An experimental study of the combined effects of *n*-hexane and methyl ethyl ketone

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ABSTRACT This study was intended to determine whether or not methyl ethyl ketone (MEK) enhances the neurotoxicity of *n*-hexane at low concentration and after long term exposure. Separate groups of eight rats were exposed to 100 ppm *n*-hexane, 200 ppm MEK, 100 ppm *n*-hexane plus 200 ppm MEK, or fresh air in an exposure chamber for 12 hours a day for 24 weeks. The body weight, motor nerve conduction velocity (MCV), distal motor latency (DL), and mixed nerve conduction velocities (MNCVs) were measured before exposure and after four, eight, 12, 16, 20, and 24 weeks' exposure. One rat of each group was histopathologically examined after 24 weeks' exposure. Exposure of 100 ppm *n*-hexane did not significantly decrease the functions of the peripheral nerve throughout the experiment. Exposure to 200 ppm MEK significantly increased MCV and MNCVs and decreased DL after four weeks' exposure, but at the later stage no significant changes were found throughout the experiment by comparison with the controls. Mixed exposure to 100 ppm *n*-hexane plus 200 ppm MEK significantly decreased DL after four weeks' exposure and decreased MCV and MNCVs after 20 and 24 weeks' exposure by comparison with the controls. On histopathological examination of the tail nerve, however, no changes were found in any of the exposed groups or the controls. These results suggest that MEK might enhance the neurotoxicity of *n*-hexane at a low concentration, and mixed exposures to *n*-hexane and MEK should be avoided.

n-Hexane is widely used in industry and is well known to be neurotoxic. It is often used with other solvents; and, as a result, many workers are exposed to mixed organic solvents containing *n*-hexane.^{1,2} The study of the combined effects of *n*-hexane and other solvents on the nervous system is important for the maintenance of the health of men exposed at work. Billmaire *et al*³ and Allen *et al*⁴ reported an outbreak of peripheral neuropathy in workers exposed to methyl *n*-butyl ketone (MBK) (a metabolite of *n*-hexane) and methyl ethyl ketone (MEK) in a coated fabrics plant. Saida *et al*⁵ reported that MEK did not produce peripheral neuropathy in the rat, but the combination of MBK and MEK did. Altenkirch and Mager⁶ reported a polyneuropathy in four youngsters who sniffed thinners containing *n*-hexane, MEK, and other solvents. Altenkirch *et al* also showed in an experimental study,⁷ that exposure to 1000 ppm MEK plus 9000

ppm *n*-hexane shortened the onset of severe polyneuropathy compared with exposure to 10 000 ppm *n*-hexane. On the other hand, Takeuchi *et al*⁸ showed that 1000 ppm toluene inhibited the neurotoxicity of 1000 ppm *n*-hexane. Ono *et al*⁹ showed that even 200 ppm *n*-hexane could affect the peripheral nerve in rats. Therefore, in the present study we intended to clarify whether or not MEK could increase the neurotoxicity of *n*-hexane in the rat, even at the low concentrations used in industry, and whether 100 ppm *n*-hexane alone could affect the peripheral nerves.

Material and methods

Thirty two Wistar strain male rats (mean body weight 318 g, SD $\pm$ 10 g) were divided into four groups of eight rats, and the separate groups were exposed to 100 ppm *n*-hexane, 200 ppm MEK, 100 ppm *n*-hexane plus 200 ppm MEK, or fresh air in an exposure chamber¹⁰ for 12 hours a day, every day for 24 weeks. Food and water were provided freely.

