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Examination of nervous system effects and other health effects in tunnel workers exposed to acrylamide and N-methylolacrylamide in Romeriksporten, Norway

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Abstract

Objectives This study examined nervous system effects and other health effects in tunnel workers exposed to acrylamide and N-methylolacrylamide during tunnel work.

Methods Symptoms and nerve conduction properties were recorded in 25 tunnel workers exposed to acrylamide and N-methylolacrylamide during grouting operations, four and 16 months after the cessation of exposure. Visual evoked response (VEP) and electroretinography (ERG) were performed 16 months post exposure. Hemoglobin adducts of acrylamide were measured and qualitative exposure indices were developed. Fifty unexposed tunnel workers served as referents. Chromosome aberrations and the distribution of Glutathion S transferase (GST) genotypes (M and T) were also examined in the 25 exposed workers, and were compared to 25 age and smoking matched referents.

Results The exposed workers reported an increased prevalence of symptoms during grouting work compared to the examination after 16 months. A statistically significant reduction in mean sensory nerve conduction velocity (NCV) in the ulnar nerve was observed 4 months post exposure when compared to the reference group (52.3 vs. 58.9 m/s, p=0.001), and mean ulnar distal delay was prolonged (3.1 vs 2.5ms, p=0.001). Both measures were significantly improved when measured one year later. By correlating outcome measurements with the qualitative exposure indices of the individual workers, exposure-related improvements from four to 16 months post exposure were observed both in the median nerve (motor and sensory NCV and F-response) and the ulnar nerve (sensory NCV, F-response). A significant reversible reduction in the mean sensory amplitude in the median nerve was also observed, while sensory amplitudes in the sural nerve were significantly reduced after 16 months. The amplitudes in the electroretinography on photoptic (30 Hz) stimulation were significantly lower in the exposed subjects compared to the referents. There were no clear differences in the neurophysiological outcome parameters related to GST genotypes M and T. Chromosome examinations showed no significant differences between the 25 exposed subjects and 25 of the referents for chromosome aberrations, chromatid breaks, chromosome breaks and chromosome gaps. A significant increase in chromatid gaps was observed in the exposed subjects, but no exposure-response relationship could be found. Subjects with GSTM1-/GSTT1 genotype had slightly higher frequencies of all effect parameters compared to referents with the same genotype.

Conclusion The results indicate slight effects on the peripheral nervous system in tunnel workers related to exposure to N-methylolacrylamide and acrylamide during grouting operations. Apart from a

possible delayed axonal effect on sensory fibres in the sural nerve, the effects seemed largely to have been reversible, with normalisation 16 months post exposure. A possible slight change in the ERG suggests subclinical effects on photoreceptors (cones) in the central part of the retina. The pattern of the chromosome results may indicate a possible slight genotoxic effect.

Stikkord:
Akrylamid, N-methylolakrylamid, injeksjonsmidler, nerveledning, perifere nervesystem, reversibilitet, symptomer, tunnel arbeidere, hemoglobin addukter, kromosomskader, glutathion-S-transferase

Key terms:
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Content

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1. INTRODUCTION

Monomeric acrylamide is neurotoxic, probably carcinogenic to humans, and may have reproductive effects on animals ((WHO, 1985; IARC, 1994). Thus, occupational exposure to acylamide may present a hazard to the workers, primarily by affecting the nervous system, including both peripheral and central nervous effects (WHO 1985). Acrylamide-containing grouts have been used in tunnel construction work for several decades, in order to prevent water leakage into the tunnel. Due to the toxic properties of acrylamide, several other grouts have been developed, among them one based on the less toxic N-methylolacrylamide (NMA).

During 1995-1997, 340 tons of an NMA-based grouting agent, Rhoca-Gil (Sipro-Gel) was used for injection in Romeriksporten, a 14 kilometer long railway tunnel from the city of Oslo to the new Gardermoen airport located north of Oslo. Reports of adverse effects from use of the same grouting agent from another tunnel project in Hallandsåsen in Sweden appeared in the Norwegian press during August 1997 (Hagmar et al., 2001). Shortly after, several Norwegian tunnel workers also complained of symptoms compatible with acrylamide effects. During the autumn of 1997, all workers engaged in the injection work in Romeriksporten were invited to a health examination at the Occupational Health Services (OHS survey) of the construction company. Subjects with suspected signs and symptoms were referred to further examinations on an individual basis.

The National Institute of Occupational Health (NIOH) initiated an epidemiological study to further examine the possible relationship between exposure to acrylamide-containing grouting solution and neurological symptoms and signs in the tunnel workers. Exposure assessment was partly performed qualitatively using detailed interviews and questionnaires, and quantitatively by measurements of hemoglobin adducts to acrylamide, a biomarker for recent exposure. Neurophysiological examinations, including neurography, visual evoked responses and electroretinography, were performed. We have also examined chromosome aberrations in exposed groups and individual factors related to biotransformation and detoxification of acrylamide (GST genotypes).

This report presents a summary of the OHS survey (Brudal et al., 1998), and reports the results from the NIOH follow-up study of 25 tunnel workers from Romeriksporten. Thus, the focus in this report is on the possible acute effects in the exposed tunnel workers, together with possible

reversibility of effects one year later, when the group was re-examined. Results from this project has also been published elsewhere (Kjuus et al., 1999, Kjuus et al., 2001, Kjuus et al., 2002).

Acrylamide-containing grouts have been used in many major tunnel projects in Norway during the early 90's. To our knowledge, there has been no report of adverse health effects. However, no systematic investigations had been undertaken to explore this relationship. We therefore initiated another epidemiological study, with the aim of examining if there could be any persistent effects of acrylamide exposure several years after the cessation of exposure to acylamide-containing grouts. That study, which also includes an examination of neuropsychological function, colour vision and vision fields, will be reported separately (Goffeng et al., 2000, Goffeng et al., 2002, in preparation).

2. BACKGROUND

2.1 Acrylamide and N-methylolacrylamide in grouts

Acrylamide grouts were introduced in the 1950's. They quickly obtained a major share of the market, owing to their low cost and superior performance properties. In the USA, the production of acrylamide grout ceased in the late 1970's owing to the producers' concern for its potential risk to humans. However, in 1989 acrylamide grouts still accounted for more than 40% of the grout use in USA.

The grout is typically injected in and around concrete, rock and soil to increase the absolute strength of the mass and to restrict the water flow through a structure or the grouted area. The majority of its use has been in sewer rehabilitation, during sewage lining repair or manhole sealing. Other uses include structural water control, e.g. during tunnel construction, and other geotechnical applications. These operations involve manual injection techniques.

In general, acrylamide grouts consist of a 19:1 mixture of acrylamide and crosslinking agent. When the grout is prepared for use, water and small amounts of catalysts, activators or accelerators and inhibitors are added. When the acrylamide grout polymerises, it solidifies into a stiff gel which is impervious to water. In the gel form, the grout contains less than 0.05% free acrylamide. An assessment of chemical grouting agents, including acrylamide, was performed by NIOH in 1982 (Bye and Lenvik,1982).

Of the less toxic grouts that were developed, one was based on N-methylolacrylamide (NMA). NMA is produced from acrylamide and formaldehyde, in aqueous solution:

Acrylamide + Formaldehyde $\leftrightarrow$ N-Methylolacrylamide.

The reaction is slightly reversible, where NMA is hydrolysed back to acrylamide. The extent to which this happens in practice is not known and is dependent on the environmental conditions. NMA may also be partly transformed to acrylamide at high pH and when sodium silicate is added. At temperatures below 5°C polymerisation of the monomer may be considerably delayed in relation to the gelling of the silicate. Consequently the grout will not adhere perfectly adhered to the fissures and may leak out again. Thus, exposure to the mixed, but not yet polymerised product may occur.

2.2 Acrylamide – kinetics and metabolism

Acrylamide is readily absorbed via all routes of administration. Thus, it has been reported that acrylamide induces neurotoxic effects in many animal species following absorption via the respiratory, dermal and oral routes (WHO, 1985). The development of neurotoxicity is independent of the route of absorption, probably because it is distributed to all body fluids. Once absorbed, acrylamide is rapidly distributed throughout the body, metabolised and excreted. After oral administration in rats of 1-100mg/kg of acrylamide, 65-80% of the dose was excreted after seven days, of which 90-95% in the urine (Sipes and Carter, 1981). Rather surprisingly, the highest concentration has been found not in the nerves, but in red blood cells. After intravenous injection, 10% of the administered dose remained relatively constant in the blood for one week. By contrast, the lowest concentration was found in nervous tissue (less than 1% of the administered dose was found in brain, spinal cord and sciatic nerve after i.v. administration). Acrylamide in relatively large quantities was found in areas having high blood flow, such as muscle, skin, liver and small intestine. Acrylamide is excreted primarily as metabolites (>90%), while less than 2% is excreted as the parent compound in the urine. (Sipes and Carter, 1981). Acrylamide is primarily conjugated with gluthathion and is excreted as mercapturic acid (n-acetyl-cystein conjugate). A minor proportion is oxidised to glycidamide through the cytochrome P-450 system. Glycidamide has high reactivity, particulary against free

thiol-groups, and is excreted in conjugated forms. The elimination curve for acrylamide follows a biphasic, first order kinetics pattern. The half-life for acrylamide ($T_{1/2}$) is approximately 2 hours in blood and 5 hours in tissue. The terminal $T_{1/2}$ for most tissues was 6-8 days, while it was found to be significantly longer for the spinal cord (approximately 24 days) (Tilson, 1981).

2.3 Nervous system effects of acrylamide

The variety of symptoms described in cases of acrylamide poisoning suggest involvement of both the central and peripheral nervous system, together with the autonomic nervous system. The reported symptoms include irritation of skin and mucous membranes, with peeling of the skin of the hands and feet, muscular weakness, paresthesia, numbness in hands, feet, arms and legs, and unsteadiness with difficulties in walking and standing (WHO, 1985). Other symptoms reported are unusual fatigue and sleepiness, dizziness and memory difficulties. Vegetative symptoms, such as excessive sweating of the hands and feet (Takahasi et al., 1971; Kesson et al., 1977), and micturation and defaecation difficulties (Garland and Patterson, 1967) have also been reported.

The clinical signs found in cases of poisoning are consistent with the reported symptoms. Thus, contact dermatitis, with blueness and sometimes redness of hands and feet (Auld and Bedwell, 1967), loss of tendon reflexes, impairment of sensation, and muscular wasting have been observed (Takahasi et al., 1971; Kesson et al., 1977)

Altogether, since the first report on acrylamide poisoning in man was presented in 1953 (Kuperman, 1957), more than 150 cases has been presented, mainly related to the primary production of acrylamide from acrylonitrile (Kuperman, 1957; Fujita et al., 1960; Morviller, 1969), or to the polymerisation of acrylamide to polyacrylamide (Auld and Bedwell, 1967; Garland and Patterson, 1967). The first report on acrylamide-related health effects in construction workers related to waterproofing was presented in France in 1970 (Graveleau et al., 1970). Later reports in tunnel workers relate mainly to the polymerisation of acrylamide in grouting operations (Kesson et al., 1977; Mapp et al., 1977). In most cases, the symptoms and signs have been shown to be reversible, with full restitution 2-12 months after the cessation of exposure (WHO, 1985). However, in severe intoxications, symptoms have persisted for several years (Myers and Mahun, 1991).

The grouts used by the workers in the aforementioned studies seem mainly to have been based on pure acrylamide, although in one study they are based on N-Methylacrylamide and Methylen-bis-acrylamide (Graveleau et al., 1970). Thus, the Swedish study (Hagmar et al., 2001) and the present study are the first to examine neurological effects in workers exposed to grouts based on N-methylolacrylamide.

2.4 Mechanisms for acrylamide-induced neurotoxicity

Analogous of acrylamide with structural modifications have been tested for the potential to induce neurotoxicity in animals. Substitutions at the amide nitrogen resulted in a decreased neurotoxic potential and saturation of the double bond abolished neurotoxicity (Edwards, 1975; Hashimoto, 1981). Covalent binding of acrylamide to central nervous system proteins may play an important role in the toxicity, resulting in inhibition of a number of enzymes and essential compounds (Carlson and Weaver, 1985). The unsaturated double bond of acrylamide is relatively reactive towards free thiol groups in glutathione (GSH) and cystein. Depletion of GSH in mice brain enhanced the neurotoxicity effects. GSH may play an important role in the detoxification of acrylamide in the nervous system (Shivakumar and Ravindranath, 1992). Although some DNA binding has been shown in vitro, acrylamide only demonstrated weak activity (Dearfield et al., 1995). This in contrast to its epoxide metabolite glycidamide, which has been positive in the Salmonella mutation assay. Typical DNA adducts from acrylamide exposure also indicate the involvement of the epoxide in genotoxicity (Dearfield et al., 1995). The glycidamide may also have neurotoxic effects.

Two principally different types of pathological changes may occur in peripheral nerves. In segmental demyelination, there is a patchy breakdown of myelin affecting individual internodal segments, while the axis cylinders remain in continuity. The primary neurophysiological finding will be a significant reduction of nerve conduction velocities due to myelin damage. This neuropathy has been found after exposure to lead, triethyltin and isonicotinic acid hydracide (Norton, 1986). In axonal degeneration, on the other hand, biopsies may show axonal degeneration of nerve fibres, a reduction of large diameter nerve fibres and /or the accumulation of neurofilaments in the axon. The conduction velocities of motor and sensory

fibers are often normal at the electrophysiological examination. However, the amplitude of sensory and motor nerve action potentials is often reduced.

Although extensively studied in animal models, the mechanisms by which acrylamide exerts its neurotoxic effect is still not clear (Miller and Spencer, 1985; Tilson, 1981; Smith and Oehme 1991). What is clear, however, is that neurons both in the CNS and PNS are targets for the insult from acrylamide. Acrylamide is capable of producing axonopathy by transsection of neurons, which leads to the degeneration of that proportion of the axon which is separated anatomically from the nerve cell body (Smith and Oehme, 1991).

Thus, the morphological effects of acrylamide on peripheral nerves are primarily axonal degeneration with secondary demyelination, predominantly in the distal part of long nerves with diameters of 8-16 um (Smith and Oehme, 1991). Sensory fibres are first affected, followed by degeneration of motor fibres and long axons of the spinal cord, leading to a reduction in the number of large diameter fibres. Accordingly, sensory nerve action potential amplitudes have been reported to be the most sensitive indicator of acrylamide intoxication (Igishu et al). The accumulation of neurofilaments and enlarged mitochondria lead to significant swelling in terminal axons of the PNS and the boutons terminaux in the CNS. Degenerative changes also occur in the autonomic nerve fibres.

An important difference exists, however, in the outcome of axonal degeneration in the CNS compared to that in the PNS: peripheral axons can regenerate whereas central axons cannot (Norton, 1986). This implies that recovery (partial or complete) can occur after axonal degeneration in the PNS, whereas effects on the CNS tend to be irreversible.

