7 µg/l). | Lanthony D15 hue desaturated panel carried out 1x with a 1200-lux lamp. CCI and total confusion index (TOTCI) calculated. | Significant increase in CCI and TOTCL in exposed workers compared with controls. Neither CCL or TOTCL were significantly correlated to TolU. |
| Muttray *et al.* 1995[4] | Toluene | Rotogravure workers (n=59) | Blood toluene concentrations measured. Toluene (<0.22-7.37mg/l). | Ishihara test, Farnsworth D15 test, Lanthony D15 hue desaturated panel, the standard Pseudoisochromatic plates part 2 and Velhagen test carried out. | No significant change in colour discrimination before and after toluene exposure. |

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| Muttray *et al.* 1999[4] | Toluene | Printshop workers (n=8) and controls (n=8) | Blood toluene conc. measured. Toluene (3.61-7.37mg/l). Exposure to 290-360ppm toluene for 30 minutes. | Farnsworth D15 test, Lanthony D15 hue desaturated panel and the standard Pseudoisochromatic plates carried out 1x under standard lighting conditions. | No significant difference in colour discrimination between workers and controls. |
| Nakatuka *et al.* 1992 | Toluene | Paintshop workers (n=174) and controls (n=120) | Toluene (46ppm) | Ishihara's test and Lanthony new colour test carried out 1x under natural sunlight or artificial lighting conditions. | No significant difference in colour discrimination between workers and controls. |
| Zavalic *et al.* 1998a[5] | Toluene | Print workers. Group 1 (n=41); Group 2 (n=32) and controls (n=83). | Ambient air measured. Blood toluene conc. measured. Urinary hippuric acid (HA) and orthocresol measured. Toluene: group 1. (11.30-49.30ppm); group 2 (66.00-250.00ppm). | Lanthony D15 hue desaturated panel carried out 1x under natural sunlight rather than standardised conditions and CCI calculated. | Significant increase in CCI and alcohol-adjusted CCI in both worker groups compared with controls. |
| Zavalic *et al.* 1998b[5] | Toluene | Print workers (n=45) and controls (n=53). | Ambient air measured. Blood toluene conc. measured. Urinary HA and orthocresol measured. | Lanthony D15 hue desaturated panel carried out 1x under natural sunlight rather than standardised conditions. Age and alcohol adjusted CCI calculated. | No significant difference in colour discrimination between workers and controls. |

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| Zavalic *et al.* 1998c[5] | Toluene | Shoe factory workers (E1; n=48), printers (E2; n=39) and controls (n=92) | Toluene concentration in ambient air measured. E1< 50ppm. E2 >10ppm. Blood toluene concentrations measured and urinary concentrations of HA and orthocresol. Significant correlation between toluene in ambient air and urinary conc. of hippuric acid and orthocresol in E2. | Lanthony D15 hue desaturated panel carried out 1x in natural daylight. | Significant increase in frequency of dyschromatopsia in exposed workers compared with controls. |
| **Perchloroethylene** | | | | | |
| Cavalleri *et al.* 1994[1] | Perchloroethylene | Dry cleaners (n=22), ironers (n=13) and controls (n=35) | Time weighted average concentrations of PCE measured in breathing zone by personal passive samplers. PCE - dry cleaners (7.27 ppm (0.38-31.19)); ironers (4.8 ppm (0.52-11.28 ppm)). | Lanthony D15 hue desaturated panel carried out 1x with a 1200-lux lamp, and CCI calculated. | Significant increase in CCI in dry cleaners compared with controls, with a dose related effect. |
| Gobba *et al.* 1998 | Perchloroethylene | Dry cleaners (n=33) and controls (n=10) | Concentrations of PCE measured in breathing zone by personal passive samplers. PCE (1.94 – 240 ppm). | Lanthony D15 hue desaturated panel carried out 1x with a 1200-lux lamp, and CCI calculated. | Significant increase in CCI in older exposed workers compared with controls. Higher CCI in workers exposed to higher concentration of styrene. |
| Till *et al.* 2003[6] | Perchloroethylene | Children from mother working in a dry-cleaning business whilst pregnant, and non-exposed children (n=3) | No measure of PCE obtained. Concentrations of PCE reported to be within regulated limits. | Visual evoked potential measured and a Minimalist test carried out. | Reduced contrast sensitivity and colour vision in exposed child compared with controls. |