The temperature and the relative humidity in the chambers fluctuated from 23–24°C and 40–60%. The *n*-hexane and MEK used were more than 99% pure, and the concentrations of vapour in the exposure chamber were measured daily by gas chromatography. The measured concentrations were 106 ± 35 ppm of *n*-hexane, 200 ± 40 ppm of MEK, and 104 ± 26 ppm of *n*-hexane plus 203 ± 26 ppm of MEK (mean $\pm$ SD, for 24 weeks). The peripheral nerve conduction velocity was measured in the tail nerve of the rat by the methods previously reported.^{8,11,12} The rat was wrapped in a towel to keep it immobilised without anaesthesia and laid on its back to allow electrodes to be inserted in the ventral part of the tail. The electrode was a stainless steel needle, 0.34 mm in diameter and about 15 mm long. Electrode A was inserted 3 cm distal from the anus, electrode C, 3–4 cm proximal to the end of the tail, and electrode B, 5 cm proximal to C. The references were inserted about 2 mm from each electrode, and the body earth was inserted between B and C. After insertion of the electrodes, the tail was immersed in a paraffin bath in which the temperature was maintained between 37° and 38°C. The conduction velocity of the tail nerve was measured more than four minutes after the immersion, and the measurement was finished within 20 minutes of the immersion. The tail nerve was stimulated by a square pulse of 0.3 msec duration, 2 c/s and supramaximal strength with an electrostimulator (SEN-7103, Nihon Koden), and the biopotentials were observed with an Addscope (ATAC-350, Nihon Koden). Points A and B were stimulated by a single stimulation and the muscle action potentials were observed at point C to obtain motor nerve conduction velocity (MCV) and the motor distal latency (DL). The tail nerve was stimulated 100 times at point C, and the action potentials at points A and B were summated to obtain mixed nerve conduction velocities (MNCVs). The formulae of these indices are as follows: MCV = distance (AB)/latency time (AC–BC), DL = latency time (BC), MNCV (CB) (distal) = distance (CB = 5 cm)/latency time (CB), MNCV (CA) (total) = distance (CA)/latency time (CA), and MNCV (BA) (proximal) = distance (BA)/latency time (CA–CB). The body weight, MCV, DL, and MNCVs were measured before exposure and every four weeks after exposure.

One rat of each exposed group and one control animal were killed after 24 weeks' exposure and pathohistologically studied. Under anaesthesia, the rat was perfused from the left ventricle with a fixative that contained paraformaldehyde and glutaraldehyde. About 1 cm of nerve tissue was dissected out from the proximal and distal portions of

the dorsal tail nerve trunk. For light microscopic examination of the teased nerve, nerve tissues were postfixed in osmium tetroxide and dehydrated in glycerin, and teased by a needle in glycerin under binocular dissection microscope. For electron microscopic examination, tissues were fixed in the same fixative and then postfixed with osmium tetroxide. After staining en bloc with uranyl acetate, the tissues were dehydrated and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (JEOL 100 CX). The present electrophysiological results were compared with those from the previous 200 and 500 ppm *n*-hexane exposure.⁹

Results

The changes in body weight, MCV, and DL are shown in fig 1, and the changes in the MNCVs in fig 2. The body weight of the rats in the exposed groups showed no significant differences from the controls throughout the experiment. No abnormal behaviour was observed in either the exposed groups or the controls throughout the experiment. In the 200 ppm MEK group a significant increase in MCV and

Fig 1 Changes in body weight, MCV, and DL. Significance level: * $p < 0.05$.

Fig 2 Changes in MNCVs. Significance level: *(week) $p < 0.05$.

MNCV (total), and a significant decrease in DL were shown by comparison with the controls after four weeks' exposure, but at the later stage no significant differences were found throughout the experiment. In the 100 ppm *n*-hexane group a significant decrease in DL after four weeks' exposure and a tendency for MNCV (total) to decrease after 24 weeks' exposure was shown, but no other significant differences were found in any electrophysiological index by comparison with the controls. In the 100 ppm *n*-hexane plus 200 ppm MEK group a significant decrease in MCV, and MNCVs (total and distal) after 20 and 24 weeks' exposure was shown by comparison with the controls. A significant increase in DL and a significant decrease in MNCVs (total and proximal) were found after 16 weeks' exposure in the mixed exposure group by comparison with the group of *n*-hexane alone; no other significant differences were found between the two groups. A significant increase in MNCV (total) was found in the mixed exposure group by comparison with the group of MEK alone after four weeks' exposure; no other significant differences were found. The results of the electrophysiological examinations are summarised in the table.

The changes in MCV of the present 100 ppm *n*-hexane, 200 ppm MEK, and 100 ppm *n*-hexane plus 200 ppm MEK, and the previous 200 ppm and 500 ppm *n*-hexane groups⁹ are shown in fig 3. A dose-dependent effect of *n*-hexane on the function of the peripheral nerve is found in 100, 200, and 500 ppm *n*-hexane exposure, and the effects of 100 ppm *n*-hexane plus 200 ppm MEK are found between the

Summary of significant differences found in neurophysiological values in exposed and control animals

		<i>n</i> -Hexane 100 ppm								MEK 200 ppm								<i>n</i> -Hexane 100 ppm + MEK 200 ppm							
		Week	0	4	8	12	16	20	24	0	4	8	12	16	20	24	0	4	8	12	16	20	24		
Control	BW																								
	MCV																								
	DL																								
	MNCV (CA)																								
	MNCV (CB)																								
	MNCV (BA)																								
<i>n</i> -Hexane 100 ppm	BW																								
	MCV																								
	DL																								
	MNCV (CA)																								
	MNCV (CB)																								
	MNCV (BA)																								
MEK 200 ppm	BW																								
	MCV																								
	DL																								
	MNCV (CA)																								
	MNCV (CB)																								
	MNCV (BA)																								

Arrows show statistically significant level ($p < 0.05$) and directions.
BW = Body weight; MCV = Motor nerve conduction velocity; DL = Distal motor latency; MNCV = Mixed nerve conduction velocity.