Several investigations have been conducted concerning the possible effects of acrylamide on axonal transport. Peripheral sensory nerves are bipolar neurons, with a peripheral axon that may extend the entire length of a limb and a central axon that projects towards the brain. The axon is capable of transferring electrically encoded information over long distances (up to two metres). Because the axoplasm is unable to synthesise lipids or proteins, the axon is dependent solely upon its neuronal cell body (perikaryon) which is located in the dorsal root ganglion. The perikaryon is the main site for protein and lipid synthesis, but the axon is capable of modifying them by phosphorylation and other mechanisms (Smith and Oehme, 1991).

The main function of the anterograde transport (down the axon) is to move various structural proteins (neurofilament triplet proteins, tubulin, actin), enzymes (glycolytic enzymes, dopamine-β-hydroxylase, acetylcholinesterase) and substance P and neurotransmitters (acetylcholine, norepinephrine) distally along the axon. Previous studies indicate that acrylamide interferes with the rapid anterograde transport of substances (down the axon), but may not significantly affect the slow transport system (Tilson, 1981). In contrast, several later studies have found a reduction in the slow anterograde transport (Smith and Oehme,1991). A Danish study suggests that although acrylamide may play a role in the slow anterograde transport late in the neuropathic process, neither fast nor slow components are involved in the initial pathological events in the distal axons (Sidenius and Jacobsen, 1981).

The purpose of the retrograde axonal transport is to transfer axoplasmic constituents for reprocessing in the perikaryon. The fast retrograde system transports a variety of substances (e.g. acetylcholinesterase, adrenergic granules, lysosomes) at a rate of 1.5-2.5 cm a day. The slow retrograde axoplasmic system transports a single protein, probably albumin, at a rate of 3-6 mm a day. Acrylamide may initiate subtle changes in the structural lattice of the axon, causing retrograde transport to decrease and eventually stop (Smith and Oehme, 1991, Miller and Spencer, 1984). This effect is dose dependent and precedes the development of clinical signs of neuropathy. Sensory neurons have been shown to be more sensitive to acrylamide than motor neurons.

It has also been shown that acrylamide may penetrate the neuron at the neuromuscular junction by pinocytosis and bind to sulfhydryl groups in the axon of the nerve, resulting in disassembly of microtubules in the axon and the disruption of retrograde transport (Smith and Oehme, 1991). This mechanism may be potentially relevant to dermal exposure, implying a potential direct toxic effect on the nerve at the neuromuscular junction, in addition to the systemic effect.

2.5 Neurophysiologic effects of acrylamide exposure.

Fullerton and Barnes presented in 1966 an electrophysiological and histological study in chronically poisoned rats, which were given repeated oral doses of 25 to 100mg/kg at various intervals. In the animals with severe clinical abnormalities, motor nerve conduction velocity was reduced by about 20% (from 55m/s to 44 m/s), gradually developing from 6 to 12 weeks

after the start of exposure (Fullerton and Barnes, 1966). Full recovery in NCV was observed five to nine months after the cessation of exposure. Histologically, degeneration of axis cylinders and myelin sheaths was found in the peripheral nerves, affecting predominantly the distal parts of the longest fibers. Similar findings have been reported in other species (WHO, 1985; Tilson 1981; Smith and Oehme, 1991).

Hopkins and Gilliatt examined nerve conduction velocities in seven adult baboons intoxicated by acryamide (Hopkins and Gilliatt, 1971). In animals given 10-15 mg/kg/day, the gradual development of a peripheral neuropathy was accompanied by a decline in amplitudes, which were correlated with a 34-49% gradual fall in conduction velocity. In the most severely affected baboon, partial recovery (to 80% of normal values) was seen 21 months post exposure. Histological examination showed degeneration of the peripheral nerves with little demyelination, suggesting that the reduced conduction velocities were most likely due to the selective loss of large-diameter fibres and not to paranodal demyelination.

With regard to studies in humans, Fullerton performed electrophysiological studies of peripheral nerves in three patients who had developed peripheral neuropathy and ataxia after exposure to acrylamide during their work. They had been exposed for 4 weeks, 6 weeks and 18 months respectively before the onset of symptoms, and the examination was performed 6, 8 and 2.5 months after cessation of exposure. Motor NCV was normal or slightly reduced, except in one nerve (23.8 m/s in lateral popliteal nerve). Muscle response to nerve stimulation was dispersed, potentials with markedly prolonged distal latencies were found, and sensory nerve action potentials were reduced in amplitude or absent. The authors suggest that the slow conduction was due to degeneration followed by regeneration of the distal part of the fibres. In 15 workers handling acrylamide for 1-8 years and with varying signs of peripheral neuropathy, the action potentials were greatly reduced while conduction velocity was not greatly affected.

Nerve conduction velocities were studied in 69chinese workers producing acrylamide. No differences in NCV were observed when compared to a reference group, except for a slight reduction in motor NCV in the peroneal nerve. In 27 subjects with neurological signs, the sensory action potentials of the median, ulnar and sural nerves were decreased significantly compared to those of the reference group (He et al., 1989).

Acrylamide has also been shown to affect the visual system. Axonal swellings in the lateral geniculate and superior colliculus have been described in intoxicated rats and cats (Schaumberg and Spencer, 1979; Canavagh, 1982), and visual evoked response abnormalities have been reported in rats (Boyes et al., 1980). A variety of pathological effects has been shown in the visual system of macaque monkeys (Merigen et al., 1985). Also, there have been reported visual effects in some of the human case histories (Mapp et al., 1977, Myers et al., 1991).

As previously described (Section 2.4) glutathione has a protective effect on the occurrence of acrylamide-induced nerve damage. Acrylamide is conjugated to glutathione by glutation S-transferases (GST's). Glutation S-transferases occur in 4 different families, but the contribution of the particular GST's to the detoxification of acrylamide is unknown. In the Norwegian population, about 50% lack GST M1 activity, and 15% lack GST T1 activity. One hypothesis would be that subjects will have a different susceptibility to acrylamide-related nervous system effects and genotoxic effects dependent on their efficiency to detoxify acrylamide compounds via glutation conjugation.

2.6 Toxicology of N-methylolacrylamide.

The toxicological data on N-methylolacrylamide (synonyms: N-(hydroxymethyl)acrylamide, N-(hydroxymethyl)2-propenamide, N-Metanolacrylamid, Monomethylolacrylamide) are more limited than for acrylamide. There is no information on the uptake from skin or the gastrointestinal tract. NMA is rapidly distributed in all body fluids after intravenous injection. T1/2 in the blood is ca. 1.5. hours in rats. From available data, the toxicodymanics are comparable to that of acrylamide, but with a reduced potential for neurotoxic damage and carcinogenic development (Smith and Oehme, 1991; IARC, 1994; Ahlbom et al., 1998). In animal experiments, the neurotoxic potential of NMA has been reported to be 20-30% of acrylamide (IARC, 1994). The carcinogenic potential of NMA has been estimated to 10 (rat) and 100 (mice) times lower than for acrylamide.

3. THE ROMERIKSPORTEN TUNNEL PROJECT

A new airport for Eastern Norway was built at Gardermoen, 50 kilometers north of Oslo during 1995-98. A high speed railway between Oslo and Gardermoen was constructed, including a 13.8 km tunnel, Romeriksporten, from Etterstad in Oslo to Stalsberg in the district of Skedsmo.

Water leakage into the tunnel became a problem due to unstable rock structures in the tunnel area. This led also to an increasing environmental and public problem as several of the small lakes in the area were involuntarily drained during the construction work. In addition, there were also discussions on the instability of house foundations in one of the suburbs, due to the type of construction and the possible reduction in the ground water level. Thus, in addition to the problems of water leaking into the tunnel, environmental issues were even more highlighted and all tunnel work was stopped for a period. Several traditional methods for stopping the water leakage were used, primarily with cement, but other injection agents, based on polyurethan (TACCS) and acrylate gel (Meyco MP 307) were also used in relatively large quantities (125 000 and 57 000 kg respectively).

During September 1995 to August 1997 340 000 kg of Rhoca-Gil was used for injection in Romeriksporten. In traditional use grouting agents are injected into wholes drilled in all directions in the rock due to be blasted, which will normally prevent water leakage into the excavated tunnel. However, owing to the unexpected water leakages, the grouts were also injected into fissures wich leaked water after blasting. Thus, the normal polymerisation of the gel was incomplete and was disturbed by the water leakage.

During August 1997, the Norwegian press reported adverse effects on the environment, cattle and humans related to the use of 1340 tons of Rhoca Gil during a short period (6-8 weeks) in a tunnel project in Hallandsåsen in Sweden. Fish died, cows were intoxicated after drinking the contaminated water, and the tunnel workers reported health effects compatible with effects earlier described for acrylamide intoxication. These observations led to a comprehensive examination, both in the environment and in the tunnel workers and the public living near potentially polluted water sources (Edling et al., 1998; Hagmar et al., 2001).

At this time it was revealed that the grouting agents used in Romeriksporten also contained acrylamide, an information which owing to incomplete labelling of the product was not known

to the workers. Several of the workers interviewed in the press reported health complaints which they related to their work. Thus, the Occupational Health Service of the construction company in charge of the project initiated a health survey of the tunnel workers.

3.1 Exposure conditions in Romeriksporten

The actual grout used in Norwegian tunnels (Rhoca-Gil 110-25 or SiproGel) was an NMA-based grout based on two solutions. Solution 1 consisted of 26-29% NMA, 0.02-0.03% of the cross linker Methylene-bis-acrylamide, 12-17% Methylic diesters (catalyst), small amounts of other catalysts and stabilizers, together with impurities and water. According to the declaration of content, Solution 1 also contained up to 1.5% acrylamide. Later information from the producer stated 2.5-5% (average 4%) acrylamide in Solution 1, while analyses on different batches from Hallandsåsen showed acrylamide concentrations between 4.0-5.4%, and NMA concentrations between 29 and 31% (Hagmar et al.,2001). When ready-mixed, the grout consisted of 3.75 parts of water. 0.125 parts Solution 1 and 0.125 parts Solution 2. Initially, Solution 1 and 2 were delivered in 30 L cans which were manually emptied into an open mixing vessel and manually stirred. During the construction period, automatic pumping of the two solutions was introduced, but the mixing was performed manually. The not yet polymerised mixture was then pumped into the rock in a closed system, through an injector gun either as a preventive measure or to stop an actual water leakage in the tunnel.

When the injecton was finished at one location, the injection gun was moved to other drilled holes or fissures. After injection, the equipment was cleaned and sometimes repaired by the operators. The normal working hours in the tunnel were 12 hours per day for one week, then one week off. However, owing to the special problems with the water leakage, the injection work often lasted until the leakage was under control. This often led to very long working hours, and some workers reported continuous work up to 24 hours in order to finish a job.

Worker exposures occurred during grout mixing, injection, equipment disassembly and clean up. The main exposure occurred when mixing and pumping the grouting solution, and the following drilling of holes in the wall when the grouting solution was injected. Frequently, the grouting solution splashed back at the worker, resulting in his clothing being covered with the acrylamide-containing solution. In addition, workers often report «showers» of leaking water contaminated with acrylamide. Dermal absorption could then take place directly through the skin on the neck or on the hands, or through wet clothes all over the body.

The production volumes used in the different time periods and in the various parts of the tunnel are shown in Figure 1 (page 22). The figure shows that the major part of the Rhoca Gil grout was increasingly used in the late construction period in the Gardermoen end of the tunnel. Furthermore, both the available water samples for measurements of acrylamide in tunnel water and the blood samples for adduct analyses were taken after the injection period had come to a halt.

3.2 Initial OHS survey

All subjects who were involved in the injection work during the actual two-year period were offered a health examination by the Occupational Health Services of the construction company (SRG, (Scandinavian Rock Group ANS)). The examinations were not compulsory, but all 73 injection workers from the company met for the examination during the period 20th October 1997 – 15th January 1998. Their average age was 40 years, with an average of 14.0 years of tunnel work (range: 2.5-31.0 years). Forty-three subjects were daily smokers , 10 had quit smoking and 20 subjects were never smokers. They were all male, and had been working for the company for 2.5 years on average. Other workers with potential exposure to contaminated tunnel water were also offered health examinations on an individual basis. The results from the OHS survey have been presented in a separate report (Brudal, 1998), but will be summarized here in short.

The health examination consisted of a semi-structured interview about the worker's previous work, tunnel experience and detailed information on his present injection work, work practices and the use of personal preventive devices. Information was obtained about present and previous diseases, and in particular present symptoms from the nervous system, skin and respiratory system. A clinical examination was performed, including lung function measurements (Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1), FEV1/FVC%, and Peak Expiratory Flow (PEF), laboratory tests (Hb, Sed.rate, Urin test) and a blood test for the later analysis of hemoglobin adducts to acrylamide in selected groups.

All subjects with skin complaints which had started or became aggravated during the tunnel work were referred to a dermatologist for further examination (Dr. Morten Sandberg, Oslo).

They were tested with standard European epicutaneous tests for allergy to acrylamide i 0.1% and 0.05% concentrations and to Rhoca Gil in 0.2-0.1% and 0.05%. Subjects with lung complaints or reduced lung function (FVC or FEV1 < 80% of expected value, or FEV1/FVC < 75%) were referred to a lung specialist (Dr. Kurt Myhre, Dept. of Occupational and Environmental Medicine, Ullevål Hospital). Traditional spirometry, together with diffusion capacity for CO, TLCO were performed. A chest x-ray was taken of all the subjects. Subjects with symptoms and signs from the nervous system were referred to measurement of nerve conduction properties (Dr. Mona Skard Heier, Dept. of Clinical Neurophysiology, Ullevål Hospital). The latter examinations were coordinated with the NIOH follow up study.

Altogether, 15 of the 73 subjects reported symptoms related to injection work with Rhoca Gil. The symptoms were burning, itching, various types of eczema and peeling of skin. At the time of examination, 13 subjects still reported symptoms, and 11 had clinical signs of skin affection. In 10 subjects examined by the dermatologist, one had a specific contact allergy to acrylamide, and he had also had skin reactions in connection with previous acrylamide contact in earlier tunnel work. Three subjects had non-allergic irritative contact eczema. At the time of the specialist examination, only two subjects had very modest skin changes. There were no signs of skin involvement in the remaining workers.

None of the 73 subjects reported acute respiratory symptoms during injection work. Some reported an unpleasant smell when mixing the solutions. However, they all reported work with other injection agents, of which several have been reported to be irritative to the respiratory system, e.g. the isocyanate-based TACCS. According to the above-mentioned criteria, 41 subjects were referred to the lung specialist. Among these, 15 subjects received a diagnosis of chronic bronchitis (87% were smokers), and seven subjects were diagnosed as having chronic obstructive lung disease (all were smokers). The neurophysiological results are included in the NIOH follow up study.

4. NIOH FOLLOW-UP STUDY

The follow-up study was designed as two cross-sectional examinations of subjects with recent exposure to NMA-based grouts during tunnel work. One main challenge was to identify relevantly exposed subjects and to examine them as soon as possible after the cessation of the injection work. Owing to the reports on possible health effects in Swedish tunnel workers, the injection of Rhoca Gil in Romeriksporten was stopped 25th August 1997. The first examination was performed during October 1997-February 1998, i.e. on average four months after the injection work was stopped. The examinations were repeated one year later.

