effects of petroleum hydrocarbons on the nervous system

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PART I

GENERAL OVERVIEW OF THE EFFECTS OF PETROLEUM HYDROCARBONS ON THE NERVOUS SYSTEM

1. INTRODUCTION

Recently it has been claimed that long-term exposure to low levels of petroleum hydrocarbons may impair behaviour and memory. This claim has led to an appraisal of the effect of these products on the nervous system. CONCAWE has therefore reviewed relevant published literature available up to 1984. This is covered in detail in Part II. A brief general overview of the subject is provided in Part I.

2. THE HUMAN NERVOUS SYSTEM

The nervous system in man is made up of two basic units - the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). The CNS consists of the brain and spinal cord. The brain is the site of intelligence, memory, emotion and other activities and is responsible for integrating the messages received from the outside world and determining the response to them. The PNS consists principally of the nerve trunks that flow from the spinal cord and the base of the brain to be distributed throughout the body. These nerve trunks carry messages between the various organs and tissues of the body and the CNS.

Both of these units may be adversely affected by hydrocarbon solvents and damage to one system may not necessarily be accompanied by damage to the other.

3. TYPES OF ADVERSE NEUROLOGICAL EFFECTS

Three types of adverse neurological effects have been described, (a) CNS depression, (b) damage of PNS and (c) CNS disease.

3.1. CNS DEPRESSION

A single exposure to a moderately high concentration of virtually any hydrocarbon solvent vapour will cause a general depression of CNS which, at high doses, will lead to unconsciousness. This property has been recognised for many years and some hydrocarbons (e.g. ethane) have been used as anaesthetics.

Controlled short-term exposure of healthy subjects (up to 1-2 weeks), by repeated inhalation, to xylene, toluene, white spirit and jet fuel has shown that at levels of exposure above 250, 150, 300 and 200 ppm respectively an impairment of concentration, and of coordination occurs. These effects are readily and completely reversible on cessation of exposure.

3.2. PNS DAMAGE

In addition to producing CNS depression n-hexane and methyl n-butyl ketone cause damage to the peripheral nerves, particularly of the feet and hands and this results in disturbances of sensation (numbness and tingling) and muscle weakness. If the damage is severe, paralysis may result; this paralysis is rarely permanent but recovery is slow. Nerve damage of this type has been found to occur in workers with a history of fairly heavy and prolonged (several months) exposure to the solvent vapour (500-1000 ppm in the atmosphere) and liquid.

A typical example is that of the shoemakers who worked for long hours in confined spaces and used a glue dissolved in n-hexane. This type of nerve damage has been shown to be due to the formation of hexane 2,5-dione from the metabolism of n-hexane and methyl n-butyl ketone and does not appear to be caused by other hydrocarbons or ketones; there is, however, evidence that substances with structures related to hexane or methyl n-butyl ketone (eg methyl ethyl ketone) can potentiate the nerve damage caused by these two hydrocarbons.

3.3. CNS DISEASE

In the last ten years or so, a number of publications has appeared, particularly from Scandinavia, which suggest that workers employed in occupations involving exposure to organic solvents suffer a deterioration in their emotional balance, memory, intelligence and powers of concentration. They also describe a higher than average incidence of headaches, dizziness and other subjective complaints. Those investigated were principally painters and lacquerers. The condition has been given various names, painters'syndrome, organic solvents disease, psycho-organic syndrome, chronic Danish syndrome and chronic organic solvent intoxication. Specialised investigations have provided no evidence of nerve or brain damage in workers affected by this syndrome.

A critical evaluation (1) of these publications revealed that the solvents principally involved are toluene, white spirit and jet fuel; xylene and styrene do not appear to have been implicated.

Toluene and white spirit are important components of many paints; in addition toluene is used extensively as a de-greasing and cleaning agent. Examination using a battery of psychological tests in workers who had been exposed for several years to toluene vapour at atmospheric levels of approximately 100 ppm failed to establish any differences from controls. Subjective complaints were, however, more frequent in exposed subjects. At very high concentrations, such as may occur in glue-sniffing, toluene has been reported to cause damage to the cerebellum (a part of the brain that controls balance).

No studies are currently available on the effects of long-term exposure to white spitit but two such studies are available on jet fuel. Psychological and psychiatric tests revealed no meaningful difference between groups of workers who had been exposed to jet fuel for several years and a matched control group who had not been exposed.

Because painters and lacquerers are exposed to a variety of solvents it is impossible to ascertain which of the solvents (or of the many mixtures available) is implicated in the painters'syndrome. More importantly, whether or to what extent exposure to solvents contributes to the cause of this disease is uncertain.

In the majority of the papers reviewed, insufficient attention has been given to the possibility that other factors could lead to the development of the findings in those exposed. The most important of these factors are alcoholism, use of psychoactive drugs, exposure to lead or mercury and advancing age. Lead and mercury are particularly relevant in this respect since they have, until recently, been important components of many paints; hence most of the painters who had been in this occupation for ten or more years must have had substantial exposure to these chemicals.

The impression gained from a review of the literature is that although it would appear that some groups of painters and others exposed to solvents show a deterioration of some important mental functions, the aberration is of a minor nature and impossible to connect causally with solvent exposure. It may equally well be attributed to other causes.

It must be recognised however that many physicians and psychologists in Scandinavia are convinced that the disease does exist and only performance of well designed epidemiological studies which avoid the failings of the studies reviewed will provide evidence to justify or refute this convinction.

4. CONCLUSIONS

Whilst there is good evidence that reversible CNS depression can result from exposure to moderately high vapour concentrations of organic solvents and that PNS damage can occur from exposures to n-hexane and methyl-n-butylketone at levels above currently established exposure standards, there is at present no conclusive evidence that brain damage is caused by long-term exposure to organic solvent vapours. However, in accordance with accepted good industrial hygiene practices, it is prudent to keep exposures to solvent vapours as far below recommended limits as is reasonably practicable.

5. REFERENCES

1. Grasso, P. et al (1984) Neuro physiological and psychological disorders and occupation exposure to organic solvents. Food and Chemical Toxicology 22, 819

PART II

DETAILED REVIEW OF THE EFFECTS OF PETROLEUM HYDROCARBONS ON THE NERVOUS SYSTEM

1. INTRODUCTION AND OVERVIEW

1.1 EFFECTS ON THE CENTRAL NERVOUS SYSTEM

Over the last ten years a series of publications have appeared on the results of studies of the functioning of the central nervous system in workers exposed to industrially-used solvents and some related petroleum hydrocarbon fuels after short- or long-term exposure periods. Studies on volunteers have supplemented the data obtained from studies on workers. The functions of the human brain are extremely complex and interrelated and thus difficult to study separately. It is known that some parts of the brain may be damaged or even surgically removed without causing obvious clinical signs or symptoms. The functions of these parts may be taken over by other parts since anatomical repair is impossible. Formation of new neurons does not occur, but new synapses may be formed. The brain has a large reserve functional capacity and diffuse brain changes have to be extensive before they become clinically evident. Classical neurological examination frequently lacks sensitivity to detect early diffuse brain changes and so behavioural methods have been developed to investigate the various functions of the brain i.e. memory tests, overall intelligence, capacity for sustained attention, dexterity and eye-hand coordination, reaction time, psychomotor function and personality or mood (1,2,3).

Though these methods may be sensitive, they lack specificity and cannot be used to differentiate between for example, senile atrophy of the brain from the after effects of episodes of cerebral anoxia or to differentiate reversible from progressive (irreversible) changes. The results of such tests are also influenced by factors such as pain, fatigue, lack of sleep, irritation. Therefore, other indices of cerebral changes have been examined in some studies, including electroencephalograms, electroneuromyograms, pneumo-encephalograms and also computerized axial tomograms.

In the seventies reports appeared on behavioural effects due to short-term and long-term exposure to various types of hydrocarbon and other solvents, to white spirits, or to aromatic compounds such as toluene or styrene. Most of these reports originated from Scandinavian countries.

They present the statistical evaluation of the results of psychiatric, psychological and psychometrical studies, sometimes supplemented by neurophysiological, neurological and radiological studies. Some of these studies reported the presence of subclinical neuropathic effects similar to those following n-hexane exposure and indeed probably due to the presence, frequently unsuspected, of n-hexane in the solvent mixture.

However, a high incidence of neurasthenic complaints and subclinical or clinical behavioural effects was found such as changes in reaction time and memory. These effects were not only found in workers after periods of excessive exposure but also reported from exposure to vapour concentrations which, until now, were not considered to have any effects. Finally, irreversible effects have been found in long-term exposed workers which clearly indicate the presence of an organic brain syndrome associated with radiological or other evidence of atrophy of the brain.

The use of questionnaires to examine the incidence of subjective symptoms is wide-spread but subject to difficulties of interpretation. A great drawback of these behavioural methodologies is the lack of standardization of tests; the selection of behavioural tests has been based often on convenience and local experience and many of them cannot be adapted to animal testing, which makes comparison with data from experiments on animals impossible.