| n-hexane | | | | | |
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| Raitta *et al.* 1978[7] | n-Hexane | Adhesive bandage factory workers (n=8) and vegetable oil extraction workers (n=7). | n-hexane measured in breathing zone of workers. n-Hexane in ambient air (factory workers, 423-3250ppm); vegetable oil workers, 10-50 ppm; due to leaks 2000-3000 ppm). Concentrations in breathing zone were lower (data not given). | Farnsworth D15 and Farnsworth-Munsell 100 hue tests. | 3/15 subjects showed normal colour discrimination according to both tests. 11 subjects showed acquired colour discrimination with one or both of the tests used, mainly in the blue-yellow spectrum. One subject had a congenital green defect. |
| Seppalainen *et al.* 1979[7] | n-Hexane | Adhesive bandage factory workers (n=8), vegetable oil extraction workers (n=7) and controls (n=10). | n-Hexane measured in the breathing zone of workers. n-Hexane (vegetable oil workers if leaking 2000-3000ppm; factory workers, 1500-3250ppm). | Visual evoked potentials (VEPs) and electroretinographs recorded. | Significantly lower VEP and ERG amplitudes in exposed workers compared with controls. |
| **Carbon disulfide** | | | | | |
| Raitta *et al.* 1981[7] | Carbon disulfide | Viscose rayon workers (n=64) and controls (n=46). | Exposure assessment not carried out. Overall carbon disulfide concentrations below the threshold limit value (data not given). | Farnsworth-Munsell 100 hue test. | Significant shift towards impaired colour discrimination in exposed workers compared with controls. |
| Ruijten *et al.* 1990 | Carbon disulfide | Viscose rayon plant workers (n=45) and controls (n=37). | Personal air sampling carried out. Carbon disulfide (1-12 ppm) | Lanthony D15 hue desaturated panel carried out 1x using two fluorescent light sources (Philips type 57), and CCI calculated. | No significant difference in CCI between workers and controls. |
| Wang *et al.* 2002 | Carbon disulfide | Viscose workers (n=271) and controls (c=133) | 8-hr personal air sampling carried out. Carbon disulfide (13.7-20.5 mg $m^{-3}$) | Farnsworth-Munsell 100 hue test under daylight conditions and error score calculated. | Significantly increased total error score of green-zone and blue-zone in exposed workers compared with controls. |

| Mixtures | | | | | |
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| Bowler *et al.* 2003 | Manganese, chromium, iron oxide. | Welders (n=81) and non-exposed controls (n=83). | None reported. | Lanthony D15 hue desaturated panel and Farnsworth-Munsell test carried out 1x (type of lamp used not stated) and colour confusion index (CCI) calculated. | Significantly higher CCI score in right eye of welders compared with controls. |
| Gobba and Cavalleri 2000[1] | Styrene, PCE and mercury. | Fibre-glass reinforced plastics (FRP) factory workers (n=39 and 30; exposed to styrene), dry cleaners (n=33; exposed to PCE), thermometer manufacturers (n=21; exposed to mercury) and controls (n=10). | Time weighted average exposures measured by biological monitoring or personal passive sampling. Styrene (13 ppm (3.1-29.2 ppm)); PCE (2.4ppm (0.01-18.8)); mercury (114 μg/g creatinine and 10 μg/g creatinine following factory modification). | Lanthony D15 hue desaturated panel carried out 1x in subjects and 10x in controls with a 1200-lux lamp, and CCI calculated. | Significant increase in CCI in FRP workers, dry cleaners, thermometer manufacturers than controls. Colour vision loss in workers exposed to Hg was reversible, compared to styrene and PCE exposed workers. |
| Gong *et al.* 2002[3] | Styrene, acetone, 2-hexanone, orthoxylene, metaxylene, paraxylene. | Fibreglass reinforced plastic boat plant workers (n-76) and sex-matched controls (n=102). | Individual air samples and urinary MA, PGA and styrene concentrations measured. Atmospheric styrene (49.9 ppm). Urinary MA (0.26 g/g creatinine); PGA (0.11 g/g creatinine); styrene 9138.6 μg/l). | Lanthony D15 hue desaturated panel carried out 1x with a 1200-lux lamp, and CCI calculated. | Significant increase in CCI in exposed workers compared with controls. |
| Gong *et al.* 2003[3] | n-hexane, acetone, methylethylketone, 1,2-dichloroethane, butyl acrylate, methylisobutylketone, isobutylacetate, toluene, n-buthanal acetone, | Furniture factory workers (n=251) and age-matched unexposed controls (n=147). | 8-hr personal air sampling in breathing zone carried out. Environmental samples - n-hexane (0.29ppm); acetone (2.11ppm); methylethylketone (0.33ppm); 1,2-dichloroethane (0.29ppm); butyl acrylate (0.26ppm); | Lanthony 15 hue desaturation panel and CCI calculated. Visual evoked potential (VEP) and visual contrast sensitivity measured. | Significant increase in CCI in exposed workers compared with controls. Decreased spatial frequency (visual contrast sensitivity) in workers compared with controls. No change in VEP. The CCI was negatively correlated to visual |