4.1 Aim of the study

The main aim of the study was to examine possible neurological effects and indications of possible genotoxic effects of acrylamide exposure in tunnel workers.

More specifically, the aims were to:

- study symptoms and clinical signs of acrylamide effects in tunnel workers recently exposed to Rhoca-Gil.
- determine levels of hemoglobin adducts to acrylamide in tunnel workers recently exposed to Rhoca-Gil.
- examine nerve conduction velocities in tunnel workers with recent exposure to Rhoca-Gil.
- explore the possible reversibility of neurophysiological effects in Rhoca-Gil exposed tunnel workers by re-examination of the workers after one year.
- examine possible visual effects of acrylamide exposure, by recording visual evoked response (VER) and electroretinography (ERG).
- study possible chromosome aberrations in subjects with recent exposure to Rhoca-Gil.
- examine whether individual characteristics of the glutathione conjugation metabolism (GST genotypes) might affect the susceptibility to possible aberrant effects of acrylamide.

4.2 Methods

4.2.1 Material

The study base consisted of the 73 exposed tunnel workers identified as those workers who had taken part in injection work during the tunnel construction. Thus, all 73 had been exposed to acylamide-containing grouting solution (Rhoca-Gil) during work in Romeriksporten from September 1995 to August 1997 and all participated in the OHS survey. Based on the

information given at the interview and in the questionnaire at the OHS survey, and several meetings with groups of the workers, the workers' safety representatives, the health and environmental chief officer at the plant and the occupational physician who had interviewed the workers, we identified the workers with the highest and most relevant exposures. Thus, the 25 most heavily exposed workers among the 73 participants in the health survey of all tunnel workers during autumn 1997 were selected for further study. All workers gave written informed consent to participate in the study. Altogether, 24 exposed subjects were included in the analyses of symptoms and neurophysiologic measurements, as one worker was excluded owing to diabetes.

In another study, we have examined the possible irreversible health effects related to the previous exposure to acrylamide-containing grout in tunnel work in 50 exposed tunnel workers and 50 referents recruited from tunnel workers in the same companies who were unexposed to acrylamide-containing grouts (Goffeng et al., 2000). The latter group also served as referents for the index population in the present study. In the chromosome studies, all 25 subjects were included, and compared to a subsample of 25 age and smoking-matched referents from the same reference group.

The 24 exposed workers were on average 43.1 (standard deviation, SD=8.6) years old (range 31-62), compared to 43.9 years (SD=9.6) in the 50 referents (range 23-60) . The exposed group had been doing tunnel construction or other construction work for 19.3 (7.8) years compared to 20.4 (10.2) years in the referents. However, the exposed group had been involved in direct tunnel work much longer than the reference group (14.8 (SD=8.3) vs. 3.8 (SD=4.0) years).

The distribution of some life style factors (smoking, alcohol consumption), and some other relevant exposure factors are presented in table 1. No major differences in exposure to these factors were observed between the two groups. In the smokers, the exposed subjects had smoked on average 12.8 cig./d, compared to 15.3 cig./d in the referents. The exposed workers had worked with vibrating tools for 6.0 (SD:5.5) years, compared to 7.9 (SD:8.5) years in the referents. The estimated annual alcohol consumption was slightly higher in the exposed subjects.

Table 1. Background factors and other exposures reported by the exposed group (n=24) and referents (n=50)

			Exposed	Referents
Age (years)	Mean		43.1	43.9
	Range		31-62	23-60
	SD		8.6	9.6
Years in construction work		Mean	19.3	20.4
		SD	7.8	10.2
Present smokers (%)			45.8	50.0
Mean no. of cigarettes			12.8	15.3
Alcohol, >5 L/year (%)			33.3	24.0
Work with solvents (%)			47.8	61.2 [a]
Previous lead exposure (%)			9.5	16.0
Vibrating hand tools (%)			100.0	91.9[a]
No. of years (Mean)			6.0	7.9
Vibration, whole-body (%)			34.8	50.0

[a] n=49

During the period October 1997 to February 1998 all the exposed subjects were examined at the NIOH and at the Department of Clinical Neurophysiology at Ullevål Hospital, Oslo, preferably on the same day. The workers received an additional questionnaire asking for more detailed information on exposure, together with information on symptoms during injection work and present symptoms (see Appendix 1). The questionnaire was self-administered, and followed by a short interview by one of the physicians in the project (VS or HK). New blood samples were taken for the analyses of gluthation S-transferase genotypes and chromosome abberations.

Although the workers experienced exposure to Rhoca-Gil from unpolymerised acrylamide/-NMA and contaminated tunnnel water during September-October 1997, the most relevant exposure came to a halt when the injection work was stopped 25th August. The NIOH examinations were performed during October 97 – February 98, thus on average 4 months «post exposure».

In order to study the possible reversibility of neurophysiological effects in Rhoca-Gil exposed tunnel workers, they were re-examined after one year. Thus, the 24 workers were re-examined 16 months «post exposure», by use of repeated questionnaires and measurements of nerve conduction velocities. We also examined possible visual effects of acrylamide exposure at 16

months “post exposure” by measuring visual evoked response (VER) and electroretinography (ERG) in the 24 workers.

4.2.2 Exposure assessment

No measurements of acrylamide or NMA in the working environment had been performed during the injection work. Thus, the exposure assessment had to be mainly based on qualitative exposure information. The two available sources for quantitative exposure information were the measurements of acrylamide and NMA in tunnel water, measured in the period after the injection work had been stopped, and measurements of hemoglobin adducts to acrylamide, analysed in blood samples taken from 2-5 months after the cessation of the injection work.

The estimated average amount of Roca-Gil (in kg) used per week are shown in Figure 1 A. This information was used to estimate the weighted injection time described in 4.2.2.2. Figure 1B and 1C shows measurements of acrylamide and methylolacrylamide in the tunnel water. Figure 1D shows time and number of blood samples collected for measurements of acrylamide-protein adducts

4.2.2.1 Measurements of acrylamide and NMA in tunnel water

Due to the technical and environmental problems with the water leakages, the tunnel construction work came to a halt on the 25 th of August 1997. Before this time, only a few sporadic measurements of acrylamide were analysed from the water draining from the tunnel. The highest concentrations were measured on the 25th of August, with 9654 μg/L acrylamide and 16600 μg/L NMA (Sverdrup et al., 1999). During September, the average concentration of acrylamide was 100-110 μg/L, and during October 1997 – April 1998 the majority of the measurements were below 50 μg/L (see Figure 1).

“Post injection» was performed with 116 000 kg of an isocyanate containing grouting agent, TACCS (45% difenylmetan-4,4-diisocyanat (MDI), 50% di-n-butylftalat and 5% heksadekyldimetylamin (HDMA)). During this work, several bore holes with unpolymerised Rhoca Gil were discovered, and very high concentrations of acrylamide were measured in these holes and in the water dripping from the adjacent tunnel wall. The highest concentration was 90.6 mg/L. These observations indicate that skin exposure through contaminated tunnel water could be of relevance to the workers, also in the period after the injection work came to a halt.

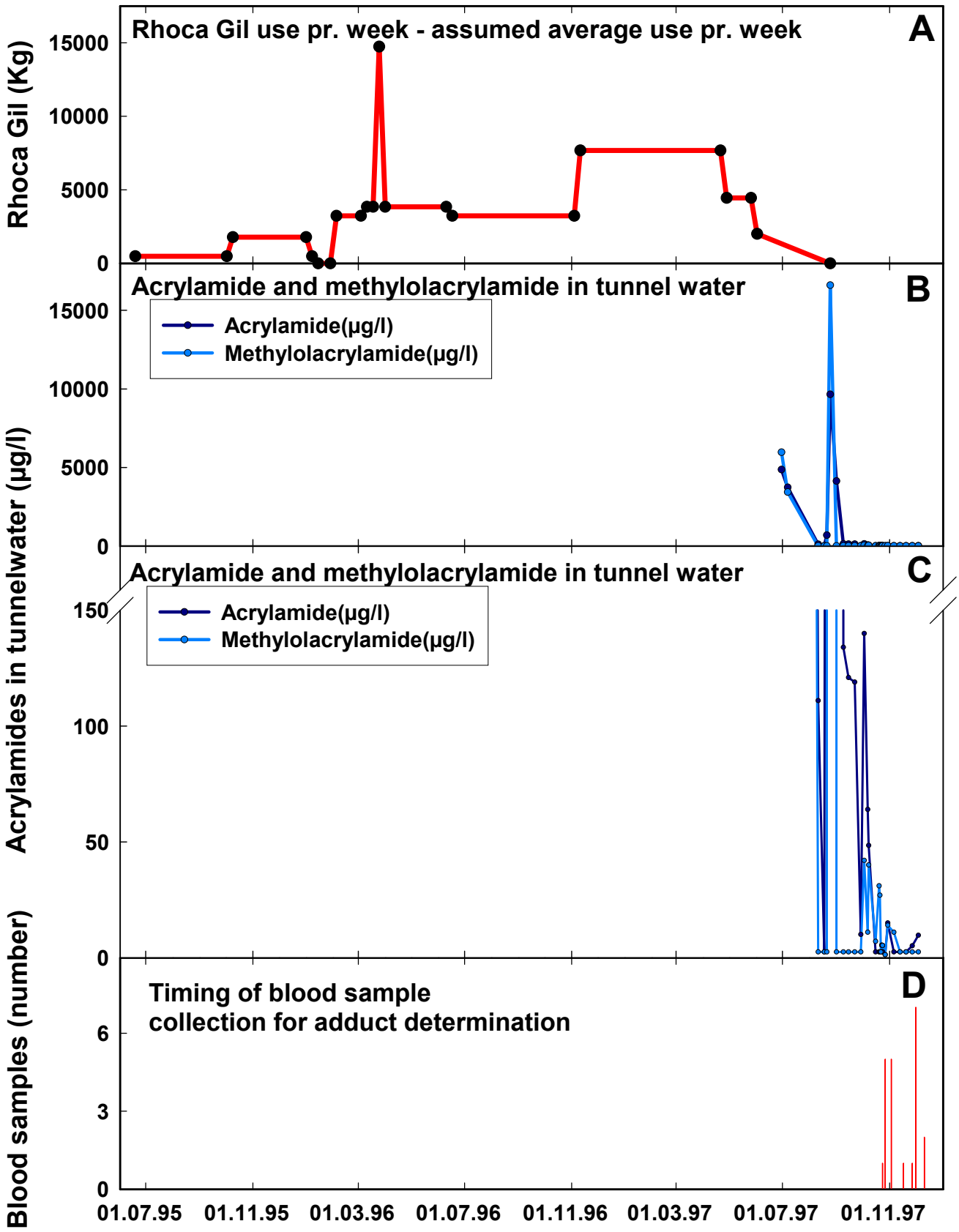

Figure 1. Acrylamide use and concentration of acrylamides in tunnel water and number and timing of blood sample collection for adduct determination

4.2.2.2 Qualitative exposure information

Based on information obtained from the questionnaires, we estimated the cumulative time for each worker had done tunnel work, together with the cumulative time of injection work during the two-year period September 1995-August 1997. We obtained information from the construction company about the amount of Rhoca Gil used in the different parts of the tunnel in the different periods (se figure 1A and B). The relevant exposure period was thus divided into three subperiods, related to the injected volume of NMA grout:

Low volume injection period (months) (LVP), from September 1995 - November 1996
High volume injection period (months) (HVP) from December 1996 - August 1997
Tunnel water exposure period (months) (TWP), from September 1997 – October 1997.
We also developed an exposure time index (ETI) which was the weighted cumulative exposure time based on information on the amount of NMA grouts used in the different periods, where the three periods were given weights of 2, 4 and 0.5 respectively.

For the analyses, we thus used a set of time-related indices:

- **Tunnel time (TT)**: Total working time in the tunnel during 1995-97(months)
- **Injection time (IT)**: Time with NMA grout injection (months)
- **Exposure time index** (ETI), based on weighted cumulative exposure time:
    - Low volume injection period (months) (LVP)
    - High volume injection period (months) (HVP)
    - Tunnel water exposure period (months) (TWP)

  where ETI was estimated as: (LVPx2) + (HVPx4) + (TWPx0.5)

Based on the questionnaires, we developed an **intensity index**, which was estimated as follows:

Injection of NMA grouts:	Often:6, seldom:2, never:0
Mixing of NMA grouts :	Often:2, seldom:1, never:0
Cleaning of NMA grouts	Often:2, seldom:1, never:0
Spill of NMA on skin	3-0 , based on 5 questions on frequency and job task
Contaminated tunnel water on skin	Yes:1, no:0
Inhalation of NMA grouts	Yes:1, no:0

Thus, the intensity index was a sum score with the range: 1-15. In the analyses, we also used a combined measure, multiplying the injection time (IT) with the intensity index.

4.2.2.3 Measurements of hemoglobin adducts to acrylamide

Analysis of hemoglobin adducts from acrylamide and N-methylolacrylamide

Hemoglobin (Hb) adducts derived from acrylamide and *N*-methylolacrylamide together with ethylene oxide adducts were measured in blood samples at the Department of Environmental Chemistry, Stockholm University. Hemoglobin (Hb) adducts of acrylamide in blood would give a valid and sensitive estimate of the average exposure during preceeding months (Bergmark 1997, Törnqvist 1994). During October 1997 – January 1998 blood samples were obtained from 24 exposed workers and 3 reference subjects, and were frozen for later analyses. Half of the blood samples were taken 60-70 days after cessation of the injection work (Figure 1). The rest were taken from 90 to 150 days after the injection work was stopped, thus somewhat late to reflect exposure during injection work.

The analysis of adducts to N-terminal valines in hemoglobin (Hb) derived from acrylamide and/or N-methylolacrylamide was performed by the N-alkyl Edman method with derivatization and detachment of adducted valines with pentafluorophenyl isothiocyanate (PFPITC) and analysis by gas chromatography tandem mass spectrometry (GC-MS/MS) as a derivative detached from the globin: pentafluorophenyltiohydantoin (PFPTH), according to Törnqvist et al. (1986), with modifications according to Bergmark et al. (Bergmark et al.,1993).

Acrylamide reacts with the N-terminal valine in Hb forming an *N*-(2-carbamoylethyl)valine adduct detached from the globin as an *N*-(2-carbamoylethyl)valine-PFPTH (Bergmark et al., 1993). *In vitro* and *in vivo* studies show that *N*-methylolacrylamide gives rise to an identical derivative (see Paulsson et al., 2002). This fact means that we cannot distinguish between acrylamide and *N*-methylolacrylamide exposure. The adduct level from an absorbed amount of *N*-methylolacrylamide is about 25 and 35% ofthe level of an equivalent amount of acrylamide in mouse and rat, respectively (Paulsson et al., 2001).