As a result of studies of individual cases or collections of individual cases, of studies on volunteers and of epidemiological studies on exposed workers, a range of symptoms, signs and syndromes varying from neurasthenia to dementia has been described, allegedly caused by repeated exposure to petroleum hydrocarbons, either as mixtures of individual compounds and to several other types of industrially-used solvents. As is well known, almost all volatile solvents have a general anaesthetic effect. The biochemical basis of this effect has, as yet, not been clarified but has been found to be related to the lipid solubility. It has been assumed that the solvent molecules are incorporated into the nerve cell membranes and thus affect ionic transfer. This may be an explanation of acute, reversible effects caused by short-term exposure, but the biochemical or histological basis of the development of permanent effects following long-term exposure is still speculative. A similar situation exists as regards the histological aspects, which scarcely have been studied. Permanent effects from anaesthesia, if occurring, were considered to be due to anoxia of the brain as a result of depression of respiration, effects on the function of the cardio-vascular system or dysfunction of the respiratory system. Another general effect of the presence of hydrocarbons in the blood was known to be an increase in the permeability of the endothelium of the small vessels, which may induce the formation of perivascular oedema and haemorrhage. This effect, in general, is only seen at high concentrations but one aromatic compound i.e. p-tertiary-butyltoluene shows this action at relatively low concentrations. In this particular case small haemorrhages in the spinal cord may cause paralysis or other permanent damage.

However, doubts have been thrown on the relatively simple concept of the neurotoxic effects of hydrocarbons.

1.2 EFFECTS ON THE PERIPHERAL NERVOUS SYSTEM

In the early sixties a series of epidemics of a peripheral neuropathy occurred among workers in the leather industry exposed to technical hexane used as a solvent.

The first was observed in Japan but subsequently in many other countries. Experimental studies on animals proved that n-hexane possesses a neurotoxic action. An epidemic of a similar peripheral neuropathy was observed among workers exposed to a hexanone i.e. methyl-n-butylketone, and biochemical studies led to the discovery that this hexanone is a metabolite of n-hexane and that both lead to the formation of a highly neurotoxic diketone, 2,5-hexanedione, which has a specific affinity for nervous tissue.

Recently it was found that the neurotoxic potency is due to the gamma-diketone structure since the heptadione or octadione are also neurotoxic if they possess the gamma-diketone structure, but not in those with an alpha-or beta-diketone structure.

Experimental neurpathological studies demonstrated that not only are the peripheral nerve fibers affected but that, simultaneously, changes in the fibers of the spinal cord, the medulla oblongata, the optic tract and manillary bodies develop.

The subject of neuropathy related to exposure to n-hexane has been extensively reviewed (9).

1.3 GLUE SNIFFER'S NEUROPATHY

Cases of a similar neuropathy were reported from the medical observation of a relatively new type of addiction in which the victims voluntarily inhale the vapours of some glue- or paint thinners containing hexane. These cases are referred to as huffer's or gluesniffer's neuropathy. Isolated cases of different effects on the central nervous system have been reported e.g. cerebellar atrophy and dementia after long-term inhalation of solvents containing, for example, toluene. Many of these reports lack, however, satisfactory data on the composition of the solvent which was inhaled.

Obviously, the victims of this addiction show changes in personality or neurological status, part of which may have been pre-existent and are not necessarily a sequel to the inhalation of the solvent.

2. HEXACARBON NEUROTOXICITY

n-Hexane ($CH_3-(CH_2)_4-CH_3$) has been demonstrated to be the agent responsible for outbreaks of a peripheral neuropathy in groups of workers in various industries and in individuals addicted to the inhalation of glue solvents or thinners.

The first epidemic was described in the early sixties in Japan (4-8) and subsequently outbreaks occurred in Italy (9-13), France 14,15), Morocco (16), the USA (17-21) and Germany (22). In 1973 a similar outbreak was discovered among workers at a plant in the USA where n-hexane was not used but where the causitive agent was found to be a related hexacarbon solvent i.e. methyl-n-butylketone (MBK) ($CH_3-CO-(CH_2)_3-CH_3$).

Studies on man and experiments on animals showed conclusively that it was the same disease caused by a neurotoxic metabolite from both n-hexane and MBK. In fact MBK is one of the metabolites of n-hexane. An excellent survey of these studies, both clinical and experimental, are found in ref. 11 and 23, on which the following description is based.

2.1 CLINICAL PICTURE

The most common, initial complaint is the insidious onset of numbness of the toes and fingers. It may remain the only complaint in the least severe cases. The distribution is symmetrical and involves only the hands and feet, rarely extending as high as the knee. There is a moderate loss of touch, pin, vibration and thermal sensation and there may be a loss of the ankle jerk. In mild cases position sense is retained and there is no sensory ataxia or periosteal pain; no cranial nerve abnormalities or evidence of autonomic dysfunction are present.

In more severe industrial cases weakness and weightloss are seen, which may be accompanies by anorexia, abdominal pain and cramps in the legs. Loss of reflexes is usually restricted to the ankle and finger jerks. Muscular weakness is usually restricted to the intrinsic muscles of the hands and to long flexors and extensors of the fingers and toes. Pinching or grasping of objects and stepping over kerbs becomes difficult.

Cases of pure motor neuropathy are unusual in the industrial setting. Vibration and position senses are only mildly impaired and the loss of pinprick and touch sensation remains confined to the hands and feet. In more severe cases weakness and atrophy increase and progress to involve the proximal muscle groups.

In very severe cases, as seen among glue sniffers, bulbar or phrenic nerve paralyses are seen. There has never been found objective evidence of visual loss but optic atrophy has been seen in a few cases. Memory loss has only been described once. There are no documented cases of seizures, cerebellar ataxia, tremor or cholinergenic symptoms.

One study (11) indicated that slowed motor nerve conduction may be seen in clinically "normal workers" in a factory where also cases of neuropathy were present.

Glue sniffers have also shown autonomic disturbances such as hyperhydrosis of the hands and feet which may be followed by anhydrosis. Blue discoloration and reduced temperature of the hands and feet and Mees' lines have occasionally been seen.

2.2 CLINICAL COURSE

In industrial cases the onset has been insidious and progression slow. Among glue sniffers the course may be subacute, leading in severe cases to quadriplegia within 2 months after the onset. A universal feature has been the progression of the disease continuing for 1-4 months after discontinuing exposure. The degree of recovery correlates usually with the severity of the disease. Mild and moderate cases usually recover completely within 10 months after cessation of exposure. In severe cases some residual neuropathy may be found e.g. hyperactive kneejerks, which is assumed to be due to a degeneration of the corticospinal tracts in the spinal cord.

In very severe cases, such as observed among glue sniffers, full strength may not be recovered. Persistent hyperpathia and autonomic dysfunction has been seen in such cases.

2.3 ELECTRODIAGNOSTIC STUDIES

By the use of electromyography changes were observed in exposed workers without any subjective or objective signs of neuropathy. This has been observed while studying the outbreak of toxic neuropathy in the USA in workers exposed to methyl-n-butylketone (23).

The changes in the tracting are usually symmetrical and greater in the distal muscles. Electromyography is suggested as an excellent screening device to detect subclinical effect.

Nerve conduction velocity (NCV) determinations show, at most, only very slight changes in cases with minimal clinical involvement. With clinical illness there is a progressive slowing and in extreme cases distal perioneal nerve conduction cannot be detected (23). This slowing of NCV has been found in many studies (11, 21, 22, 24). Changes have been observed in visual evoked potentials and electroretinograms indicating that there may be axonal degeneration in the central nervous system visual tracts (24).

Also the H-reflex response may be changed in cases of polyneuropathy (23).

2.4 LABORATORY DATA

Examination of the cerebrospinal fluid usually shows no changes in protein amount or cell count. An elevation of the protein content can occur when nerve fiber degeneration has ascended to the spinal roots. Clinical laboratory findings reveal no consistent changes. In one study (23) there was a decrease in blood cell cholinesterase accompanied by an elevation of plasma butyrocholinesterase. This finding was, however, not correlated with the presence of neuropathy.

2.5 NEUROPATHOLOGICAL DATA

Teased myelinated nerve fiber preparations of nerve biopsies of typical cases show paranodal, giant, axonal swellings accompanied by myelin retraction (12, 20, 22, 25). These axonal swellings, lying just proximal to the nodes of Ranvier, consist of accumulations of 10-nm neurofilaments (21).

Demyelination, remyelination and regeneration are also present. In experiments on animals similar changes have been caused by exposure to both n-hexane and methyl-n-butlyketone (26).

This central-peripheral axonopathy is further characterized by distal and retrograde axonal degeneration occurring in long and large fiber tracts in the peripheral as well as in the central nervous system. As regards the central nervous system, experimental studies have shown that the long ascending and descending spinal tracts are first affected. Changes have been found in both the cerebellar white matter and the distal optic nerve and also in the mamillarly bodies.

2.6 BIOCHEMICAL DATA

Biochemical studies have shown that the neurotoxic aliphatic hexacarbon diketones derived metabolically from n-hexane inhibit glycolytic enzymes and bind to proteins which may impair the axonal transport system.

Recently a disturbance in lipid metabolism was found, in particular in sterol metabolism, in the peripheral nerves of n-hexane fed rats (27). Whether this is the cause or consequence of other changes is not clear.