Fig 3 Changes in MCV (percentage of the exposed groups in the controls). Significance level: * $p < 0.05$, ** $p < 0.01$.

exposure of 100 ppm and 200 ppm *n*-hexane. Microscopic examination of the teased nerves showed no changes in the tail nerves of rats in the present 100 ppm *n*-hexane, 200 ppm MEK, 100 ppm *n*-hexane plus 200 ppm MEK groups, or the controls.

Discussion

The present study showed that exposure to 100 ppm *n*-hexane plus 200 ppm MEK impaired the function of the peripheral nerve in the rat and that exposure to 100 ppm *n*-hexane alone did not. The differences between the mixed exposure group and the 100 ppm *n*-hexane group were not as clear as those that Altenkirch *et al*⁷ showed at high concentrations. These results suggest that mixed exposure to *n*-hexane and MEK might be more toxic than *n*-hexane alone even at the low concentrations used in industry, and that exposure to this mixture should be avoided.

The present experiment showed no significant changes in the 100 ppm *n*-hexane group except for DL after 16 weeks' exposure by comparison with the controls throughout the experiment, although MNCV (CA) (total) showed a tendency to decrease after 24 weeks. On the other hand, Ono *et al*⁹ and Kitoh *et al*¹³ reported that exposure to 200 ppm *n*-hexane affected the peripheral nerve of the rat in conditions similar to those of the present experiment, and that changes could be detected by both electrophysiological and histopathological examination. The present histopathological examinations of the tail nerve trunks showed no changes in either the exposed groups or in the controls. From these results it might be inferred that the changes of the peripheral nerve in rats can be detected earlier by

electrophysiological examination than by histopathological examination. And as the dose of 100 ppm *n*-hexane for 12 hours a day every day is equivalent to that of 175 ppm for eight hours a day, six days a week, it might also be inferred that the present electrophysiological methods can detect impairment of the function of the peripheral nerve in rats exposed to the concentration between 175 to 350 ppm of *n*-hexane for eight hours a day, six days a week for 24 weeks. Sanagi *et al*¹⁴ reported that workers exposed to 58 ± 41 ppm (time weighted average for eight hours) *n*-hexane developed impairment of the nerve conduction velocities. And, according to the previous review of the dose-response relationship of *n*-hexane,¹ it can be speculated that workers exposed to about 600 ppm (TWA for eight hours) *n*-hexane could develop severe polyneuropathy with muscle atrophy and gait disturbance, whereas it would require an exposure of about 4700 ppm (TWA for eight hours) *n*-hexane for rats to develop the same. Judging from these results, it may be inferred that man is several times as sensitive to *n*-hexane as rats at both low and high concentrations.

In the 200 ppm MEK exposure group the MCV and MNCVs significantly increased and DL significantly decreased by comparison with the controls after four weeks' exposure, but these changes were not found at the later stage of the experiment. Savolainen and Seppäläinen¹⁵ reported that a significant increase in MCV and in axonal membrane protein was noted in rats exposed to 300 ppm toluene for three weeks by comparison with the controls, and that these changes could not be found at the later stage. These results suggest that the 200 ppm MEK exposure might also affect the axonal membrane and transiently increase the nerve conduction velocity similar to 300 ppm toluene at the earlier stage of exposure, although the chronic mixed effects of MEK and *n*-hexane are opposite to those of toluene and *n*-hexane.⁸ The mechanism by which MEK enhances the neurotoxicity of *n*-hexane is interesting but, as yet, not clear. Abdel-Rahman *et al*¹⁶ reported that the combined exposure of 225 ppm MBK and 750 ppm MEK enhanced the neurotoxicity of MBK and increased MBK and 2, 5-hexanedione (a common metabolite of *n*-hexane and MBK) in the blood of rats by comparison with 225 ppm MBK alone. Couri *et al*¹⁷ reported that the combined administration of MBK/MEK (40/120 mg ip) increased the urinary excretion of 2-hexanol and 2, 5-hexanedione compared with the administration of an equal dose of MBK alone. These results suggest that MEK itself is not toxic to the peripheral nerve but that it might modify the biotransformation of, and enhance the neurotoxicity of, *n*-hexane.

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