Sample preparation

Red blood cells were separated from the blood plasma by centrifugation (3000 x *g*) and 1.5 volume of water was added for lysis of the cells. The globin was precipitated by the addition of the hemolysate to 50 mM HCl in 2-propanol, followed by pelleting by centrifugation (3,000 x

g), first of the cell membrane and then, following addition of ethyl acetate, of the globin. The precipitated globin was then dissolved in formamide and treated with the PFITC reagent at near-neutral pH (at room temperature overnight and then at 45°C for 1 h). The alkylated N-terminal valines, which detached from the globin as PFPTH`s, were separated by ether extraction (Mowrer et al. 1989; Törnqvist, 1994).

Gas chromatography mass spectrometry analysis

The PFPTH derivatives were analysed by using a GC (Varian 3400) coupled to a triple quadrupol MS (Finnigan TSQ700) in the chemical ionisation negative ions mode(NICI) (*i.e.* electron capture ionization). The samples were injected via a septum equipped temperature programmable injector (SPI, Varian) with temperature programme: 1 min at 100°C, 20 °C/min to 240 °C, 10 °C/min to 320 °C, and then 320 °C for 7 min. The column used was a 30 m (0.32 mm i.d., 1.0 μm thickness) fused silica capillary column (DB-5MS, J&W Scientific). The GC carrier gas was helium. The gas in the reaction chamber was methane (ion source temperature 150 °C and ionization energy 70 eV), and the collision gas was argon (collision chamber pressure: 1.06 mTorr). For identification and quantitation of the PFPTH derivatives, MS/MS analysis was performed by single reaction monitoring (SRM), using the product ions m/z=303, 304 and 319 formed from the precursor ion m/z=375, $[M-20]^-$. For calibration and quantification, an acrylamide alkylated reference globin and a synthesised deuterated internal standard (*N*-(2-carbamoylethyl)[$^2H_7$]valine-PFPTH were used. These standard compounds were kindly provided by E. Bergmark (Bergmark et al., 1993).

4.2.3 Examination of health effects

4.2.3.1 Symptoms

Symptoms related to the actual tunnel exposure were recorded for each participant at the initial examination through a detailed questionnaire (Appendix 1). The information obtained here was compared to the corresponding information obtained at the examination one year later.

4.2.3.2 Neurophysiological measurements. Initially, during October - December 1997, all participants underwent neurophysiological measurements (nerve conduction velocity, NCV) at the Neurophysiological Laboratory, Ullevål Hospital, Oslo (Dr. Mona Heier and Hans Sjöholm). Neurography was performed with measurements of motor nerve conduction velocity, amplitude and F-latency in the right median, ulnar, peroneal and posterior tibial nerves.

Sensory nerve conduction velocities and amplitudes were measured in the right median and ulnar nerves with orthodromic stimulation in the palm and recording 7 cm from the stimulation site at the volar side of the wrist. The sensory nerve conduction velocity and the amplitude of the sural nerve were measured with antidromic stimulation approximately 10 cm. proximal to the distal insertion of the Achilles tendon with the recording electrode placed below the lateral malleolus. F-latencies were measured as the shortest latency from 20 successive optimal stimuli. Nerve stimulation and the recording of nerve- and muscle action potentials were performed with bipolar surface electrodes according to established, standardised procedures using a 4 channel «Dantec Keypoint». All subjects participated at both examinations. Owing to technical reasons, some measurements were not successful in a few of the subjects.

4.2.3.3 Visual evoked response (VER)

Visual evoked responses were recorded with pattern reversal stimulation of each eye separately, with the other eye covered. The cortical evoked response was recorded from surface electrodes located at standard EEG-placements (O1, Oz and O2) over the occipital region, with a frontal reference (Fz). Latency to the maximum positive deviation from the baseline (P100) was measured, as well as the latencies to the onset (N75) and end (N120) of this potential.

4.2.3.4 Electroretinography (ERG)

Electroretinography was recorded with a surface electrode at the zygomatic arch just behind the retinal plane with a reference at the lower eye lid in front of the retinal plane. Photoptic stimulation was given as flashes of white light with the frequencies 2 Hz and 30 Hz, with normal indoor light conditions. The amplitudes of the positive B-wave which is generated by the photoreceptor cell of the retina and the adjoining bipolar cells were measured at both frequencies.

4.2.4 GST genotyping of subjects and kinetic characteristics of different GSTs.

All the 25 exposed subjects and 50 referents were genotyped for the deletion polymorphism in GSTM1 and GSTT1 and for the ile/val variation in codon 104 of the GSTP1 gene. DNA were extracted from 100 µl blood and the polymorphic regions were amplified by PCR using

previously described methods (Ryberg et al., 1997; Abdel-Rahman et al., 1996). The PCR products were analysed by capillary electrophoresis using internal standards.

This genotyping was preceded by laboratory studies at our Toxicological Department, where we examined kinetic parameters for glutathion transferase conjugation with different acryl-amide compounds (acrylamide, bis-acrylamide, methylol-acrylamide) and glycidamide. Kinetic parameters were determined for the following recombinant human glutathione S-transferase isoenzymes using acrylamide and several acrylamide-like compounds as substrates: GSTM1-1, GSTA1-1, GSTP1-1 and GSTT1-1. The reaction was initiated by the addition of [^{35}S] GSH (10 mM) and terminated by the removal of enzyme by centrifugation through Centriflex protein-removal cartridges after 1 minute. The supernatants were immediately analysed by high-pressure liquid chromatography (HPLC). The eluted peaks were collected in separate tubes and radioactivity measured in a liquid scintillation analyser. Control incubations without enzyme were run in each experiment. All kinetic data were corrected for the background chemical reaction.

4.2.5 Chromosome studies

Chromosome studies were performed in the 25 exposed workers and a subsample of 25 tunnel workers not exposed to Rhoca-Gil, matched for age, sex and smoking habits. Since it was impossible to collect blood samples from the exposed worker and matched control on the same day, seven additional subjects were recruited from the staff at the NIOH. One of them was available for blood sampling each sampling day in order to control for possible postal and culture factors. They were labelled laboratory controls.

Blood samples of 10 ml heparinized blood were collected from two exposed or control subject together with one laboratory control on the same day and posted for delivery to Telemark Central Hospital the next day. Four whole blood cultures for each subject were cultured for 50-53 hours in Hams F10 medium with foetal calf serum, L-glutamin, gentamycin sulfate and phytohemaglutinin. Colcemide was added to the cultures two hours before hypotonic treatment with 0.75 M HCl and fixation with 3:1 metanol: acetic acid. Slides were stained in Giemsa and chromosome damage was specified and scored in 200 metaphases per person on coded slides.

All subjects answered a questionnaire at the time of the blood sampling on smoking habits, use of mediccation, recent X-rays, allergy and infections, i.e. factors which may influence the results.

All participants signed a declaration of consent to participate in the study.

4.2.6 Statistical methods

McNemar test for two related samples was used to test the difference between reported symptoms (dichotomous variables) during exposure and 16 months post exposure (table 6-9). When comparing the difference between neurographic measures in the exposed group and the reference group, we used t-test for two unrelated samples (table 10,11,14,18-21). In order to test for change in neurographic measures from 16 to 24 months post exposure, we applied the t-test for two related samples (table 12-13). In the further assessment of the exposure-related reversibility of outcomes, Spearman correlation coefficients were estimated in order to test the association between the semi quantitative exposure indices and the change (in %) in the neurographic measurements from four (T_1) to 16 months (T_2) post exposure (T_1 - T_2) (table 15-17). Mann-Whitney's non-parametric ranking test was used for comparison between groups in the chromosome studies. A step-wise linear regression test was also applied. The level of statistical significance was set two-tailed at $p<0.05$. The SPSS Statistical Package was applied on a personal computer.

4.3 RESULTS

4.3.1 Reported work tasks and exposures

All 24 index workers had taken active part in the injection of grouting agents (table 2). Four of the workers had been exposed to NMA-based grouting agents in previous tunnel work. The tunnel workers had worked in Romeriksporten for 19.4 months on average (range 8-24 months). Twenty-one had injected Rhoca-Gil, 19 had mixed Solution 1 and 2, and 22 had taken part in cleaning the equipment. We also asked how many days/shifts they injected Rhoca-Gil, but the majority could not give information at such a detailed level. Seventeen of the 24 workers had done injection work for more than 18 months.

Table 2. Reported work tasks during tunnel grouting (N=24)

	Yes, total		Yes, often	
Work task	n	%	n	%
Injection work	24	100.0	21	87.5
Inj. of acrylamide grout	21	87.5	15	62.5
Mixing Solution 1+2	19	79.2	15	62.5
Cleaning of equipment	22	91.7	19	79.2
Repairing inj.equipment	14	58.4	10	41.7
Sampling	11	45.9	7	29.2
Mining	19	79.2	18	75.0
Drilling	18	75.0	13	54.2
Loading	9	37.5	7	29.2
Other	10	41.7	9	37.5

It can be seen from table 2 that the tunnel workers were involved in a variety of other work tasks in the tunnel. Thus, the work organisation was such that all the workers were expected to be able to perform the various tasks in the tunnel.

Table 3. Reported skin contact with acrylamide grout or contaminated tunnel water

		Yes, total	
Exposure	(N)	Number	%
Skin contact with acrylamide grout	(24)	19	79.2
Skin contact with tunnel water	(23)	23	100.0
Completely wet from tunnel water	(23)	23	100.0

Nineteen of the workers reported direct skin contact with Rhoca Gil, while all workers had experienced skin contact with tunnel water (table 3). In fact, all workers reported that they had been »completely wet» from tunnel water.

Table 4. Reported work tasks involving skin contact with acrylamide grout (N=24)

	Yes, total			Yes, often	
Skin contact when:	n	%		n	%
Work with solution 1	14	58.3		5	20.8
Mixing before injection	16	66.7		7	29.2
Injection	17	70.8		6	25.0
Moving injection pins	15	62.5		4	16.7
Unpolymerised acrylamide grout	11	45.8		2	8.3
Drainage work	2	8.3		0	-

The majority of the workers reported tasks involving skin contact with Rhoca Gil during injection (70.8%)and during mixing before injection (66.7%) or when moving injection pins (62.5%) (table 4).

Table 5. Use of protective clothing/equipment

		Yes, total		Usually		Sometimes		Seldom	
	(N)	n	%	n	%	n	%	n	%
Rain trousers	(22)	18	81.8	11	45.8	5	20.8	1	4.2
Rain jacket	(22)	19	86.4	7	29.2	10	41.7	1	4.2
Gloves	(23)	23	100.0	18	75.0	3	12.5	1	4.2
Changed wet clothes	(24)	10	41.7	1	4.2	3	12.5	5	20.8
Respiratory protection	(24)	12	50.0	1	4.2	4	16.7	6	25.0

All workers used gloves, while only 18 reported regular use (»usually») (table 4). Eleven and seven of the workers «usually» wore rain trousers and rain jackets, respectively. Only one subject "usually" changed wet clothes before the end of the shift, and only one reported regularly use("usually") of respiratory protection.

4.3.2 Hemoglobin adducts to acrylamide

The first blood samples from the 24 workers were taken in October 1997, two months after cessation of the injection work. Most samples were taken later, from 2 to 5 months after the injection work was stopped. The mean value of acrylamide adducts in the exposed group was 0.16 nmol/g hemoglobin (n=24, SD=0.17)) and 0.11 nmol/g hemoglobin (n=3, SD 0.08) among the unexposed referents. Only three workers had adduct levels exceeding 0.20 nmol/g

hemoglobin (0.25, 0.28 and 0.89 nmol/g Hb, respectively). For these three workers, the samples were collected 8-9 weeks after the last use of acrylamide-containing grouts.

Figure 2A shows acrylamide adducts plotted as a function of days from 24th October 1997, the first date of blood sample collection. The 3 measurements marked red are from individuals most likely exposed to acrylamide. In Figure 2B acrylamide adducts are plotted against ethylene oxide adducts together with the regression line for the data shown as filled circles. The three measurements marked red were not included in the regression computation. Since smoking results in acrylamide adducts (Bergmark 1997), the regression line represents an average background of acrylamide adducts due to smoking. Acrylamide adduct level for exposed workers sampled 60 –70 days after cessation of the injection work (N=12) had a mean adduct level of 0.20 (SD=0.23) compared to 0.12 among those sampled later.

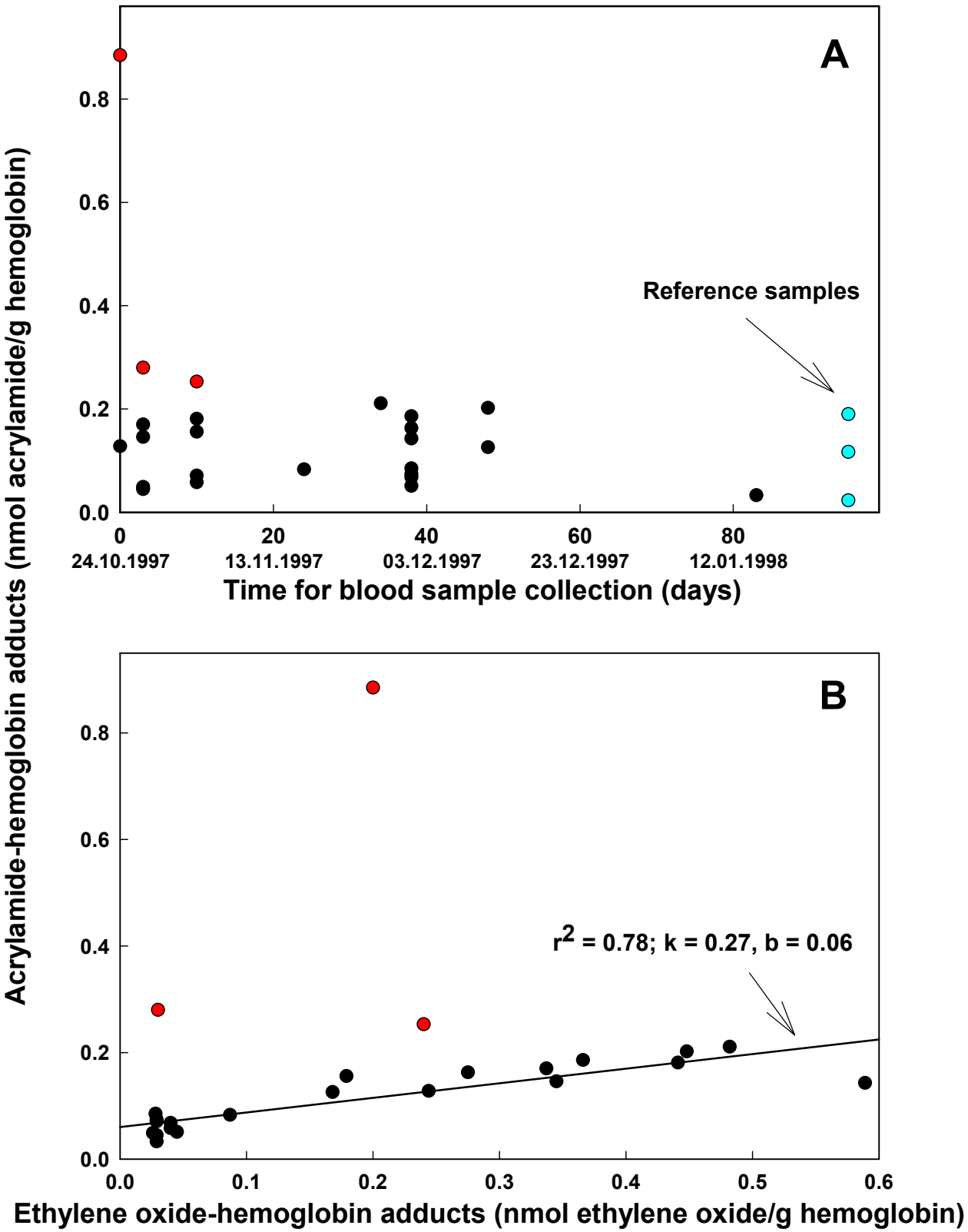

Figure 2. Acrylamide adducts and ethylene oxide adducts in blood samples from exposed operators

4.3.3 Symptoms

We repeated the symptom questionnaire at the 12 months follow-up examination, and were able to compare the reported symptom prevalence during grouting work with the corresponding figures 16 months post exposure. Table 6 shows the reported symptom prevalence related to the peripheral nervous system at the two points in time.