2.7 POTENTIATION OF THE EFFECTS OF N-HEXANE BY OTHER SUBSTANCES

The possibility of potentiation of the neurotoxic action of n-hexane by other components of solvent systems is obviously of great importance. There is clinical, epidemiological and experimental evidence that methylethylketone (MEK) when administerd with n-hexane, a decrease of the toxic effects of n-hexane increases the neurotoxic action (22, 28).

In a recent study on rats, exposed to a mixture of toluene and n-hexane, a decrease of the toxic effects of n-hexane was found (29).

Future studies will clarify the exact mechanism by which potentiation or antagonism is occurring. The interaction may involve an effect on the uptake or, more probably, the metabolism of n-hexane. It is known from in vitro studies that n-hexane is devoid of neurotoxicity but that 2,5-hexanedione, a metabolite, which n-hexane shares with methyl-n-butylketone, is the ultimate neurotoxicant (30). MEK may increase the concentration of this neurotoxic metabolite by increasing its formation or decreasing its breakdown.

An antagonism may be explained e.g. by competitive inhibition of the enzymatic oxidation, by stimulation of the breakdown or by interference in respiratory uptake or elimination. Many more studies will have to be done before this important field of investigation has been well studied.

2.8 DOSE-RESPONSE RELATIONSHIP

The conditions of exposure causing the epidemics of neuropathy in Japan and Italy have been unusual since the activities were of the house-hold type, involving long working hours (up to 12 per day) and poor ventilation especially in winter time. Air levels as high

as 2500 ppm must have been present. From the experience in Japan it could be calculated, that exposures to n-hexane at a level of 500-1000 ppm, 8 hours per day cause a clinically evident neuropathy in 3-7 months (7).

The levels of exposure in most cases have been in the range of 250 ppm or more. Skin exposure must also have been present but the significance of its contribution to the total exposure cannot be defined.

Experimental data from rat studies indicate that at vapour exposure levels of 100 ppm or less no evident neuropathy will develop.

In a recent study on a small group of workers, where skin exposure was excluded and vapour exposure averaged 58 ppm, 8 hours/day over an average of 5 years, no clinical signs or symptoms of neuropathy were found but a statistically significant difference was found in the results of a study of the neuropsychological condition as judged by questionnaires, neurological examination and neurophysiological measurements when compared to that of an age matched control group (24). Since the groups were small, the differences slight and the exposure mixed (in this case with acetone) it may not be concluded that the difference is real. It indicates that more extensive studies should be done.

The present, generally accepted standard of maximum acceptable exposure to n-hexane for an eight hour working day, 5 days per week for a working lifetime is 100 ppm, based on epidemiological evidence and on experimental studies on the rat. In several countries is thas recently been proposed to lower this standard e.g. in the USA (31) and The Netherlands (32). In any case, skin exposure should be minimized, since skin exposure has been reported to cause neuropathy (33).

Since it is known that at least one compound (MEK) may enhance the appearance of neuropathy in n-hexane exposed workers, it is important to know the composition of the mixtures containing n-hexane and to measure the exposure to the various components. Conceivably also drugs such as phenobarbital, stimulating the microsomal oxidation system of the liver cell, may show interaction. As far as is known, the effect of alcohol consumption has not been studied.

2.9 DISCUSSION AND CONCLUSIONS

The clinical observation that workers exposed to n-hexane or methyl-n-butylketone may develop a neuropathy has induced a series of interesting studies in various fields: clinical, biochemical, electrophysiological, behavioural and neuropathological.

A disease entity with typical clinical, electrophysiological and neuropathological findings has now been defined to be caused by exposure to n-hexane and methyl-n-butylketone and found to be due to a common metabolite, 2,5-hexanedione. In man and also in many animals species (with the exception of pigeons), exposure to these two compounds has caused a similar neuropathy.

Experimental studies with a few other gamma-diketones with longer straight carbon chains (7 and 8 carbon atoms) have demonstrated that the neurotoxic activity is related to the gamma-diketone structure. However, under practical conditions of exposure, only the 2-5 gamma-diketone has been shown to present this activity. It is assumed that the quantity of the toxic gamma-diketone formed during the metabolism of compounds of chainlengths other than those with six carbon atoms is too small to be effective or alternatively are not neurotoxic.

It is not advisable at the present moment to rely too strongly on this assumption for the following reason. Methylethylketone (MEK) has been shown to increase the neurotoxic effect of the hexacarbons considerably, by a biochemical mechanism which is not understood. Whether MEK has the same effect when administered together with a 7 or 8 carbon atom containing alkane has not been studied. The possibility that other substances may have a similar effect of enhancing toxicity, has not been excluded. Toluene is probably devoid of this synergistic effect and may even offer some protection.

A study of the literature on this type of neuropathy due to exposure to petroleum hydrocarbons indicates that n-hexane or the related compound methyl-n-butylketone has always been present or at least their presence could not be completely excluded.

The contribution from skin absorption to the total absorption by occupational exposure is only poorly understood. This is partly due to the lack of experimental studies on percutaneous absorption in man as well as in experiments on animals and partly to the fact that the measurement of total exposure is, as yet, impossible. In principle, the measurement of urinary metabolites or the levels in the blood of n-hexane and/or its metabolites should offer a possibility, but under practical conditions of exposure this has not provided any useful data.

In the clinical description of the neuropathy induced in man by exposure to n-hexane or methyl-n-butylketone the effects of the damage to the peripheral nervous system have been the most striking in severe cases after discontinuation of exposure and final recovery. Some evidence of the central nervous system involvement may be found but even in these cases the residual signs and symptoms have been of a different nature than those which have been described as the "chronic-painters syndrome" which is discussed in Section 3.

There is no evidence, clinical nor experimental, that a central nervous system effect persists without a preceding, clinically evident peripheral effect.

If any such central effect is demonstrated in man exposed to petroleum hydrocarbons it is unlikely to have been caused by the presence of n-hexane.

3. NEUROTOXICITY FROM "NON-HEXACARBON" HYDROCARBONS

3.1 STUDIES ON ALKANES, ALKANE MIXTURES AND RELATED COMPOUNDS OTHER THAN N-HEXANE.

Several studies strongly indicate that hexacarbon alkanes free from n-hexane and other alkanes such as n-pentane and n-heptane are not neurotoxic (34,35).

On the other hand it has been recently found that 2,5 heptanedione but not 2,6-heptanedione, given in high oral doses (1000 or 2000 mg/day) produces clinical signs and neuropathological findings identical to those caused by 2,5-hexanedione. This finding strongly suggests that it is the gamma-diketone structure which is necessary for neurotoxicity (36).

In a study on 2 groups of rats, one inhaling 700 ppm methyl-n-butylketone and the other a similar level of ethyl-n-butylketone for 11 and 24 weeks respectively, only the rats inhaling methyl-n-butylketone developed clinical and neuropathological evidence of neurotoxicity.

Ethyl-n-butylketone is believed to be metabolized to 2,5-heptanedione and in fact this metabolite was demonstrated in the serum of the rats but at very low concentrations (6.8 ± 4.0 µg/mg) while in the rats inhaling methyl-n-butylketone the serum levels of 2,5-hexanedione were 133.2 ± 36.7 µg/mg (37).

In another study (38) rats, receiving as their drinking water, aqueous solutions of 0.5% 2,5-hexanedione or 2,5-hexanediol over a period of 12 weeks, developed clinical and neuropathological changes characteristic of neurotoxicity but rats receiving similar solutions of heptane-2-one, heptane-3,5-dione, hexane-2,4-dione, hexane-2,3-dione, hexane-1,6-dione, or a solution of 0.05 or 0.1% to butane-1,4-diol, of 0.25% glutaraldehyde or 0.5 or 1.0% acetone showed no changes.

Summarizing these studies, only the gamma-diketone structure appears to be responsible for a neurotoxic effect since only hexane-2,5-dione and heptane-2,5-dione have shown neurotoxicity. The 3,5-diones, 2,4- or 2,3-diones failed to show activity. The 3 carbon compound acetone, the 4 carbon compound 1,4-butanediol and the 5 carbon compound glutaraldehyde ($O{=}CH{-}CH_2{-}CH_2{-}CH_2{-}CH{=}O$) were also inactive.

3.2 EXPOSURE TO WHITE SPIRIT

3.2.1 Short-term exposure to white spirit

An excellent group of studies in which the pulmonary uptake and resulting concentrations of the aliphatic and aromatic components of white spirit in blood were measured and the subjects examined by a small series of behavioural tests has been published by a group of Swedish authors (39,40). The same group of workers has studied the effects of exposure to other substances, in a similar way, some of which are discussed in the following sections (toluene and styrene).

These studies were carried out on groups of 12-14 healthy volunteers who were exposed at intervals of 7 days to successive 20 minute periods of stepwise increased concentrations. In the case of white spirit these concentrations were 625, 1250, 1875 and 2500 mg/m^3. The white spirit used consisted of 83% aliphatic and 17% aromatic compounds. The latter included pseudocumene (1,2,4-trimethylbenzene), n-propylbenzene, mesitylene and 1,2,3-trimethylbenzene. This is a so-called low aromatic white spirit, presumably with a boiling range of 161-197°C, containing mainly C_9 - C_{11} aliphatic hydrocarbons. It is similar to the so-called Stoddard solvent of which the inhalationed toxicity has been studied in man and in experimental animals (41) and for which a TLV/TWA of 160 ppm (575 mg/m^3) has been set by the A.C.G.I.H. (31) based on the result of these studies.