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| | ethylbenzene, xylene, styrene, cyclohexanone. | | methylisobutylketone (0.37ppm); isobutylacetate (0.37ppm); toluene (6.43ppm); n-buthanal acetone (1.83 ppm); ethylbenzene (2.55ppm); xylene (1.94ppm); styrene (1.15ppm); cyclohexanone (0.33ppm). Urinary solvents and metabolites – xylene (0.06 l/g); hippuric acid (0.36 l/g); methylhippuric acid (0.04 l/g); mandelic acid (0.04 l/g). | | contrast sensitivity. |
| Mergler *et al.* 1991[2] | Chlorofluorohydrocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, ethyl alcohol. | Microassembly workers (n=54) and controls (n=54) | Not stated | Lanthony D15 hue desaturated panel carried out 1x using a 150-lux lamp, and CCI calculated. | Significant increase in CCI in exposed workers compared with controls, only in one eye. |
| Muttray *et al.* 1997[4] | Xylene, toluene, ethyl benzene, propyl benzene, ethyl toluene, methyl ethyl ketone, methyl isobutyl ketone, perchloroethylene. | Automobile workers (n=24) | Personal air sampling carried out. PCE (below limit of detection). | Ishihara test, Farnsworth D15 test and Lanthony D15 hue desaturated panel carried out 1x using a 900-lux lamp, and CCI calculated. | Significant increase in CCI in exposed workers compared with controls. |
| Nakatsuka *et al.* 1992 | Toluene, xylenes, isopropyl alcohol, tetrachloroethylene | Paint production workers (n=261) and controls (n=120) | Time-weighted average solvent vapour concentrations in breathing zone measured. Toluene (46ppm), tetrachloroethylene (13ppm) or mixture of tetrachloroethylene (12ppm) and trichloroethylene (7ppm). | Lanthony and Ishihara's colour vision test, carried out 1x using a 1150-lux lamp or natural daylight. | No significant loss of blue-yellow colour vision observed in workers. |
| Sharanjeet *et al.* 2004 | Polyethylene, polystyrene, | Plastic products plant workers (n=39 and | Not stated. | Ishihara plates, Lanthony D15 hue | Increase in CCI in exposed workers compared with |

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| | perchloroethylene | n=40), dry-cleaning workers (n=14) and non-exposed controls (n=27 and n=29) | | desaturated panel and Farnsworth-Munsell 100 hue test Confusion index and total error score calculated. | controls. Increased total error scores in workers. |
| Till *et al.* 2001[6] | Benzene, toluene, methane, ethane, trichloroethylene, methyl chloride, methanol, ethanol, methyl ethyl ketone, petroleum fuels, naphthalene, glycols, ethers | Children from mothers exposed to solvents whilst pregnant (n=36) and controls from unexposed mothers (n=27) | Questionnaires used to ascertain exposure. | Minimalist test carried out and visual acuity measured | Decreased colour discrimination and visual acuity in exposed children compared with controls. |
| Till *et al.* 2005[6] | Halogenated solvents, aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, aldehydes, ketones | Children from mothers exposed to solvents whilst pregnant (n=321) and controls from unexposed mothers (n=27) | Questionnaires used to ascertain exposure. | Visual evoked potential technique | Decreased contrast sensitivity in exposed children compared with controls. |
| Valic *et al.* 1997 | Trichloroethylene, perchloroethylene, toluene, xylene | Workers (n=138) and non-exposure controls (n=100) | Urinary concentrations of trichloroacetic acid as a measurement of trichloroethylene/ perchloroethylene exposure. TCA (1.55 mg $L^{-1}$). | Lanthony D15 hue desaturated panel carried out 1x using a 1200-lux lamp, and CCI calculated. | Colour vision was not affected by solvent exposure or alcohol intake. |

1 – Same group from France; 2 - Same group from Canada; 3 - Same group from Japan; 4 - Same group in Germany; 5 - Same group from Croatia; 6 - Same group from Canada; 7 - Same group from Finland.