Table 6. Reported symptoms related to the peripheral nervous system (PNS) during grouting work and 16 months post exposure among 24 acrylamide exposed tunnel workers

Symptoms related to PNS	During exposure			16 months post exposure		
	n	No.	%	n	No.	%
Paresthesia in hands	22	9	40.9	24	4	16.7
Pain in hands	21	5	23.8	24	2	8.3
Weakness in hands	24	3	12.5	24	4	16.7
Paresthesia in feet	23	7	30.4	23	5	21.7
Pain in feet	21	4	19.0	24	3	12.5
Cramps in feet	22	4	18,2	24	3	12.5

There was a lower prevalence of reported paresthesia in both hands and feet at the follow-up examination, from 40.9 to 16.7% and from 30.4 to 21.7% respectively. There was also a reduction in the prevalence of pain in hands from 23.8% to 8.3 %. However, none of these differences were statistically significant.

Table 7. Reported symptoms related to the central nervous system (CNS) during grouting work and 16 months post exposure among 24 acrylamide exposed tunnel workers

Symptoms related to CNS	During exposure			16 months post exposure		
	n	No.	%	n	No.	%
Headache	24	11	45.8	23	4	17.4
Nausea/dizziness	24	9	37.5	23	1	4.3*
Concentration problems	23	5	21.7	23	2	8.7
Eye problems when reading	24	6	25.0	24	3	12.5

*p< 0.05

For symptoms related to the central nervous system (CNS) (table 7), there was a statistically significant reduction in the reported prevalence of nausea and dizziness from 37.5% during grouting work to 4.3% 16 months post exposure. There was also a non-significant reduction in the reported prevalence of headache, concentration problems and eye problems when reading.

Table 8. Reported symptoms related to the autonomous nervous system (ANS) during grouting work and 16 months post exposure in 24 acrylamide exposed tunnel workers

Symptoms related to ANS	During exposure			16 months post exposure		
	n	No.	%	n	No.	%
Sweating, hands and feet	22	3	13.6	24	2	8.3
Diarrhoea	23	3	13.0	24	3	12.5
Voiding problems	24	1	4.2	24	0	0.0
Tachycardia	24	1	4.2	24	3	12.5

We also asked questions related to the autonomous nervous system, including sweating, diarrhoea, voiding problems and tachycardia. No statistically significant differences were observed (table 8). Other symptoms are presented in table 9.

Table 9. Other symptoms reported during grouting work and 16 months post exposure in 24 acrylamide exposed tunnel workers

Symptoms	During exposure			16 months post exposure		
	n	No.	%	n	No.	%
Cough	21	8	38.1	24	5	20.8
Throat irritation	22	7	31.8	24	3	12.5
Nose irritation	23	6	26.1	24	3	12.5
Eye irritation	22	4	18.2	24	4	16.7
Wheezing in chest	21	4	19.0	23	4	17.4
Breathlessness		22	6 27.3		23	4 17.4
Eczema, skin irritation	23	8	34.8	24	2	8.3*
Peeling of skin on hands	23	8	34.8	24	4	16.7
Attacs of white fingers	22	4	18.2	24	3	12.5
Attacs of white toes	22	2	9.1	24	0	0.0

*$p < 0.05$

The prevalence of reported symptoms from mucous membranes and upper airways were consistently, but non-significantly reduced at the follow-up examination (cough, throat irritation, nose/eye irritation). As the workers are exposed to many skin and airway irritants during tunnel work, also including other grouting agents, these trends could just as likely be

due to other exposure factors than Rhoca Gil. Skin irritaion is most relevant in acrylamide exposure. This was significantly reduced: from 34.8% during grouting work to 8.3 % at the follow-up examination. Another typical symptom reported among workers with skin contact with acrylamide is peeling of the skin on the hands. The reported prevalence was 34.8% during grouting work, and 16.7% at the follow-up.

4.3.4 Nerve conduction velocities

Nerve conduction velocities (NCV) measured 4 months post exposure compared to corresponding measurements in the reference group are shown in table 10. Statistically significant differences in the measured mean NCV's were observed in the ulnar nerve only. There was a statistically significant lower velocity in the exposed group compared to the controls (52.3 vs. 58.9 m/s, p=0.001), and motor distal delay in the ulnar nerve was significantly prolonged (3.1 vs. 2.5ms, p=0.001).

Table 10. Mean nerve conduction velocities (NCV) in 24 acrylamide exposed workers 4 months after the cessation of exposure compared to 50 referents.

	Exposed (n=24)		Referents (n=50)		
	Mean	SD	Mean	SD	p-value
Median nerve					
Motor NCV (m/s)	54.7	4,1	55.7[c]	5.9	0.46
Sensory NCV (m/s)	53.5	5.5	53.6	8.3	0.94
Distal delay (ms)	3.8	0.8	3,8	0.7	0.91
F-response (ms)	24.5	1.8	24.5	2.9	0.98
Ulnar nerve					
Motor NCV (m/s)	57.9	6.1	55.9	6.8	0.23
Sensory NCV (m/s)	52.3[a]	5.8	58.9[d]	8.5	0.001
Distal delay (ms)	3.1	0.7	2.5	0.4	0.001
F-response (ms)	26.0	2.2	25.5[e]	2.4	0.42
Sural nerve					
Sensory NCV (m/s)	47.0[b]	6.7	48.3[e]	5.8	0.41
Tibial nerve					
Motor NCV	44.2	4.3	43.2	4.7	0.37
F-response (ms)	50.3[a]	6.2	50.4[e]	6.6	0.97
Peroneal nerve					
Motor NCV (m/s)	46.5	4.7	45.8[e]	4.4	0.50
F-response (ms)	48.1[a]	5.3	49.8[f]	5.3	0.21

[a]n=23 [b]n=21 [c]n=48 [d]n=46 [e]n=49 [f]n=44

The results from the corresponding amplitudes showed reductions in the sensory amplitude of the median nerve (p=0.01) in the exposed, while there was a significantly elevated amplitude in the motor amplitude of the median nerve compared to the referents (table 11). Sensory amplitudes were not measurable in three exposed subjects and two referent subjects, thus scored as zero. Standard deviations were large.

Table 11. Mean amplitudes in 24 acrylamide exposed workers 4 months after the cessation of exposure compared to 50 referents.

	Exposed		Referents		
Amplitudes	Mean	SD	Mean	SD	p-value
Median nerve					
Motor (mV)	9.1	3.0	6.7	3.2	0.004
Sensory (μV)	16.9	11.5	31.2	36.3	0.01
Ulnar nerve					
Motor (mV)	7.9	3.7	6.5	2.0	0.09
Sensory (μV)	9.7[a]	6.9	13.1[c]	11.7	0.19
Sural nerve					
Sensory (μV)	12.0[b]	9.6	8.1 [a]	5.7	0.07
Tibial nerve					
Motor (mV)	4.3	2.6	4.4	2.2	0.83
Peroneus					
Motor (mV)	3.5	1.7	3.6	2.1	0.98

[a] not elicited in one subject [b] not elicited in 3 subjects [c] not elicited in 2 subjects

When comparing the neurographic results from 4 months post exposure with the corresponding measurements one year later (table 12) there was a statistically significant improvement in mean sensory NCV in the ulnar nerve from 52.9 m/s to 57.5 m/s (p=0.01), with a statistically significant reduction in distal delay in the same nerve. In addition, a reduction in NCV was seen for the sensory NCV in the sural nerve (p=0.045), and also a prolonged F-response in the tibial nerve (p=0.01).

Table 12. Comparison of mean nerve conduction velocities 4 and 16 months after the cessation of acrylamide exposure among 24 tunnel workers

	4 months post exposure		16 months post exposure		
	Mean	SD	Mean	SD	p-value[1]
Median nerve					
Motor NCV (m/s)	54.7	4.1	54.5	6.3	0.92
Sensory NCV (m/s) (n=23)	53.4	5.5	52.5	7.0	0.47
Distal delay (ms)	3.8	0.8	3.7	0.6	0.11
F-response (ms)	24.5	1.8	25.0	2.0	0.10
Ulnar nerve					
Motor NCV (m/s)	57.9	6.1	58.1	7.7	0.90
Sensory NCV (m/s) (n=22)	52.9	5.3	57.5	7.4	0.02
Distal delay (ms)	3.1	0.7	2.7	0.5	0.001
F-response (ms)	26.0	2.2	26.5	2.0	0.19
Sural nerve					
Sensory NCV (m/s) (n=20)	47.6	6.4	45.1	3.5	0.05
Tibial nerve					
Motor NCV	44.2	4.3	45.5	5.1	0.32
F-response (ms) (n=23)	50.3	6.2	51.9	5.7	0.01
Peroneal nerve					
Motor NCV (m/s)	46.5	4.7	46.4	4.8	0.91
F-response (ms) (n=23)	48.1	5.3	48.3	6.8	0.75

[1]Paired samples T-test. Paired differences.

Table 13. Comparison of mean sensory (uV) and motor (mV) amplitudes among 24 acrylamide exposed workers 4 and 16 months after cessation of exposure.

		4 months post exposure		16 months post exposure		
Amplitudes	No of pairs	Mean	SD	Mean	SD	p-value
Median nerve						
Motor (mV)	24	9.1	3.0	8.4	3.5	0.36
Sensory (μV)	24	16.9	11.5	35.5	35.1	0.01
Ulnar nerve						
Motor (mV)	24	7.9	3.7	5.7	1.9	0.01
Sensory (μV)	24	9.7	6.9	9.5	7.7	0.94
Sural nerve						
Sensory (μV)	24	12.0	9.6	7.2	5.3	0.02
Tibial nerve						
Motor (mV)	24	4.3	2.6	4.3	2.1	0.94
Peroneus						
Motor (mV)	24	3.5	1.7	3.0	1.6	0.15

When amplitudes from four and 16 months post exposure were compared, the sensory amplitude in the median nerve was significantly improved after one year. However, a significant reduction of the sensory amplitude in the sural nerve and motor amplitude of the ulnar nerve was also observed.

When the exposed workers were divided according to exposure time into a long time exposed group (> 18months of injection work, n=17) and a short time exposed group (<18 months of

injection work, n=7), an exposure response pattern was observed with significant differences between the high exposed group and the references both for sensory NCV and distal delay in the ulnar nerve 4 months post exposure (table 14). One year later, the measurements in the exposed group approached the reference values, but only partially for distal delay.

Table 14. Sensory NCV and distal delay in the ulnar nerve in 17 long time exposed workers (≥18 months of injection) and 7 short time exposed workers (<18 months of injection) 4 and 16 months after the cessation of exposure compared to 50 referents.

	4 months post exposure	16 months post exposure
	Mean	Mean
Ulnar nerve sensory NCV (m/s)		
High exposed (n=17)	51.8**	58.2
Low exposed (n=7)	53.4	55.0
Referents (n=46)	58.9	
Ulnar nerve distal delay (ms)		
High exposed (n=17)	3.14* *	2.70
Low exposed (n=7)	2.99*	2.64
Referents (n=50)	2.55	

*p<0.05, **p<0.01, compared to referents

We have further examined the correlation between nerve conduction velocities measured 4 months post exposure, and the qualitative exposure indices (tunnel time, injection time, exposure time index, intensity index and (injection time x intensity index)). For velocities (measured in m/s), a negative correlation indicates an exposure-related reduction in NCV. For distal delay and F-responses (measured in ms), a positive correlation indicates a prolonged response. These results are shown in table 15.

Table 15. Correlation (Spearman correlation coefficients) between indices of acrylamide exposure and neurographical measures 4 months post exposure (T_1) in 24 tunnel workers

	Tunnel time	Injection time	Exposure time index	Intensity index	Inj.time x intensity index
Median nerve					
Motor NCV (m/s)	-0.28	-0.16	-0.20	-0.09	-0.21
Sensory NCV (m/s)	0.18	-0.02	-0.01	-0.003	0.004
Distal delay (ms)	-0.10	-0.11	-0.09	-0.03	-0.01
F-response (ms)	-0.21	0.08	0.10	0.11	0.13
Ulnar nerve					
Motor NCV (m/s)	-0.07	-0.19	-0.19	0.004	-0.10
Sensory NCV (m/s)	-0.21	-0.19	-0.18	0.06	-0.08
Distal delay (ms)	-0.02	-0.11	-0.09	-0.19	-0.14
F-response (ms)	-0.05	0.18	0.20	0.28	0.29
Sural nerve					
Sensory NCV (m/s)	-0.14	-0.33	-0.35	-0.22	-0.31
Tibial nerve					
Motor NCV (m/s)					

NCV: Nerve conduction velocity

The correlation coefficients were low for all measures, none were statistically significant.

To assess the possible exposure-related reversibility of NCV, we have examined the correlation between indices of acrylamide exposure and the change (in %) in neurographical measurements between 4 and 16 months post exposure (T_1 - T_2). These are shown in table 16.

If a nerve conduction velocity, measured in meters/second, was reduced at 4 months post exposure (T_1) and then returned to normal after one year (T_2), the T_1 - T_2 difference will be negative. The more negative it is, the more improvement there is. If this improvement is related to the level of exposure, a negative correlation coefficient will indicate an exposure-related improvement during that year. Correspondingly, for distal delay and F-responses, which are measured in milliseconds, an improvement in the time period T_1 to T_2 will result in a positive T_1 - T_2 difference, and a positive correlation coefficient will indicate exposure related improvement from 4 to 16 months post exposure.

Statistically significant negative correlation coefficients in the range of 0.45 – 0.54 were observed between the change in both motor and sensory nerve conduction in the arms and several of the surrogate variables for exposure level (tunnel time, injection time, exposure time index and (injection time x intensity index)). This indicates that those with the highest exposures had the largest improvement in NCV from 4 to 16 months post-exposure.