In the studies on concentrations in alveolar air and in blood it was found that the aromatics, being the most soluble, continued to accumulate in the blood during those periods when the blood level of the less soluble aliphatics tended to level off. During exercise the level of both series of compounds increased markedly. In the case of the aromatics the increase was 15-fold when exercise was at the highest intensity, probably due to the increase in pulmonary ventilation.

At these concentrations no effects were found in the subjective response or in the results of the behavioural tests when the subjects were at rest. In a separate series of exposures to 4000 mg/m^3 for 30-40 minutes, a prolonged reaction time and possibly an impaired short-term memory were observed. At this concentration of white spirit in inspiratory air an alveolar air-concentration was found which corresponded to values observed in exposure to 2500 mg/m^3 during light exercise.

Thus some effects on simple reaction time and on short-term memory can be expected at concentrations approximately 4 times the TWA/TLV (ACGIH) in subjects doing light exercise. It is likely

that, with a heavy work load, similar concentrations in alveolar air may be reached at lower concentrations in the atmosphere.

These studies have shown that at the end of an inhalation period of 30 minutes, with the subject at rest, the arterial blood concentrations of both the aliphatic and aromatic components tend to level off, but when the subjects are exercising, the blood concentrations of the aromatic compounds continue to rise during this episode while the aliphatics albeit at a higher level, again tend to level off during the end of the episode. The question of whether or not the aliphatic or the aromatic compounds are the main contributors to the neurotoxic effects has not been studied. There are no data on tissue concentrations.

It appears likely that the concentrations in brain tissue will bear the closest correlation with the neurotoxic effects. The only studies of this type have been done many years ago on other hydrocarbon compounds to find a correlation between tissue concentrations and the LC_{50} in rats and mice. In these studies, in which styrene was included and found to be the most toxic of the hydrocarbons studied (the others were isoprene, butadiene, isobutylene, butane and two hexene isomers), the hydrocarbon concentration in the brain was found to correlate well with the degree of nervous system depression (42).

Animal studies of this type should be done to establish saturation levels in arterial and venous blood and in tissues at various levels of exposure. It is recommended to establish the correlation between these levels and the effects on behavioural tests, comparable to those done on volunteers. Carpenter et al. (41) in their inhalation studies of 3 months duration on hydrocarbon solvents in animals (dogs and rats) established a no-effect level with respect to behaviour of 190 ppm (1100 mg/m^3), while at an inhalational level of 330 ppm no histological changes in the central nervous system or in the sciatic nerves of dogs or rats were found.

3.2.2 Long-term exposure to white spirit

A series of reports have appeared from Scandinavia over the last 4 years which appear to throw doubt on the absence of irreversible effects on the central nervous system of occupationally exposed workers, mainly of painters, who use mineral spirits (terpentine) extensively together with other solvents (3,43,44,45,46,47). Browning has described some reports of a casuistic nature about permanent cerebral effects of long-term exposure to mineral spirits (48).

It is difficult to put these studies in a proper perspective. The effects described are in many respects similar and independent of the type of solvent or mixture of solvents studied.

It is generally assumed that physochological (behavioural) tests are more sensitive in detecting early disturbances of the central nervous system than traditional neurological examinations. Moreover, it is suggested that the stated effects are due not to the reversible general anaesthetic effects which are common to most solvents but are associated with diffuse brain damage ultimately resulting in what has been called the "chronic painters' syndrome" (45).

Arlien-Soeborg et al (45) described a study of 50 house painters who had been referred to hospital, suspected to be suffering of chronic solvent intoxication or dementia. Other aetiological factors such as head injuries, epilepsy, perinatal trauma, alcoholism or other diseases were excluded. The patients were studied by neuropsychological examinations i.e. behavioural studies, electroencephalography (EEG) studies and neuroradiological examinations such as fractional pneumoencephalography and cranial computerized tomography. Their mean age was 47 years (range 24-63) and the mean exposure time 27 years (range 8-50 years). The exposure free interval before the study was from 5 days to 5 years.

Most subjects reported subjective complaints such as forgetfullness, fatigue, inability to concentrate, irritability, headache, dizziness, apathy, nervousness, depression, bursts of perspiration, alcohol intolerance, abdominal distress (nausea, vomiting, pain), reduced libido or impotence and blurred vision. Based on the neuropsychological tests 39 of the 50 were considered to have intellectual impairment varying from a slight to a moderate to severe degree. The EEG was found to be slightly to moderately abnormal in 9 subjects only; in 9 subjects evidence of a peripheral neuropathy was found and 12 patients studied by pneumoencephalography showed atrophy of the brain; central, cortical or both combined. Among 38 of the painters subjected to computerized tomography 19 were found to have atrophy.

The authors tend ascribe these changes to exposure to white spirit. Though white spirit is widely used as a diluent and cleansing agent, commercial paints contain many other volatile solvents and compounds. In a comparable retrospective study (51) on patients hospitalized for pneumoencephalography on account of suspected brain injury due to organic solvents, 37 subjects were evaluated, 19 of which had been exposed to a mixture of solvents; the others had been exposed to carbon disulphide, trichloroethylene, styrene, a not further-defined thinner (probably methylene chloride), toluene, methanol or carbon tetrachloride. Since they had been hospitalised because of suspected brain injury it is not surprising that all patients showed personality changes and psychomotor disturbances. Clinical neurological findings comprised slight psychoorganic alterations, cerebellar dysfunction and peripheral neuropathy. The pneumoencephalograms of 63% of these patients suggested brain atrophy. Other possible causes of the observed changes were, as far as possible, excluded. Most of these patients

seemed to have had extensive contact with the solvents in question over long periods but quantitative data are not available and it is not stated whether episodes of acute intoxication had preceded the changes.

A different approach has been followed in another publication (50), a case-referent study on neuropsychiatric disorders among workers exposed to solvents, using the data from a pension fund register which afforded all necessary data on the duration of employment and the medical diagnosis. This study suggests that painters carry a risk 1.8 as great as that of non-exposed workers of developing a disability due to neuropsychiatric disease.

An excellent study, showing the complexity of exposure, was reported in 1980 by a large group of Swedish scientists, on occupationally exposed car and industrial spray painters (3). A total of 20 different solvents was present and workers were often exposed to 8-10 solvents at the same time; moreover there was some exposure to dust, lead and chromium. Toluene was the solvent most frequently occurring in the highest concentration but high concentrations of xylene, trichloroethylene and white spirit were also common. Among the other solvents were also methylethylketone, methyl-n-butylketone, methyl-iso-butylketone, various alkyl acetates and methylene chloride.

Though the average inhalation exposure was low and all levels measured had been below the then (in Sweden) acceptable levels of exposure (less than half), total exposure including skin exposure could not be measured.

It is stated that workers used solvent to clean tools and soiled skin. It is not mentioned which solvent was used for this latter purpose. It is well worth presenting the summary of this study in full:

> "In the present epidemiologic study 80 car or industrial spray painters with long-term low level exposure to organic solvents were examined and compared with two matched reference groups of non-exposed industrial workers (80 persons in each group). The aim of the study was to investigate the possible effects of the solvent exposure on health. The investigation included psychiatric interviews, psychometric tests, neurological, neurophysiological and ophthalmologic examinations, and computed tomography of the brain. The painters' previous and present exposure was carefully assessed by interviews and on-the-job measurements both at the modern places of work and in a reconstructed model of a 1955 workshop. On the basis of the psychiatric interviews the psychiatric symptoms were rated according to a specially designed scale of 46 different items, graded in seven steps of increasing severity. The psychological performance was assessed by a battery of 18 tests. The neurological and neurophychological examinations comprised visual evoked responses (VER), electroencephalography

(EEG), and computerized EEG analysis for the central nervous system and electroneurography, the estimation of vibration sense thresholds, and a quantified neurological examination for the peripheral nervous system. The ophthalmologic examination concentrated on the condition of the lens. Statistically significant differences between the exposed individuals and referents were found for psychiatric items indicative of a slight cerebral lesion (i.e., a neurasthenic syndrome). The psychometric tests revealed statistically significant differences between the groups with respect to reaction time, manual dexterity, perceptual speed, and short-term memory. No differences were found with respect to performance on verbal, spatial, and reasoning tests. Significant differences between the groups were also found for the majority of the neurophysiological parameters measuring peripheral nerve functions, the most pronounced occurring in the long, sensory fibers.Moreover EEG and VER showed some differences between the groups, as did the results of the ophthalmologic examination and the computed tomography. Finally, it should be emphasized that the exposure levels, as measured at modern places of work and in the reconstructed workshop from 1955, were found to be considerably lower than the valid threshold limit values in Sweden."