Correspondingly, the F-responses in the median and the ulnar nerve show significant positive correlations for the same exposure indices, indicating an improvement in this sensitive measure of peripheral nerve conduction. No statistically significant correlations were observed for nerves in the lower extremities. It can further be seen that the time-related exposure indices (tunnel time, injection time, injection time index) are those with the highest correlation coefficients, while the correlations with the questionnaire-based intensity index were very low.

Table 16. Correlation (Spearman's rank correlation coefficients) between indices of acrylamide exposure and the improvement (in %) in neurographical measures from 4 to 16 months post exposure.

		Improvement (in %)		Tunnel time	Injection time	Exposure time index	Intensity index
	N	Mean	SD				
Median nerve							
Motor NCV (m/s)	24	0.3	14.2	0.45*	0.35	0.39	0.33
Sensory NCV (m/s)	23	-1.5	10.4	0.49*	0.54**	0.54**	0.08
Distal delay (ms)	24	-2.8	11.4	0.02	-0.08	-0.06	0.23
F-response (ms)	24	2.1	5.8	0.22	0.53**	0.53**	0.16
Ulnar nerve							
Motor NCV (m/s)	24	0.7	11.6	0.13	0.36	0.36	-0.14
Sensory NCV (m/s)	22	9.5	15.9	0.54*	0.53*	0.53*	-0.04
Distal delay (ms)	24	-11.7	14.7	0.03	-0.08	-0.06	-0.13
F-response (ms)	24	2.7	9.1	0.40	0.48*	0.50*	0.34
Sural nerve							
Sensory NCV (m/s)	20	-4.1	9.5	0.05	0.33	0.32	0.26
Tibial nerve							
Motor NCV (m/s)	24	3.4	13.1	0.29	0.10	0.12	0.31
Peroneal nerve							
Motor NCV (m/s)	24	0.1	8.8	0.06	0.19	0.19	0.22
F-response (ms)	23	2.1	5.8	-0.01	0.22	0.19	0.01

NCV: Nerve conduction velocity * $p<0.05$ ** $p<0.01$

From table 16 it can also be seen that the mean change in measured values (in %) from time T_1 to T_2 goes in both directions, which corresponds with the results in table 12. For the F-responses there are significant positive correlation coefficients with exposure, indicating an improvement after one year in those with the highest exposures, even if the mean difference is slightly in the opposite direction.

Table 17. Correlation (Spearman's rank correlation coefficients) between indices of acrylamide exposure and the change (in %) in amplitudes between 4 and 16 months post exposure (T_1 - T_2). (a negative correlation indicates improvement from T_1 to T_2)

Amplitudes	N	T_1 - T_2 (in %) Mean	SD	Tunnel time	Injection time	Exposure Time index	Intensity index	Inj.time x intensity index
Median nerve								
Motor (mV)	24	-1.2	45.9	-0.47*	-0.38	-0.40	-0.06	-0.31
Sensory (μV)	24	-139.0	186.5	0.18	0.11	0.14	0.30	0.27
Ulnar nerve								
Motor (mV)	24	17.6	36.0	0.04	-0.02	-0.003	0.01	0.02
Sensory (μV)	23	-18.2	105.1	-0.31	-0.39	-0.38	-0.10	-0.21
Sural nerve								
Sensory (μV)	21	39.9	42.2	0.14	-0.09	-0.08	0.12	0.05
Tibial nerve								
Motor (mV)	24	-40.5	147.5	-0.13	-0.02	-0.06	-0.41*	-0.34
Peroneal nerve								
Motor (mV)	24	-0.85	63.4	0.05	0.11	0.09	0.10	0.12

The corresponding amplitudes are shown in table 17. There are relatively large mean differences between the two measurements, with corresponding large confidence intervals and correlation coefficients which are both negative and positive. The only statistically significant correlations were observed for the median motor nerve (tunnel time) and tibial motor nerve (intensity index), indicating exposure-related improvements in the amplitude response after one year.

4.3.5 Visual evoked response and electroretinography

At the examination 16 months post exposure, visual evoked responses (VEP) and electroretinography (ERG) were measured in all 24 workers, and compared to the 50 unexposed tunnel workers. The measured P100 VEP latencies and ERG amplitudes at 2 and 30Hz stimulation for both eyes are shown in table 18.

Table 18. Mean visual evoked response (VEP) latency and electroretinography (ERG) amplitude in 24 workers 16 months after cessation of acrylamide exposure, compared to 50 referents

	Exposed			Referents			
	Mean	N	SD	Mean	N	SD	p-value
Visual evoked response							
P 100 right (ms)	105.3	24	11.1	102.7	50	7.9	0.26
P 100 left (ms)	105.1	24	11.6	101.9	50	6.9	0.14
Electroretinography							
2 Hz right (uV)	12.4	22	3.2	13.4	49	4.9	0.32
2 Hz left (uV)	11.8	22	3.5	13.4	49	6.2	0.18
30 Hz right (uV)	7.3	22	2.6	9.5	48	4.7	0.02
30 Hz left (uV)	7.4	22	2.6	9.7	49	4.8	0.04

For the P100, the latency is slightly longer in among the exposed subjects, however, it is not statistically significant. The amplitudes measured in the ERG were also lower in the exposed subjects than in the referents. The differences with the 30 Hz stimulation frequency were statistically significant for both eyes.

4.3.6 Glutathion S-transferase genotypes.

Prior to the susceptibility study the kinetic parameters of glutatione S-transfereases were determined in vitro using recombinant enzymes (see Methods). The uncatalyzed reaction between the metabolite glycidamide and glutathione was very high, which made the determination of the parameters impossible. For acrylamide the specific enzyme activity was highest for GSTA1-1 (5.3 μmol/min/mg), followed by T1-1 (4.6 μmol/min/mg), M1-1 (1.3 μmol/min/mg) and P1-1 (1.1 μmol/min/mg). The GSTT1-1 enzyme had the highest affinity for acrylamide (Km = 5.4 mM) and the other GSTs about 30% lower (Km 30% higher).
The gluthathion transferase gene for the M1 and T1 enzyme were genotyped in all 25 exposed subjects. In the general Norwegian population, 50% lack M1 and 15% lack the T1. In this small sample, 12 of the subjects were M1- and only 4 were T1-. All T1- subjects were also M1-
.

The results from selected nerve conduction velocity measurements, stratified for Glutathion S transferase (GST) genotypes M1± are shown in table 19.

Table 19. Neurographical results in 24 acrylamide exposed workers 4 months after cessation of exposure, stratified for Glutathion S transferase genotype M1±.

	Exposed M1- Mean (n=12)	SD	Exposed M1+ Mean (n=12)	SD	p-value
Median nerve					
Motor NCV (m/s)	55.5	5.0	53.8	3.1	0.33
Sensory NCV (m/s)	54.4	6.3	52.6	4.5	0.44
Ulnar nerve					
Motor NCV (m/s)	57.6	6.2	58.2	6.2	0.83
Sensory NCV (m/s)	52.9[a]	6.7	51.8	5.0	0.66

[a]n=11

On the group level, no differences in the nerve conduction velocities, as measured 4 months post exposure, were observed when the 12 exposed tunnel workers with genotype M1- were compared to the 12 exposed workers with genotype M1+. Neither were any differences observed for the T genotypes (not presented, owing to the small over all figures, together with the skewed distribution of the T genotypes).

We have also compared the change in selected neurographical measures between 4 and 16 months post exposure for the M1- and M1+ subjects respectively. No statistically significant differences were observed between the two groups (not shown).

The results from the visual evoked response (VEP) and electroretinography (ERG) measurements, stratified for Glutathion S transferase (GST) genotypes M1± are shown in table 20.

The P100 were slightly longer among the M1+ subjects, but no differences between the groups, neither in VEP or ERG were statistically significant. The corresponding results for GST genotypes T1± are shown in table 21.

Table 20. Visual evoked response (VEP) and electroretinography (ERG) in 24 workers 16 months after the cessation of acrylamide exposure, stratified for GST - M genotype (M1±)

	Exposed M1- Mean (n=12)	SD	Exposed M1+ Mean (n=12)	SD	p-value
Visual evoked response					
P 100 right (ms)	102.7	6.1	107.8	14.4	0.28
P 100 left (ms)	102.3	8.0	108.0	14.1	0.23
Electroretinography					
2 Hz right	12.9	3.2	11.8[a]	3.2	0.42
2 Hz left	12.5	4.4	11.2	2.3	0.35
30 Hz right	7.6	2.7	7.0[a]	2.7	0.61
30 Hz left	7.2	2.5	7.7[a]	2.8	0.68

[a]n=10

Table 21. Visual evoked response (VEP) and electroretinography (ERG) in 24 workers 16 months after the cessation of acrylamide exposure, stratified for GST - T genotype (T1±)

	Exposed T1- Mean (n=4)	SD	Exposed T1+ Mean (n=20)	SD	p-value
Visual evoked response					
P 100 right (ms)	100.6	4.4	106.2	11.9	0.37
P 100 left (ms)	98.2	3.2	106.5	12.2	0.19
Electroretinography					
2 Hz right	13.0	0.8	12.3[a]	3.5	0.46
2 Hz left	16.1	3.5	11. 0	2.9	0.01
30 Hz right	9.4	1.8	6.9[a]	2.6	0.08
30 Hz left	8.7	1.7	7.1[a]	2.7	0.27

[a]n=18

Reduced amplitudes were observed in the ERG in the T1+ subjects compared to T1- subjects for 2 Hz, left eye, and for 30 Hz, right eye respectively. The figures are small, however, particularly in the T1- stratum.

4.3.7 Chromosome studies

Chromosome examinations were performed for all the 25 exposed subjects, 25 age and smoking matched controls (unexposed tunnel workers) and seven laboratory controls. The chromosome results which are reported are the number of cells with aberrations (excluding gaps), the number of chromatid breaks, the number of chromosome breaks, the number of chromatid gaps and the number of chromosome gaps, all as group means per 200 cells. Comparisons between all the exposed subjects and the control workers and laboratory controls are shown in table 22. Seven laboratory controls were sampled altogether 31 times and the mean values are reported. There were no striking differences between the groups. Only the number of chromatid gaps was significantly different between the exposed subjects and the matched controls.

Table 22. Chromosomal aberrations for all the exposed workers with the controls and the laboratory controls. Mean number per 200 cells with standard deviation in brackets.

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid gaps	Number of chromosome gaps
Exposed	25	2,9 (1,7)	1,4 (1,1)	1,2 (1,1)	10,6 (5,6)[b]	1,5 (1,5)
Controls	25	2,4 (1,6)	0,9 (1,2)	0,9 (0,9)	6,4 (4,4)	1,4 (1,7)
Lab. Controls	31[c]	2,7 (1,4)	1,2 (1,1)	1,3 (1,1)	8,6 (4,7)	1,6 (1,2)

a Excluding gaps.
b Mann-Whitney's non- parametric test between exposed and control. p = 0,004 (2 tailed).
c Seven persons sampled repeatedly, 31 times in all, as a control of the method.

In order to look for a possible skewed distribution of aberrations between the groups we compared the number of persons with aberrations ≥ 3, as shown in table 23.

Table 23. Number of exposed workers compared to laboratory controls sampled at the same time, and number of controls compared to laboratory controls sampled at the same time with scores ≥3 for three groups of chromosomal aberrations.

	Number of cells with aberrations [a] N	Number of chromatid breaks N	Number of chromosome breaks N
Exposed workers	14	4	2
Lab. Controls	8	3	3
Controls	9	3	1
Lab. Controls	8	1	2

a Excluding gaps

A slightly higher number of exposed persons was found with the number of cells with aberrations above the cut off point. As the controls and laboratory controls show similar results, we are fairly confident that we have no differences due to methodological factors. In the following, therefore, only comparisons between exposed workers and matched controls are shown.

Eighteen workers were exposed to more than 500 days with injection of Rhoca-Gil. We compared these workers to their matched controls and to the seven workers with lower exposure, as shown in table 24.

Table 24. Chromosomal aberrations in the long time exposed workers (≥ 18 months of injection work) and short time exposed workers (<18 months of injection work) and matched control subjects. Mean number per 200 cells with SD in brackets.

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid gaps	Number of chromosome gaps
Long time exposed	18	2,9 (1,7)	1,3 (1,0)	1,1 (1,1)	10,5 (6,0)[b]	1,2 (1,4)
Matched controls	18	2,9 (1,7)	1,2 (1,3)	1,1 (1,0)	6,5 (5,0)	1,7 (1,9)
Short time exposed	7	2,9 (1,7)[b]	1,6 (1,4)	1,3 (1,1)	10,7 (4,7)[b]	2,1 (1,8)
Matched controls	7	1,3 (0,5)	0,3 (0,5)	0,4 (0,5)	6,0 (2,8)	0,9 (0,7)

a Excluding gaps
b Mann-Whitney's non-parametric test between exposed and control: p= 0,05 (2 tailed)

There were no differences between the high and low exposed workers. Only for chromatid gaps there was a significant difference between the workers and their matched controls. All the exposed workers are treated as one group in the following.

Comparing exposed smokers and non-smokers to their respective controls (table 25) did not reveal any significant differences except for chromatid gaps.

Table 25. Chromosomal aberrations for exposed workers and controls grouped as smokers and non-smokers. Mean numbers per 200 cells with SD in brackets.

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid gaps	Number of chromosome gaps
Exposed smokers	12	2,5 (1,7)	1,2 (1,1)	0,8 (0,9)	10,2 (6,5)	1,3 (1,2)
Control smokers	14	2,6 (1,6)	0,8 (1,0)	0,9 (0,9)	6,8 (4,0)	1,7 (2,2)
Exposed non – smokers	13	3,3 (1,7)	1,5 (1,1)	1,5 (1,2)	10,9 (4,8)[b]	1,6 (1,8)
Control non – smokers	11	2,2 (1,6)	1,1 (1,4)	0,9 (1,0)	5,8 (5,1)	1,1 (0,7)

a Excluding gaps
b Mann-Whitney's non-parametric test between exposed workers and controls: p= 0,007 (2 tailed)

We also grouped the exposed workers according to smoking habits and GSTM 1+ and GSTM 1- (table 26). The number of chromatid gaps was only significantly different for the group of workers with GSTM1- compared to their GSTM1- controls As shown in the table this was mainly due to the difference found between the exposed non-smokers and their non-smoking controls.

Table 26. Chromosomal aberrations in exposed workers and controls grouped according to genotype GSTM1 ± and smoking. Mean numbers per 200 cells with SD in brackets.