All the subjects were examined in the hospital during two successive day from Tuesday-Friday. It is reported that they had not been exposed during 18-24 hours before examination. General mecical examination included a questionnaire concerning the previous and present state of health including general, neurological and psychiatric symptoms and a blood analysis. The exposed workers had a slightly higher evidence of neurological and psychiatric symptoms than the referents. The blood analysis merely showed a difference in the levels of alkaline phosphatases which were slightly higher in the exposed group (but still within normal limits).

The investigation showed that complaints such as fatigability, learning problems, irritability, inner tension, short- and long-term memory problems, nausea, epigastric pain, headache and failure in precision movement; moreover they had more complaints about worrying over trifles, concentration difficulties, vertigo, dizziness, dyspnea and mood liability were more common among the painters.

The psychological examination included a host of behavioural performance tests (18 in total) such as on verbal comprehension, reasoning and spatial relations, perceptual speed, numerical ability, memory, manual dexterity, simple reaction time and motor speed. The effects found seem to be of the same kind as those found in the volunteer studies already discussed. Of great interest is that no dose-response relationship could be demonstrated i.e. the differences between the exposed and referent group did not show and increase with age or length of exposure. Age itself increased poor

performance of the tests in all groups. The greatest difference between the exposed and reference group was found in the reaction-time test. Also the performance on the memory tasks appeared less well in the exposed group. Of great interest are obviously the results of the neurological and neurophysiological examinations. The latter included electroencephalography, visual evoked responses (VER), electroneurography and vibratory perception.

The neurological examinations showed only minor differences and were not considered of medical significance. Also the changes in the electroencephalograms were only subtle and showed no dose-response relationship. The study of the VER showed no statistically significant differences. The electroneurography study indicated a reduced function of the peripheral nerves in the exposed group, but the difference was small and all the mean values were within normal limits. There was a slight decrease in conduction velocity and a slightly higher vibration threshold. It is worthwhile to point out here that among the solvents methylethylketone and methyl-n-butylketone were present though at low concentrations. The neuroradiological examination by computer tomography scanning showed an increased relative brain volume. One can only speculate about the significance of this finding (oedema?).

An ophthalmological examination on increased frequency of micro opacities of the lenses among the exposed group was found, not associated with any reduced vision or with cataract formation.

Part of the findings, especially those of the psychological examination may be due to an acute solvent effect since it is known that certain of the solvents have an in viro half-time of days (51). The slight difference between the results of the electroneurographical studies may be ascribed to the action of methyl-n-butylketone acting in synergism with methylethylketone. Regrettably in this case, a reversibility study has not been done. In other studies it has been shown that reversibility of an effect to the results of the simple reaction timetest exists. In a longitudinal study on a group of steelworkers (46), exposed to vapour concentrations of solvents exceeding the threshold level, and repeated 6 and 15 months after the improvement of ventilation, resulting in a dramatic decrease in exposure, the results of the simple reaction time test improved considerably. The workers were exposed to "organic solvents and solvent-based paints but figures of actual concentrations were only given for methylethylketone since these were considered excessive (440 mg/m^3 with peaks 8-10 times higher)".

Two other studies, one on psychological function changes among house-painters (43) and one on the neurophysiological effects of long-term exposure to a mixture of organic solvents (mainly toluene, xylene, butylacetate and white spirit) (44), are worth discussing.

In the first study (43) a random sample of house-painters (52 persons) was compared with a group of industrial workers with a corresponding age distribution and similar backgrounds. The painter group had a significantly lower performance on the memory test and the reaction time test. Again no correlation could be established between "painter years" and the severity of the change.

The exposure period varied from 4 to 42 years. The health interview indicated that eczema and experiences of impaired memory were more frequent among the painters than in the control group. There was also an over representation of complaints about diffuse chestpain, extreme fatigue, and of longer periods of sick leave. The only other difference was a slightly lower mean haemoglobin concentration among the painters though the values were all within normal limits.

The testing took place more than 15 hours after cessation of the last exposure. Here again there are no data on exposure (though exposure to several hundred parts per million of hydrocarbon solvents was estimated) and no mention is made of episodes of acute intoxication.

The other study (44), was on a group of 102 car painters and a control group of 102 age-matched railway engineers. The mean age was 35 years and the exposure time ranged from 1-40 years (mean 14.8 ± 8.5 SD). The mean concentration of the solvent in the garages was low i.e. 31.8% of the Finnish TLV but the range of the separate components varied from 4-122% of the respective TLVs.

No difference was found in the incidence of EEG anomalies. Abnormally slow motor and sensory nerve conduction velocities were found among 12 of the 59 car painters studied for this index but no changes were found in the 53 engineers. Methyl-n-butylketone and methylethylketone were not mentioned in the mixture of solvents, the mean concentration of white spirit was only 4.9 ppm, while toluene showed a mean concentration at the breathing zone level of 30.6 ppm. All studies were done at least 16 hours after the last work period.

In a study in rats, guinea pigs, rabbits, dogs and monkeys inhaling continuously over a period of 90 days concentrations of mineral spirits up to 127 mg/m^3, no histological changes in the brain were reported though guinea pigs showed weight loss and an increased mortality and monkeys showed weight loss only (52).

3.2.3 Conclusion

Animal experiments over 3 months periods (dogs and rats) have indicated a no-effect level of 190 ppm (1100 mg/m^3) by the usual experimental indices of effect.

Studies on man exposed to white spirit alone have been of a short-term nature and have shown that levels of 2500 mg/m^3, in persons doing light exercise, changes may be expected in simple reaction time and short-term memory. These changes are reversible.

Other studies on workers do not include one where workers have been exposed to white spirit alone. In the most extensive study (3), the concentrations of 20 different solvent compounds i.e. white spirit have been measured.

In general the studies on workers report an increased incidence of a variety of subjective complaints which frequently are called neurasthenic such as headache, giddiness, lack of concentration, sleep disturbances, depression, vague abdominal complaints such as nausea, vomiting, and pains and an increased incidence of sick leave. This neurasthenic syndrome has been reported also from studies on workers with exposures to e.g. carbon disulphide, styrene, toluene or xylene. Behavioural studies have shown only a higher incidence of increased simple reaction time and some decrease in short-term memory. These appear to be the most sensitive indices of exposure but are not specific since they show changes under conditions of sleep disturbances, fatigue of hangover, and under the influence of drugs such as diazepam.

Neurophysiological studies have shown that some indices of peripheral neuropathy may be affected but at least in one study both methylethylketone and methyl-n-butylketone were present in the atmosphere (35).

In none of the studies has it been possible to estimate total exposure i.e. atmospheric concentration and skin exposure. In the few investigations done for the purpose of studying the reversibility of the effect in relation to the simple reaction time, there was a clear indication that it was reversible.

There are a few reports on a group of painters, who after years of exposure have developed clinical changes indicative of an organic brain syndrome, frequently even with dementia and atrophy of the brain. In none of these cases are exact data on exposure given, though it is usually mentioned that work place/conditions had been poor and that intoxications had frequently been experienced. This syndrome has been called by one author the "chronic painters' syndrome". The fact that the epidemiological studies have failed to give any evidence of progression with the time of exposure makes it difficult to connect directly the relatively minor changes found in these epidemiological studies with the severe changes of the chronic painters' syndrome.

3.3 JET FUEL

A series of studies have been done on workers from a jet engine factory who were exposed to the fumes of jet fuel (53,54,55). The jet fuel which had been used since 1972 had the following composition (54): aromatic hydrocarbons, 12 vol% (of which benzene amounted to 0.3 vol% and toluene to 1.0 vol%); olefin hydrocarbons, 0.5 vol%; and saturated hydrocarbons, 87.5 vol%.

The carbon numbers of the components will have varried from C_5 to C_{16} and a certain amount of n-hexane may have been present.

3.3.1 Clinical observations

In the first study (53) twenty nine workers with at least 5 years exposure were divided in two groups, a heavily and a less heavily exposed group. The results were compared with a reference group not exposed to solvents or fuel, taken from a heavy metal industry. In a subsequent review of this study figures of 500 ppm in two workrooms and 3100 ppm at one workplace are given (56). All 13 persons in the heavily exposed group and 7 of the 16 less heavily exposed had repeatedly experienced acute symptoms consisting of dizziness, headache, nausea, respiratory tract irritation, palpitations or a feeling of pressure on the chest. A high rate of symptoms indicative of neurasthenia was found in the exposed groups compared with the reference group. They consisted of dizziness, respiratory tract symptoms (feeling of suffocation or pain on inhalation) palpitations, pressure on the chest, depression, anxiety, sleep disturbances, headache, memory impairment and irritability. The incidence seemed related to the intensity of exposure. Similarly, symptoms and signs indicative of polyneuropathy were more frequent but the mean conduction velocities were not significantly different from the controls though there seemed to be a tendency in the exposed groups to show more prolonged velocities. There was, however, a higher incidence of a high threshold of vibration sensation in the exposed group.

The first study led to the following two studies (54,55). The second study (54) comprised 30 exposed workers and a carefully matched control group of unexposed workers from the same location. The exposure duration was 17 years and the exposure was roughly calculated to be 300 mg/m^3 as a time weighted average.

Only 4 persons of the exposed group had never experienced any symptoms of acute over-exposure. This study showed a higher incidence and prevalence of psychiatric symptoms, a difference in the results of psychological tests especially those on attention and sensorimotor speed and in the results of electroencephalographical readings. No difference was found in the occurrence of symptoms or signs of polyneuropathy.