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid gaps	Number of chromosome gaps
Exposed workers GSTM1 -	13	2,7 (1,5)	1,5 (1,1)	1,0 (0,9)	12,2 (6,9)[b]	1,6 (1,9)
Controls GSTM1 -	15	2,3 (1,6)	1,0 (1,2)	0,7 (0,7)	6,5 (6,5)	1,2 (1,2)
Exposed workers GSTM1 +	12	3,2 (1,9)	1,3 (1,2)	1,3 (1,3)	8,8 (2,9)	1,3 (1,1)
Controls GSTM1 +	8	3,1 (1,6)	1,0 (1,2)	1,5 (1,1)	6,3 (5,5)	2,0 (2,8)
Exposed smokers GSTM1 -	6	2,0 (1,4)	1,2 (0,8)	0,5 (0,5)	13,0 (8,3)	1,0 (1,3)
Control smokers GSTM1 -	7	2,6 (1,7)	1,0 (1,0)	0,7 (0,8)	8,7 (3,9)	1,0 (1,0)
Exposed smokers GSTM1 +	6	3,0 (1,9)	1,2 (1,5)	1,2 (1,2)	7,3 (2,5)	1,7 (1,2)
Control smokers GSTM1 +	5	3,4 (1,3)	0,8 (1,1)	1,4 (0,9)	4,6 (3,8)	3,0 (3,2)
GSTM1 – exposed non – smokers	7	3,3 (1,4)	1,7 (1,3)	1,4 (1,0)	11,6 (6,1)[b]	2,1 (2,2)
GSTM1 - control non – smokers	8	2,0 (1,5)	1,0 (1,4)	0,6 (0,7)	4,6 (3,7)	1,4 (0,5)
GSTM1 + exposed non – smokers	6	3,3 (2,1)	1,3 (1,0)	1,5 (1,5)	10,2 (2,8)	1,0 (0,9)
GSTM1 + control non – smokers	3	2,7 (2,1)	1,3 (1,5)	1,7 (1,5)	9,0 (7,8)	0,3 (0,6)

a Excluding gaps
b Mann-Whitney's non-parametric test between exposed and control: p= 0,02 (2 tailed)

The corresponding results for GST1± are shown in Table 27. We did not stratify the participants according to smoking habits for genotype GSTT1, however, as the number of persons was so small.

Table 27. Chromosomal aberrations in exposed workers and controls grouped according to Genotype GSTT1 ±. Mean numbers per 200 cells with SD in brackets

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid Gaps	Number of chromosome gaps
Exposed workers GSTT1-	4	3,8 (1,3)	1,8 (1,3)	1,8 (1,0)	16,3 (2,2)	2,3 (2,6)
Controls GSTT1-	5	2,0 (1,7)	0,8 (1,3)	0,8 (1,1)	9,6 (5,9)	1,0 (1,2)
Exposed workers GSTT1+	21	2,8 (1,7)	1,3 (1,1)	1,1 (1,1)	9,5 (4,1)[b]	1,3 (1,2)
Controls GSTT1+	18	2,7 (1,6)	1,1 (1,2)	1,0 (0,9)	5,6 (4,0)	1,6 (1,9)

a Excluding gaps
b Mann-Whitney's non-parametric test between exposed and control: p= 0,005 (2 tailed)

From table 27 it can be seen that the highest frequencies for all effect parameters are found for the exposed workers with GSTT1- compared to the matched controls even if the differences are not significant. The same trend is observed in Table 28 where the participants are grouped according to both GSTT1 and GSTM1.

Table 28. Chromosomal aberrations in exposed workers and controls grouped according to the combination of genotypes GSTM1 ± and GSTT1 ±. Mean numbers per 200 cells with SD in brackets.

	N	Number of cells with aberrations [a]	Number of chromatid breaks	Number of chromosome breaks	Number of chromatid gaps	Number of chromosome gaps
Exposed workers GSTM1- GSTT1 -	4	3,8 (1,3)	1,8 (1,3)	1,8 (1,0)	16,3 (9,2)	2,3 (2,6)
Controls GSTM1- GSTT1 -	3	1,3 (0,8)	0,3 (0,6)	0,7 (1,2)	8,7 (4,2)	1,7 (1,2)
Exposed workers GSTMI- GSTTI+	9	2,2 (1,4)	1,3 (1,0)	0,7 (0,7)	10,4 (5,3)[b]	1,3 (1,5)
Controls GSTM1- GSTT1+	12	2,5 (1,7)	1,2 (1,3)	0,7 (0,7)	6,0 (4,3)	1,1 (0,7)
Exposed workers GSTM1+ GSTT1+	12	3,2 (1,9)	1,3 (1,2)	1,3 (1,3)	8,8 (2,9)[b]	1,3 (1,1)
Controls GSTM1+ GSTT1+	6	3,2 (1,3)	0,8 (1,0)	1,7 (1,0)	4,7 (3,4)	2,7 (3,0)

a Excluding gaps
b Mann-Whitney's non- parametric test between exposed and control: p= 0,07 and 0,02 (2 tailed)
No exposed workers with GSTM1+ and GSTT1 -.

Owing to the small numbers in the groups there are no significant differences between GSTM1-/GSTT1- workers and controls, but the trend is similar for all the effect parameters studied. The only significant differences are found in the number of chromatid gaps.

Stepwise linear regression analysis was performed with the parameters exposed/controls, smoker/non-smoker, use of medication (yes/no), GSTM1+/GSTM1-, GSTT1+/GSTT1- in the model. Exposure and genotype GSTT1 came out as significant for the number of chromatid gaps only with $p=0.002$ and $p=0.004$ respectively. More controls than exposed persons used medication regularly, but for both groups the people using medicine had lower frequencies of all types of chromosomal aberrations than the people not using medication.

The other variables asked for in the questionnaire such as X-ray exposure, infections and allergy, could not be included in the analysis because of too many missing values. However, the missing values were equaly distributed when comparing the exposed workers and the controls. Two persons in each group reported allergy problems. One had a high number of aberrations and the other had low number of aberrations.

4.4. Discussion

The results from this study indicate slight effects on the nervous system related to exposure to N-methylolacrylamide and acrylamide in 24 tunnel workers during grouting operations in tunnel work. Based on examinations and measurements performed 4 and 16 months after the cessation of the most relevant exposure, the effects seemed largely to be reversible, with normalisation after one year. Indications of a possible cytogenetic effect, expressed as an increased number of chromatide gaps among the exposed workers was also observed. However, several aspects related to the design, the exposure conditions, and the specific results of this study need to be further discussed.

4.4.1 Validity aspects

The study was performed as two cross-sectional examinations of those 25 exposed tunnel workers judged to be among the highest exposed from a study base of 73 eligible tunnel workers who participated in the OHS survey of the construction company. The participation in the OHS survey was complete for all relevantly exposed subjects, independent of symptom status. However, the selection of the 25 subjects for the follow-up study was performed after the OHS survey was initiated, and was thus particularly vulnerable to outcome dependent selection. The use of a thorough selection procedure, based on detailed information from the company, the workers and their representatives, and interviews and questionnaires from the OHS survey makes us fairly convinced that the subject selection was based on exposure criteria, exclusively, and not on subjective symptoms or other effects. Three workers who did not fullfill the exposure criteria were also examined with the same methods as the index group at their request, but they were not included in the epidemiological study. Subjects with known neurological disease, diabetes mellitus, or alcoholism were not eligible for the study. However, one subject who during the examinations was discovered to have diabetes, was excluded from most analyses, but was maintained in the genotoxic part of the study.

Several procedures were chosen in order to establish relevant contrasts in the study of exposure-outcome relationships. One was the establishment of an external reference group. Another was the performance of internal comparisons within the exposed group related to surrogates of exposure level. For the study of the possible reversibility we measured the same response in each individual at two different points in time.

The optimal reference group is comparable to the index group, except for the actual exposure under study. Other tunnel workers, who did not perform injection work with NMA-containing grouts would fullfill these requirements. Thus, the 50 reference tunnel workers selected for the other study on possible late effects of NMA-exposure (Goffeng et al., 2000) were also used as referents in the neurophysiological part of the study. For the chromosome studies, a subsample of 25 unexposed reference subjects was selected, matched on age (+/- 3 years) and smoking status (present/former/never). The matching criteria were not so strict for two subjects, but we believe that this does not influence the comparability between the groups.

Tunnel workers are exposed to a variety of exposure factors, which could in part confound the examined effects. Thus, exposure to other neurotoxic agents, such as organic solvents, lead or other metals, together with vibration exposure could have influenced the observed effects. In addition, their exposure to other factor related to life style, such as smoking, might also be different from the general population (Ulvestad et al., 2000). The distribution of these factors was comparable between the groups (table 1). Thus, we believe that the reference group secured the necessary comparability between the groups.

Owing to previous reports on the potential reversibility of acrylamide-related effects, the second examination was performed 12 months after the first examination. In these analyses, intra- individual comparisons were performed, thus avoiding between-group confounding. All subjects participated at both examinations. However, owing to technical reasons, some measurements were not successful in a few of the subjects.

4.4.2 Exposure assessment

When evaluating both the positive and non-positive results from this study, a crucial issue is to assess the «dose», i.e. the time-related concentrations of NMA and acrylamide monomer that the workers were exposed to. However, no reliable quantitative measurements of exposure were performed in this study. Thus, no information on such quantitative dose-response relationships can be given. Firstly, no measurements of acrylamide or NMA in the working atmosphere were performed. In fact, the workers themselves were not informed that the grout contained acrylamide when performing the injection work, due to incomplete material safety data sheets.

The concentrations of acrylamide and NMA in tunnel water immediately after the injection was stopped could provide a crude indicator for recent exposure, not only during injection work, but also during other tasks. Thus, all workers reported contact with tunnel water, and that they were completely wet from tunnel water. The observation of very high concentrations of acrylamide (90.6 mg/L) in water dripping from bore holes of unpolymerised grouting agent in January 1998 indicates the possibility of relevant exposure also after the injection period. However, it is believed that the exposure after the cessation of the injection work was radically reduced for most workers. Another rather unexpectedly source of exposure was reported by some of the workers. It was a common assumption that tunnel water was pure, and consequently the workers used this water for making their coffee during breaks inside the tunnel. To what degree this was common practice is unclear, but it is not believed that this oral intake of potentially polluted water contributed much to their cumulative exposure.

Hemoglobin (Hb) adducts of acrylamide in blood will give a valid and sensitive estimate of the average exposure during the preceding months (Bergmark, 1997, Törnqvist et al., 1994, Hagmar et al., 2001). This critical time window of up to 4 months post exposure is related to the about 120 days average life span of the erythrocytes. The first blood samples in the 25 workers were taken in October 1997, two months after cessation of the injection work. Most samples were taken later, from 2 to 5 months after the injection work was stopped. Due to the uncertainty with regard to the possible exposure to acrylamide in tunnel water after the grouting work came to a halt, we still chose to analyse all the samples. When adjusted for the levels of acrylamide that can be attributed to smoking, only 3 subjects had elevated adduct values, of 0.25, 0.28 and 0.89 nmol/g Hb, respectively. All these samples were taken in October.

Bergmark has recently shown that acrylamide adducts are associated with smoking (Bergmark 1997) and ethylene oxide has also been shown to be associated with smoking (Törnqvist et al., 1986). When “adjusting” for the potential effect of smoking on the acrylamide adduct levels, by the use of corresponding etylene oxide adducts (Figure 4), only 3 subjects had “unexplained” elevated acrylamide adduct levels. It is therefore reasonable to suggest that the 3 elevated measuremets are related to occupational exposure. An alternative, but less likely explanation would be related to diet, which recently has been in focus as a potential source for acrylamide exposure (Tareke et al., 2002). We have no information on diet habits among the participants,

however. The observation of generally low adduct levels in the samples taken more than 70 days after cessation of the injection work, strengthen the suggestion that the tunnel water was not an important source of exposure after the injection work was stopped.

It is also of interest to compare the exposure conditions in the Swedish and Norwegian tunnel projects. In Hallandsåsen in Sweden, about 1500 tons of the NMA grout was used during 6-8 weeks, while in Romeriksporten, 340 tons were used during a two-year period. In the 210 Swedish workers, 36 had Hb adduct values between 0.30-1.00 nmol/g Hb, and 38 had >1.00 nmol/g Hb, with the highest value measured to 17.7 nmol/g Hb (Hagmar et al., 2001). Their blood samples were taken 1 to 5 weeks after the cessation of exposure.

Assuming that exposure had been going on for four months or more, i.e. with a steady-state Hb adduct level been established, the Hb adduct level will decay in a quadratic function (Formula 7 in Granath et al., 1992). For instance a sample collected 38, 83 or 100 days after cessation of exposure contain 49, 12 and 5 %, respectively, of the steady-state adduct level of a even continuous exposure. The results indicate that exposures have not been high and that the exposure levels during injection work were higher at the tunnel construction in Hallandsåsen than in Romeriksporten.

The results show, however, that some of the examined persons reach total acrylamide adduct levels (including contribution from background exposure and smoking) near or above the no-effect threshold level of about 300 pmol/g globin estimated for light, mostly reversible, neurotoxic symptoms (Törnqvist et al., 1998 with data from Calleman et al., 1994; Hagmar et al., 2001). In agreement with this, light neurotoxic symptoms have been observed in the present study, a fact that underlines the earlier finding that the no-effect threshold in humans for light neurotoxic symptoms may be much lower than the threshold value estimated from animal experiments. The value 300 pmol/g globin (from an exposure lasting more than 4 months) corresponds to an uptake of acrylamide of about 10 µg/kg and day (Törnqvist et al., 1998 with parameter values from Calleman 1996). From animal experiments a no-effect threshold corresponding to 500 µg/kg and day is estimated (Johnson et al., 1986; WHO, 2002).

In lack of reliable quantitative exposure data, we aimed at optimising the use of qualitative exposure data. Thus, a set of surrogate variables was developed based upon the available

information from the company, the occupational health service, from interviews and several questionnaires. When comparing the different approaches, it seemed that the information on time with injection work (in months) was the best predictor of the NCV reversibility outcomes. This was only marginally improved when the different time periods were given weights according to the amount of NMA grout used (Kjuus et al., 2001). The intensity index, based upon questionnaire data on the frequency of the different working tasks, did not at all predict the outcome. As the workers were not able to answer how many shifts they had performed injection work, the main qualitative source of exposure, days with injection was obtained only in four categories, making the exposure characterisation even cruder. However, this was the best we could perform under the given circumstances.

The main reason for the use of the NMA-based grout, was its low toxicity compared to acrylamide. An important question is, therefore, to what degree the workers were also exposed to pure acrylamide monomer during the grouting operations. According to information provided by the producer and the Swedish analyses of Solution 1 used in Hallandsåsen, acrylamide concentrations were between 2.5-5.4%.

However, the actual content of acrylamide in the NMA grout during unfavourable conditions in tunnel work is very unclear, partly because of protracted polymerisation of the grout in the cold tunnel, and partly due to the reversibility of the reaction, where NMA is hydrolysed back to acrylamide. Under favourable conditions, the polymerisation should be completed after 6-12 hours. Polymerisation experiments have shown delayed polymerisation of up to one week after the mixing of the components (Ødegård and Ringstad, 1999). The observation of very high acrylamide concentrations (90.6 mg/L) as late as 5 months after the injection work, demonstrates how uncertain the degree of exposure to pure acrylamide was in these workers.

Measurements in the tunnel water after the cessation of the injection work indicated a 2:1 ratio of the concentration of NMA to acrylamide, compared with 5-10:1 in the grout. Thus, exposure to the mixed, but not yet polymerised product may have led to higher concentrations of acrylamide than previously believed. Still, there is insufficient information available on how these substances actually interact.