The behavioural tests included a simple reaction time test which showed no clear difference between the two groups: memory tests and manual dexterity showed no changes. Three of the tests, demanding high attention and sensorimotor speed appeared affected.

Electroencephalographic studies showed definitely abnormal patterns in 5 of the exposed and 4 of the control group. However, there seemed to be a significant difference in the patterns of the exposed groups especially as regards alpha activity.

The electroneuromyograms showed no consistent pattern in the results and the difference in the thresholds of vibration sensation was statistically not significant.

The last study (55) gave special attention to the extent of neuropsychiatric ill health among 30 exposed workers and 60 matched controls.

Again a higher incidence of neurasthenic symptoms was recorded among the exposed workers. The main symptoms were fatigue and emotional instability (including anxiety and depression), lack of initiative and dizziness, followed by some somatic symptoms. A high incidence of phobias and compulsions was noted.

3.3.2 Discussion

This interesting series of studies on workers exposed over long periods of time to jet fuels leaves many questions unanswered. There are no data on progression with the duration of exposure, nor has the reversibility been studied. The question may also be posed as to whether or not the symptoms observed may be related more to the repeated occurrence of episodes of a slight intoxication or to the sleep disturbances which were experienced than to the long-term toxic effects of the vapour. The tendency to show some signs and symptoms of peripheral neuropathy could be due to the n-hexane component only.

The CONCAWE Toxicology Subgroup is not aware of any experimental studies on the effects of exposure to jet fuel in animals but may refer to the experimental studies of Carpenter et al. (57) which cover most of the components in jet fuel.

3.4 TOLUENE

3.4.1 Metabolism and toxicity

Surveys of the general toxicology and the metabolism of this extensively used solvent may be found in various texts (48,58,59,60,61). The pulmonary uptake, retention, distribution, biotransformation and excretion are well known.

Hippuric acid excretion in 24 hour urine is in general considered a reasonable index of total absorption since approximately 68% of absorbed toluene is excreted in up to 18 hours after exposure (62). This is only true if there is no great intake of benzoic acid from other sources. In the mouse 14C-labelled toluene is excreted completely within 24 hours since no radioactivity can be detected in any of the tissues or excreta after that period. After absorption into the blood toluene is rapidly distributed around the body fat, bone marrow and spinal nerves; some radioactivity is found in the brain and spinal cord and also in the liver and kidney. In the latter two organs it is present as metabolites. Toluene is rapidly cleared from the nervous tissues since 30 minutes after inhalation only traces remain in the white matter. One hour after inhalation no traces are left. Benzoic acid, the main metabolite, is also rapidly eliminated via urine and bile (60).

In short-term inhalation studies on man, low concentrations (200 ppm) of toluene induce mild upper respiratory irritation, at 400 ppm it causes mild eye irritation and hilarity, at 600 ppm lassitude, hilarity and slight nausea, and at 800 ppm rapid irritation of the upper respiratory pathway, eye and nose irritations, a metallic taste, drowsiness and impaired balance (58). The present TLV (ACGIH 1981) is 100 ppm (31).

In a recent 24 month inhalation study in rats (63), no toxic effects were found at concentrations up to 300 ppm. This study included a histopathological study i.e. of the brain, cerebellum, spinal cord and various peripheral nerves. The histological techniques were however not of the type used in the n-hexane studies (see previous section on hexacarbon toxicity).

3.4.2 Behavioural and neurophysiological studies and animals

A 3 week study on rats inhaling 4 hours/day toluene concentrations from 500-800 ppm was done many years ago (64). Avoidance response tests were undertaken two hours after the onset of exposure. The effect on learning of the exposed rats was not different from that in non-exposed animals and also in the so-called consolidation and extinction phases, no difference was noted.

More recently, the neurotoxicity was studied on rats by examining electroencephalographic changes in rats exposed to various concentrations (65). The concentrations used were high, 1 000, 2 000 and 4 000 ppm, inhaled over periods of 4 hours. The changes in EEG components were observed to be characteristic for different concentrations.

The authors conclude that the result of this experiment suggest that the changes in the basic EEG rhythms are significant findings in both humans and animals exposed to high concentrations of toluene vapours. It is regrettable that the effect of lower concentrations and long-term exposure have not been studied (65).

One study has been reported on the effect of long-term vapour exposure to the peripheral neuromuscular system of rats.

Groups of rats were exposed to 2 000 ppm for periods of 8, 16 or 41 weeks (6 days per week, 8 hours per day) and to 100 and 200 ppm for 49 weeks. No effect was observed on strength capabilities in any of the exposed groups but the ratio of the threshold stimuli (that of the extensors to that of the flexors) increased in the 2 000 ppm groups exposed for 41 weeks. The maximal conduction velocity decreased more in the 200 ppm group than in the 100 ppm and the control groups due to an increase in the latency from stimuli applied at the upper point of the femur to the appearance of the muscle twitch but not due to any change in the speed at which the stimulus reached the muscle when the nerve was stimulated at the kneepoint (66). This appears to be an effect different from that caused by e.g. n-hexane.

3.4.3 Studies on human volunteers

The effect of the inhalation of various concentrations of toluene on reaction time and perceptual speed was studied in a group of 12 healthy male volunteers (67).

The levels of exposure were 100, 300, 500 and 700 ppm. A statistically significant impairment of the reaction was noted at 300 ppm and this increased at the higher levels of exposure. A statistically significant decrease in perceptual speed was seen at the highest level of exposure only. The periods of exposure did not exceed 20 minutes and the subjects were studied only at rest. It is known that with an increase in pulmonary ventilation such as at work the alveolar concentration increases considerably and the blood concentration rises (68).

3.4.4 Studies on addicts and toluene exposed workers

Summaries of the effects of toluene used as a "pleasure giving" drug are given in (62,69,70). It is remarkable that from the observation of series of addicts no uniform pathology has emerged. There are a few cases of severe pathology which have been quoted repeatedly as representing the final outcome of severe addiction as e.g. a case of permanent encephalopathy (71,72), some cases of cerebellar ataxia and one case of optic neuropathy (73,74,75) but in general even severe abuse has not been associated with definite neurological changes.

There have appeared a few studies on workers exposed to mixtures of solvents of which toluene was one of the components (44,76,77,78,79). Most of these studies come from Finnish groups of investigators using a battery of tests. These tests had been developed in previous studies on workers exposed to carbon disulfide or to lead.

In the first study (78) a group of workers suspected of chronic intoxication by different solvents (42 workers of whom 4 exposed to toluene only) and a group of 126 workers exposed but not suspected of intoxication (of which 22 were exposed to toluene) were compared with a group of 50 workers with carbon disulfide intoxication and a group of 50 healthy workers. The duration of exposure varied in the two groups from 0.1 to 30 years; the mean age and the range of age in the 4 groups were comparable.

The clearest difference appeared in the time-limited visual speed performances. The group differences in psychomotor performance were not statistically different. In the two solvent exposed groups the accuracy of visual perception and the left hand performance in the Santa Ana test (which tests co-ordination between eyes and hands/wrists/fingers), the sensorimotor speed and especially the performance of the psychomotor Mira test (which tests behaviour and ability) were disturbed. Comparing the two groups which were solvent-exposed, the groups suspected of chronic intoxication showed the worst results in the performance tests demanding sensorimotor speed. In the study it is impossible to separate the effect of toluene from that of the other solvents, mainly trichloroethylene and tetrachloroethylene. No conclusions can be drawn.

The following four publications (44,76,77,79) describe the results of a study on a group of car painters (total 102), selected from various repair garages in Helsinki (27 in total). Measurements of atmospheric concentrations were done in only 6 randomly selected garages and it was assumed that in the remaining garages exposures were not different since they were all similar and well-ventilated. Toluene was found to be the main component with a mean concentration of 30.6 ppm and a range from 5-249 ppm. The other components (xylene, butylacetate, white spirit, methyl-iso-butylketone, isopropanol, ethylacetate, acetone and ethanol) occurred at much lower concentrations of which the means varied from 1.7 to 6.8 ppm.

It is recorded that for at least 10 years the same paint solvents had been used and that the work conditions had not changed over that period. There is no discussion of possible skin contamination and no data are given on the urinary excretion of metabolites. Levels in the blood have not been determined. There are no data on possible sources of exposure outside the garages and in the homes. Carbon monoxide levels in the garages were measured and found to be low and considered negligible.

The control group was matched as far as possible regarding i.e. age, smoking habits, alcohol use, use of drugs and level of intelligence. This group consisted of railway engineers and assistants, which differed from the exposed groups in the type of work, the irregularity of working hours, the fact that in their work they are subjected to vibrations and there are slight differences in general education.

The authors state that both groups were equal in regard to the results of liver function tests which were included in the examination (79).

The first of the three studies (76) is on the behavioural effects. The battery of tests to which the workers were subjected contained one on verbal intelligence, three visual tests, four memory or learning tasks, four tests of psychomotor performance and the Rorschach test. The results in a small group of workers could be compared with the results of some psychological tests, previously done during the military service when they were 20 years of age (intelligence tests only). The period between this military testing and that in the study was 7.4 years (± 4.1 SD).

The results of this battery of tests indicated mainly impairments of visual intelligence and verbal memory and a reduction in emotional reactivity. The authors could not exclude the possibility of higher exposures in the past.

In this study the reaction time was not found to be a sensitive measure of the effects of solvent exposure.

In general the effects of exposure were considered slight and the individual results within the normal variation limits.

The second publication (44) reports the result of the neurophysiological study, which included electoencephalograms of all exposed and control workers and electroneuromyographic studies on 59 of the painters and 53 controls. Abnormally slow motor conduction velocities or sensory conduction velocities were found in 12 of the 59 painters but in none of the controls. There was no increase in EEG abnormalities in comparison with the reference group. All examinations were performed at least 16 hours after the last exposure.

The third publication (77) gives more details about clinical neurologic findings in the exposed and the reference group.

The authors report a statistically increased incidence of the psycho-organic syndrome, a decrease in the sense of light touch and pain and an increase in the vibration threshold among the exposed workers and conclude that the sensory functions seem to be the most vulnerable part of the nervous system. The psycho-organic syndrome is defined as "changes indicating impaired judgement, comprehension, memory attention, speed of response and the ability to give relevant answers to simple questions.

The fourth publication (70) discusses the result of the questionnaire study regarding symptoms occurring during the work period and more permanent symptoms, also experienced during work-free periods.

Symptoms of fatigue and disturbances in memory and vigilance occurred more often in the exposed group. Acute symptoms such as irritation and "prenarcotic" symptoms were reported significantly more often in the exposed group. The occurrence of acute symptoms seemed to be related to age. This difference was slight and cannot be used to conclude that there is any progression in the severity of symptoms with the duration of exposure.

In view of the nature of this study data on reversibility are absent. Also lacking are any data on absenteeism and the prevalence of certain diseases. Twenty years ago a Swiss author (80) published the results of a study of 2 groups of toluene exposed printing workers, one exposed to an average level of 300 ppm and the other to ± 430 ppm, with an average exposure duration of 18 and 12 years respectively.

The workers were examined medically and by means of the Rorschach test and several other tests. The groups examined were stated to be mainly exposed to toluene and consisted of 110 workers. A control group was not used. According to the author there was a linear relationship between the prevalence of an organic psychosyndrome and the duration of exposure. He calculated that 50% of the toluene exposed workers of the 300 ppm group would develop an organic psychosyndrome after 33 years of exposure. Obviously the workers were heavily exposed. The lack of details in the presentation and the absence of a control group do not allow any conclusions to be drawn.

One study from Japan has been found reporting a higher incidence of subjective symptoms of weakness, dysmenorrhoea, uneasiness and of a difference in the performance of neuromuscular and muscular function tests in female shoemakers exposed to toluene concentrations between 60 and 100 ppm, when compared to a control group. In this case the hippuric acid excretion in the urine was also measured and was found to be 3.26 ± 0.82 mg/ml in the exposed group and 0.35 ± 0.24 mg/ml in the controls when corrected to a specific gravity of 1024. This rather high excretion of hippuric acid indicates the total exposure must have been higher that the atmospheric levels indicate (81).

Discussion

Exposure to toluene has been studied experimentally rather extensively in animals as well as in volunteers. Obviously the volunteer studies were of short-term only and indicated that at an atmospheric level of 300 ppm the reaction speed became impaired. At 100 ppm no effect was noted with the subjects at rest, at higher levels symptoms of irritation of the mucous membranes became increasingly severe. In a life time study on rats an atmospheric level of 300 ppm was well tolerated and did not lead to obvious histological changes in the central or peripheral nervous system.

Behavioural and neurophysiological studies on animals have not been done at atmospheric levels around the presently accepted TLV but at higher levels over short periods electroencephalographic changes have been observed, characteristic for different concentrations (from 1 000 - 4 000 ppm).

One study on rats indicated some changes in maximal conduction velocity in rats exposed to 200 ppm or higher over a period up to 49 weeks which were of a different nature from those seen e.g. in n-hexane studies.

Reports on the effect of toluene used as a "pleasure giving" drug have suggested that in a few individual cases severe brain injury occurred, but in general the effect of addiction to toluene has not been deleterious to the nervous system.

There are some studies on workers exposed to toluene as a component of mixtures of solvents. One group of studies from Finland on workers exposed for years to low vapour concentrations of toluene as the main component of a solvent mixture suggest that there may develop an increased prevalence of a "psycho-organic syndrome" associated with some changes in neurophysiological, neurological and psychometric findings. These studies are lacking in data on total exposure, fail to indicate a dose-response relationship or an increase with the duration of exposure. Obviously since these studies are done on workers exposed to many different solvents it is difficult to single out one component. The only conclusions which may be drawn safely are that acute exposures to toluene, exceeding the TLV, may be associated with a diminished reaction speed and that there is experimental evidence of neurophysiological changes at high exposures (1 000 ppm and higher). Toluene as a pleasure giving drug in general has been tolerated rather well but in a few cases has been associated with cerebral defects. The causal relationship has not been established.

Finally, in workers exposed to relatively low vapour concentrations of toluene together with other solvents behavioural and neurological studies have shown some difference with control groups. A dose relationship and a progress with the duration of exposure have not been established and the reversibility not studied.

3.5 XYLENE

3.5.1 Metabolism

Xylenes are widely used in industry as solvents and their toxicity has been studied extensively over many years (48,50,59). It is well absorbed via the lungs as well as via the skin. Total exposure can be measured by determination of metabolites in the urine. The chief

metabolites of the three xylenes are the toluic acids, which are excreted conjugated with glycine as methylhippuric acids. By far the greatest amount of absorbed xylenes is excreted within 8 hours of exposure (± 72% of the 24 hour excretion) but trace amounts of metabolites may still be found in the urine 4-5 days after a single exposure.

3.5.2 Studies on animals

In a recent study (82) rats were exposed to vapour concentrations of 50, 400 and 750 ppm (5 days/week, 6 hours/day) of m-xylene for a period of 2 weeks. m-Xylene concentrations were measured in brain tissue and renal fat after the first and second week. At the same time several enzyme activities in the brain tissues were determined i.e. NADPH diaphorase, azoreductase and superoxide dismutase. At the two highest dozage levels, (400-750 ppm) the first two enzymes appeared to increase in activity after the second week, while the levels of the third enzyme showed a decrease. Reversion to normal levels was seen 2 weeks after cessation of exposure. Only the cerebral RNA was still found to be exceeding the control level at that time. Xylene was not present in the brain 2 weeks after exposure.

A relationship was found between the m-xylene concentrations in the brain and those in the renal fat. Interestingly, the xylene concentrations in the brain after the second week of exposure were almost 1.5 times those after the first week and the same applied to the concentrations in the renal fat. At all three levels of exposure decrease in the cerebral glutathione content was present but the decrease showed no obvious dose-relationship. The authors mention from previous studies that extension of the exposure over 9 weeks causes a gradual decrease in the tissue levels of m-xylene after the initial rise.

In a study of the effects of technical xylene inhalation (300 ppm) on rats consuming high levels of ethanol at the same time, ethanol was shown to increase the metabolism of xylene and decrease the body burden of xylene, probably due to activation of the monoxygenase system in the liver. Moreover, ethanol potentiated the behavioural effects caused by the solvent inhalation as measured by an open field situation test (83). This latter study was undertaken specifically because of the high alcohol intake of the working population in Finland, which however is not quantified.

3.5.3 Studies on human volunteers

An interesting study (51) forming a background for behavioural studies on the uptake, distribution and elimination of technical xylene containing mainly m-xylene (± 50%) and also ethylbenzene (± 40%) has been published recently. This is the only known study

in which the concentrations in subcutaneous fat have been determined, during and after exposure and the influence of workload and the amount of body fat on the body burden studied.

The data on uptake and pulmonary elimination are in fair agreement with those of previous studies i.e. approximately 95% of the total excretion took place within 19 hours after exposure and approximately 4-5% of the uptake was exhaled during the following 9 hours. The uptake was 63% at 100 ppm and 70% at 200 ppm.

Blood levels tended to level off after 2 hours of exposure in the volunteers on a light (50 W) load.

During the first 22 hours after exposure, to the mean concentration of m-xylene and p-xylene and ethylbenzene in adipose tissue did not change significantly. The estimated mean amount of m- and p-xylene and ethylbenzene retained in the total fat deposits of the body 22 hours after exposure was 5% of the amount taken up in the organism and of the order of 6-8 ppm at exposures to 100 and 200 ppm. Based on unpublished observations on workers the author estimated the half-life of m-xylene in adipose tissue at 64 ± 26 hours (51).

In a further study six male volunteers were exposed to m-xylene at concentrations of 100 to 200 ppm, 6 hours/day during 2 weeks (84). The effects on equilibrium, reaction time and critical flicker fusion were measured. The subjects acted as their own controls. During the second week the exposure was periodically varied up to 400 ppm.

Exposure to 100 ppm of non-adapted persons had an effect on equilibrium and reaction time. But adaptation developed after the first day. After the weekend the effect appeared again more pronounced at the peak concentrations to 400 ppm. The effect seemed to be related to the level of xylene in the blood.

A similar study (85) was done on volunteers undergoing exercise. The previous observation of an effect on the equilibrium reaction time and manual coordination was confirmed. Adaptation developed again but was lost during the weekend. Exercise at the beginning of exposure seemed to antagonize the effects of exposure. Differences were found at an exposure level as low as 90 ppm in non-adapted subjects. This time no dose-response relationship could be found between blood m-xylene level and the effects of exposure. Exposure to varying levels of m-xylene caused some changes in the EEG but not when the level of exposure was constant.

According to the authors these changes indicate a lowering of the vigilance level (79).

In a study on volunteers inhaling 300 ppm over periods of 10 minutes no effect on nervous functions, numerical ability, reaction time (simple and choice), short-term memory and critical flicker fusion was found with the subjects at rest but there was an effect

when physical exercise was combined with exposure. The greatest difference was found in the more complex and cognitive tests of the test battery. The simpler psychomotor and perceptual tests showed no clear difference, possibly because the physical exertion affected the arousal (86).

In other volunteer studies with mixed xylenes, the inhalation of 230 ppm caused slight mucous membrane irritation and some "light headedness" (87). Behavioural studies of the type similar to those for workers exposed to jet fuel or paint solvents have not been found in the available literature.

3.5.4 Discussion

There is some discrepancy between the results obtained by two groups of workers on human volunteers. In one study some effect was found at an exposure to 90 ppm but adaptation developed rapidly. In a second study an effect was found only at a higher level (300 ppm) but only when exposure was associated with exercise and thus with an uptake two to three times higher than at rest. Moreover the effects observed in this second study were of a different character than those of the first study.

The difference may be partly explained by the longer duration and the repetition of exposure in the first study. The half life of xylene has been estimated at approximately 64 hours and thus the level of xylene in the tissues may have been higher.

Studies on animals have shown some biochemical changes - in the activity of various enzyme systems at exposure levels of 400 ppm and higher, and a potentiating effect of ethanol consumption on the behavioural effects of xylene exposure.

No studies have been found on the effects of long-term exposure to animals. The TLV (31) has been established at 100 ppm and carries a skin notation.

4. CONCLUSIONS

Two developments have caused increased attention to be paid to the effects of exposure to petroleum hydrocarbons on the nervous system. The first development was the detection of the neurotoxic effect of n-hexane and its metabolite, methyl-n-butylketone. This effect, occurring mainly on the long nerve tracts in the peripheral and central nervous system, has been extensively studied.

A clear though not complete picture has emerged of the pathogenesis of the neuropathy caused by n-hexane regarding the metabolic, biochemical and histological aspects.

Still lacking are data on the contribution of skin contamination to the total exposure. Furthermore, biological monitoring of exposed workers at or near the levels of acceptable exposure should be developed. Another important area of study is that of the interaction by exposure to other solvents such as methylethylketone (MEK) which appears to increase significantly the susceptibility to the neurotoxic effect of n-hexane by an as yet unclarified mechanism. Until now only MEK has been found to exhibit this interaction with hexane but other compounds have scarcely been studied.

The interaction of MEK with heptane and octane, both of which may also be metabolized to a gammadiketone structure, has also not been studied as yet.

As a result of the experimental and epidemiological studies on n-hexane, the no-effect level of occupational exposure to n-hexane alone has been more or less defined to be at a level of 50-100 ppm.

The second development is associated with that of behavioural toxicology and the medical observations of possible neurotoxic effects of long-term exposure to various solvents such as toluene on addicts to the pleasure-giving effect and on occupational exposed workers (the chronic painters' syndrome). The studies on addicts to pleasure-giving drugs have demonstrated the well-known neurotoxic effects of over exposure to n-hexane but moreover resulted in finding some isolated cases of sometimes permanent effects such as cerebellar ataxia or cerebral atrophy in addicts to toluene. Most of the victims of addiction however, have been remarkably free of demonstrable permanent effects to the nervous system. It has been known for many years that the volatile petroleum hydrocarbons have a non-specific general anaesthetic action as a result of their relative easy penetration into the brain. Some of the simple hydrocarbons were used as general anaesthetics for a number of years but this use has been discontinued because of the flammability hazard. It was, however, generally assumed that this effect was completely reversible. By the use of behavioural tests on volunteers exposed to individual hydrocarbons it has been found that subclinical effects on

performance capability may be found at exposure levels frequently below those which until now were considered to be free from any effects. These effects were found to be completely reversible on discontinuation of exposure of volunteers. An important side result of these studies was the finding in the case of aromatics, that the uptake of these compounds by the lungs was strongly dependent on physical exercise. Thus at relatively low levels of exposure during exercise, the total uptake which is directly related to the effect on ther nervous system, may be as high as that of a higher exposure during rest. This applies specifically to the aromatic compounds which are more soluble in blood than the straight chain compounds. This is an important finding since it demonstrates that monitoring of the levels in respiratory air alone may be insufficient to estimate the uptake by the worker. Ideally in these cases biological monitoring e.g. the determination of metabolites in the urine over 24 hours or the level in blood or fat tissues should be done. The importance of the adverse effect on the performance of behavioural tests at low levels of exposure, of which the subject is usually unaware, is difficult to translate in practical terms. Conceivably, it could be associated with accident proneness or with a lack of precision in performing difficult tasks. It may be argued that any effect which affects the efficiency of an exposed worker should be avoided, even if it is rapidly reversible. A different question is whether this apparently reversible effect may be a warning sign of the development of more permanent effects to the human brain. This may be the case when the compound, to which exposure occurs, is a known neurotoxic compound such as carbon disulphide.

Until now there has been no experimental evidence that the petroleum hydrocarbons with the exception of n-hexane, may cause anatomical or physiological changes of ther nervous system.

An answer to this question can only be obtained from two sources i.e. epidemiological studies on exposed workers and long-term experimental studies on animals involving the use of adequate methods of study (behavioural, neurophysiological, neurohistological and neurochemical). The experimental studies on animals with petroleum hydrocarbons have, until now, not shown any effect on the nervous system with the exception of those causing the gammadiketone neurotoxic effect. There is scarcely any doubt that severe effects such as brain atrophy would have been found in these studies, though more subtle effects could have been overlooked since in classical toxicology studies the methodology, specific for this purpose, has not been used.

The studies of workers, occupationally exposed to petroleum hydrocarbons and other solvents, have suggested that long-term exposure may be associated with effects such as the more frequent occurrence of subjective symptoms which are grouped together as neurasthenia and a higher frequency of deficits in psychophysiological performance similar to those observed in volunteer studies but present also in periods when the acute effects of exposure should have disappeared. A few studies indicate that these latter effects may be reversible, when exposure is discontinued or significantly

decreased in intensity. Those studies which have included the study of matched control groups have until now failed to indicate a clear progression of the effect with the increase in the years of exposure. Age may effect the performance of some behavioural tests in unexposed as well as in exposed workers.

There are a few studies which suggest that long-term exposure to paint solvents, among which white spirit is an important one, may lead to clinical evidence of brain atrophy associated with signs and symptoms of dementia. In these cases the history of exposure is not well recorded and may have been due to several substances. Periods of slight or more severe intoxication due to over exposure usually occurred frequently in the past.

The extent of exposure in even the more carefully done studies is not well-known, since exposures were commonly to mixtures of substances and the contribution of skin contamination has never been measured. Several of these studies give only an indication of the level of concentrations in the air over a few years or the assumption is made that concentrations in various locations and in previous years will not have been different from those determined at the time of the study.

It should be appreciated that the methodology used in behavioural toxicology allows an assessment of the psychophysiological performance, which may be influenced by uncontrolled factors. This performance may vary e.g. by factors such as sleep disturbances, fatigue, worries, changing of workshift, intercurrent diseases and thus a failing performance may be caused indirectly be the work situation as a whole and need not only be caused by a direct toxic action of exposure. It may also be expected that in studies of this type especially when a large variety of tests is used (up to twenty) one or a few of these tests by chance may suggest an effect.

Moreover it is probable that only those studies which have shown a possible effect on the function of the nervous system have been published. The fact that the incipient effects of long-term exposure to different substances such as to the known neurotoxic compounds carbon disulphide or trichloroethylene and to simple petroleum hydrocarbons appear similar, is probably related to the limitations of the methods applied rather than by similarity of action on the nervous system.

Epidemiological studies of the type described in the previous chapters can scarcely be expected to give a definite answer to the many questions raised. There is a need for standardization of the tests in use which should be comparable to tests used in animal experiments; there is a need for studies on animals on the long-term effects on the nervous system of individual compounds and on the non-specific effects of repeated episodes of anaesthesia or sub-anaesthesia.

With the present state of knowledge, there is no evidence that long-term exposure to the widely used petroleum hydrocarbon solvents at or below the presently accepted standards of exposure will have any progressive effects to the nervous system of man. It is possible that especially under conditions of heavy physical exertion some involvement of the central nervous system may occur. It is only prudent to pay more attention to conditions which may give rise to over-exposure (especially by the combination of vapour exposure and skin contamination) and to avoid episodes of intoxication, as has frequently occurred in the past.

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