In conclusion, the 25 workers seem to have been relevantly exposed to NMA and acrylamide monomer during injection work from 8 to 24 months, with potential, but most probably limited exposure from the tunnel water after the grouting injection had come to a halt. The Hb adduct levels in subjects with the most recently taken blood samples indicate elevated levels, but these are definitely lower than those that have been reported in the workers in the Swedish tunnel project. Based on the task performed, and indications from the Swedish study (Hagmar et al., 2001), it is assumed that the most important route of exposure has been through the skin.

The knowledge on which doses of acrylamide that are necessary for development of clinical nervous system effects in humans is limited. However, a «no observed adverse effect level» (NOAEL) related to Hb adduct levels in the range of 0.3-1.0 nmol has been reported for short time exposure (Törnqvist et al., 1998). Although great uncertainties exist, only 3 subjects in the present study among those 11 subjects with blood samples taken less than 70 days after cessation of injection work, seem to have had adduct levels above the lower level of this range.

4.4.3 Health effects

The outcome measures of the present study were partly subjective, based on information from repeated questionnaires, and partly objective, based on measuring a wide range of neurophysiological parameters. Glutathion S transferase genotypes M and T and cytogenetic outcomes were measured in blood samples.

4.4.3.1 Subjective symptoms

With regard to the subjective reports of symptoms, we could not make valid comparisons between the index and the reference group, as the questions were formulated slightly differently in the two groups, and were not related to the same periods of time. However, when assessing the potential reversibility of symptoms in the index group, the prevalence was in general higher at the first examination 4 months post exposure compared to the examination one year later. No statistically significant differences were observed, however, neither for symptoms related to the peripheral nor to the autonomous nervous system. Of symptoms in the CNS, nausea and/or dizziness was significantly reduced after one year. Also for symptoms in the upper airways and in the skin, the prevalence was higher at the first examination compared to one year later. Peeling of skin, a typical sign related to acrylamide exposure, was reported by 34.8% 4 months post exposure, compared to 16.7% one year later. Eczema and skin irritation

was also significantly reduced after one year. These results indicate some reversibility of symptoms which are most probably related to NMA/acrylamide exposure. However, exposure to other airway and irritants during the actual period 4 months post exposure, compared to the work performed one year later may also have contributed to some of the observed differences. In addition, one cannot completely exclude that the massive media focus and anxiety in the workers during the immediate period post exposure may have influenced on the reported symptom prevalence.

4.4.3.2 Neurophysiological measurements

Previous reports on acrylamide effects in man have mainly been related to the primary production of acrylamide from acrylonitrile or to the polymerisation of acrylamide to polyacrylamide (WHO 1985). Later, similar effects were reported in tunnel workers related to grouting operations (Graveleau 1970, Kesson 1977, Mapp 1977). In most cases, the symptoms and signs have been shown to be reversible, with full restitution 2-12 months after the cessation of exposure (WHO 1985, Garland 1967, Tilson 1981). However, symptoms have persisted for several years in severe intoxications (Kesson 1977, Myers 1991).The grouts used by the workers in the aforementioned studies seem mainly to have been based on pure acrylamide, or in one study on N-Methylacrylamide and Methylen-bis-acrylamide (Graveleau et al 1970). Thus, the Swedish study (Hagmar et al 2001) and the present study are, to our knowledge, the first to report neurological effects in workers exposed to grouts based on N-methylolacrylamide.

Animal studies and previous clinical studies of acrylamide neurotoxicity have shown an axonal lesion of the distal "dying back " type, which is typical for several neurotoxic substances (Tilson, 1981, Smith and Oehme 1991). An axonal lesion is characterised by the reduction of motor and/or sensory amplitudes. The mechanism causing this effect is not completely understood, but a direct neurotoxic effect at the nerve endings where the "blood-nerve-barrier" is weak, or a toxic effect on neuronal metabolism and protein synthesis have been suggested as possible explanations. Previous studies have indicated that acrylamide both affects motor and sensory nerve fibres, where long, sensory fibres seem to be the most vulnerable (Smith and Oehme, 1991).

Most neurographical measurements in this study showed no significant differences in the mean results, neither when compared to the reference group nor over time. There was, however, a

slight, but significant reversible effect in mean sensory nerve conduction and motor distal delay in the ulnar nerve and also a reduction of mean sensory nerve conduction and mean sensory amplitude in the sural nerve on examination after 16 months. By using a set of exposure variables, exposure-related improvements were observed both in the median nerve (motor and sensory NCV and F-response) and the ulnar nerve (sensory NCV, F-response), which indicates a reversible neurotoxic effect, although small and possibly subclinical.

The findings in the upper limbs showed reductions of conduction velocities, which indicates a toxic effect on myelin, and only a slightly significant reduction in sensory amplitude in the median nerve. This finding is in contrast to previous studies (Fullerton 1969, He et al 1989, Takahashi et al.1971) on acrylamide exposure, which have rather consistently shown reduced amplitudes as a sign of axonal degeneration. Considering the large random variation in the amplitude measurements, the intermittent NMA/acrylamide exposure over a relatively long time (two years) may not have been sufficient for detecting any effect on the amplitudes in such a small group of workers 4 months or more after the cessation of exposure.

The early, reversible changes were found in the upper and not in the lower extremities, which is uncharacteristic for polyneuropathies. A possible explanation may be that the effect could be caused by direct contact after diffusion through the skin. This may possibly cause a direct, myelinolytic effect on distal nerve fibres which is different from the toxic effect on neuron metabolism causing axonal damage found in other studies of acrylamide. However, somewhat surprisingly, there was an apparent reduction in mean sensory nerve conduction and mean sensory amplitude in the sural nerve from four to 16 months post exposure. The type and localisation of this lesion is more in accordance with previous reports of acrylamide neurotoxicity. The delayed findings, if not a random error, could indicate that the changes may have taken a longer time to develop and have persisted for a longer time than one would expect.

All the time-related exposure indices (tunnel time, injection time, exposure time index and (injection time x intensity index)) were highly correlated to improvements in NCV. Few group differences were observed in the outcome parameters at the group level. Thus, these results illustrate that qualitative data can be optimised to create relevant exposure contrasts, and give results which otherwise could have been overlooked. Another point is that the more

sophisticated weighted or combined indices gave no better prediction of the outcomes than injection time (no. of days with injection work). The intensity index, based on frequency of tasks was not at all a good predictor of any reversibility outcome.

4.4.3.3 Effects on the visual system

The results from the visual evoked response (VEP) measurements did not differ significantly between the exposed group and the reference group. However, a non-significant prolongation of mean P 100 was observed. One worker in the present study reported visual disturbances during ongoing exposure, with great problems with reading. His visual evoked response was pathological with prolonged latency suggesting demyelination at the first examination, and was normalized after one year. This may have been be a neurotoxic effect of acrylamide, but other pathological mechanisms, for instance retrobulbar neuritis of other origin cannot be excluded. In animal studies, acrylamide has been shown to affect the visual system (Schaumberg and Spencer 1979, Canavagh 1982, Merigen et al 1985, Eskin et al 1985), including visual evoked response abnormalities (Boyes et al 1980).). Human case histories have also reported visual effects (Mapp et al 1977, Myers et al 1991).

Electroretinography (ERG) with stimulation frequency 30 Hz showed significantly reduced amplitudes among the exposed, compared to the reference group. With photopic stimulation the function of the cones located in the fovea and central parts of the retina is tested. Rapid, 30 Hz stimulation is more sensitive to slight changes than stimulation with 2hz frequency. Our findings indicate a slight lesion of the cone photoreceptors. The cones are necessary for colour vision and sharp vision of details.

To study the function of the rod photoreceptors 20 minutes of darkness adaptation is necessary before scotopic stimulation. The rods are located in the periphery of the retina and responsible for night vision. Scotopic stimulation with darkness adaptation was not performed in our study but will be of interest in later supplementary studies.

In another study of tunnel workers with past exposure to NMA grout, we have indications of possible irreversible effects on colour vision in the blue green area and also slight effects on the vision field (Goffeng et al., 2000, Goffeng et al., 2002, in preparation).

4.4.3.4 Effects on the autonomous nervous system

We did not perform any tests with a specific focus on autonomous nerve functions. No clear differences were observed between the groups from the symptom questionnaire (table 7). However, one of the workers reported acute problems with voiding and signs of urinary retention during an extreme period of injection work.

4.4.3.5 Examination of glutathion S transferase genotypes

About 50% of the population lack GSTM1 activity and 15% has very low GSTT1 activity due to genetic defects. These individuals might in theory have increased risk for injuries caused by acrylamide. Since many GSTs are potentially involved in the detoxification, there is only a weak effect if one of them is lacking. Therefore, lager study groups may be necessary to demonstrate increased susceptibility associated with a particular genotype. Furthermore, the kinetic parameters of different GSTs are not sufficient to evaluate their importance *in vivo*, since the relative expression level in the target cells which is an important determinant, still is an unknown factor. The interpretation of the data analysed in relation to GST genotypes should therefore be done with care. The most consistent finding here is the effect of GSTM1 and T1 genotypes on the number of chromatid gaps in acrylamide exposed workers (Table 26, 27,and 28). These data may indicate that individuals with intact genes (active enzymes) are less prone to such chromosomal aberrations when exposed to acrylamide.

4.4.3.6 Examination of genotoxic effects.

Measurements of hemoglobin adducts of acrylamide will give an indication of the exposure of the subjects under study, but information on the biological effects of exposure cannot be obtained with this method. A direct measure of damage to the cells after exposure can be obtained by chromosome studies. An association between a high amount of chromosome damage in lymphocytes and cancer has been shown (Hagmar et al., 1994), indicating the relevance of the method for possible health effects. Lymphocyte populations in humans seem to have a longer life-span than previously considered (Gundy et al., 1992; Bogen, 1993), which gives the possibility of scoring effects of recent, but not prevailing, exposure. In addition the metabolite compound, glycidamide, is a relatively stable epoxid with long half-life *in vivo*, evenly distributed in tissues of animals (Dearfield et al.1995). Some studies have suggested

that this metabolite is the active chromosome damaging agent. These arguments were the background for studying chromosomal aberrations in the Rhoca-Gil exposed workers. In previous studies any relevance to possible health effects is shown only for the increased frequencies of number of cells with aberrations including chromatid and chromosome breaks. In the present study no significant differences were found between the exposed workers and their matched controls for most of the comparisons with these three effect parameters. Only for less exposed workers compared to their matched controls (table 24) was there a significant difference in the number of cells with aberrations. The median value for this parameter was 3 for the exposed group compared to 2 for controls indicating that more exposed workers were in the higher range. However, the three subjects with clearly elevated adduct levels, did not have higher levels of chromosome aberations than the remaining of the exposed subjects. When grouping the participants according to genotype, the numbers decreased drastically and it would thus be difficult to demonstrate any significant differences. But for the exposed workers with GSTM1-/GSTT1- a trend was indicated with higher frequencies for all effect parameters for the exposed group compared to controls with the same genotype (table 28). It is also of interest that GSTT1, which in the kinetic study had the highest affinity for acrylamide (see 4.3.6) was the only genotype which came out as significant for the number of chromatid gaps. We have also included this parameter since the results are so consistent for all the comparisons done, and it has recently been recommended that such analyses should be included (Paz-y-Mino et al., 2002). Although chromatid gaps may be indicative of damage to the DNA, we have no documentation for a connection between this parameter and possible health effects.

4.4.4 Preventive measures

How can it happen that a toxic compound that has been studied in depth for decades can still lead to occupational and environmental effects during construction work both in Norway and Sweden, two countries which both have a strong tradition of protecting the health and safety of workers? This question has recently been raised elsewhere (Kjuus 2001), but will also be shortly discussed here.

Both in Hallandsåsen and Romeriksporten tunnel projects the water leakage became unexpectedly high due to the extremely unstable rock structures in the tunnel area. In the Norwegian project, several of the small lakes in the area were almost emptied during the construction work. These environmental problems, with the potential disturbance of the ground water level, came in addition to the possible health risk to the workers. It is believed that some of these unexpected problems could have been avoided if the projects had been planned better.

Thus, one may also ask if large construction projects of this type are subject to so tight time limits owing to time pressure that crucial stages both in the planning and implementation of the project may suffer, and that this has direct implications for the workers. For example, the traditional grouting technique with cement is time-consuming. It is easy to use more of the «fast» grouting agents, such as those based on acrylamide when pressed for time. When grouting agents are used in tunnel projects the common practice has been that they are injected into the rock before blasting, thus preventing water leakage. However, due to the unexpected water leakages and the pressure on the time schedule for completing the tunnel work, the grouts were also injected into fissures that leaked water after blasting. Thus, the normal polymerisation of the gel was incomplete and was disturbed by the water leakage. As the injection work often lasted until the leakage was under control, this could lead to very long working hours. Some Norwegian workers reported continuous work of up to 24 hours in order to finish a job. One would believe that less NMA grout could have been used under a different time schedule.

As the predominant uptake of NMA from the grout is believed to be by the dermal route, another challenge in this kind of injection work is to prevent skin contact with the grouting agent. The lack of proper information to the workers in Romeriksporten due to incomplete

material safety data sheets may have led to less regular use of personal protection devices than otherwise would have been used. Stricter routines were introduced when this was revealed.

However, all 25 workers reported having used gloves during injection work, and the majority used rain jackets and trousers. Still, 80% reported skin contact with the grouting agent, and all reported skin contact with contaminated tunnel water and that they were «completely wet» from tunnel water. Correspondingly, Hagmar et al. report that none of the workers had used appropriate personal protection devices (Hagmar et al., 2001). By conducting hand rinses on the workers and analysing the rinse water, other studies have also shown that nearly all the grouters had hands contaminated with acrylamide despite wearing of gloves (Hills et al., 1986, McHugh 1987). It was not determined whether the acrylamide contamination was due to wearing gloves over previously contaminated hands or due to permeation of acrylamide through the glove material. Thus, even when proper protection with gloves and rain jackets is worn, it is still difficult to avoid skin contact with the grout. One solution to this problem would be to substitute the NMA grout with a less toxic agent. When the use of NMA-based grout was stopped in August 1997, one of the alternative grouts that were used contained isocyanates. Thus, the choice between alternative substitutes is not easy.

4.5. Conclusion

In 25 tunnel workers exposed to acrylamide and N-methylol acrylamide during grouting work in Romeriksporten in the period 1995-1998 who were examined 4 and 16 months after the cessation of the injection work, and compared to 50 unexposed tunnel workers, the main findings were the following:

- Slightly more symptoms related to the nervous system were observed in the exposed subjects during grouting injection work, compared to one year later.
- Sensory nerve conduction velocity and distal delay in the ulnar nerve were reduced/prolonged compared to the referents, with normalisation after one year.

When reversibility was further examined, exposure-related improvements were observed, both in the median nerve (motor and sensory NCV and F-response, motor amplitude) ulnar nerve (sensory NCV, F-response) and tibial nerve (motor amplitude). The findings were more marked

in the upper than in the lower extremities with signs of demyelination being more prominent than axonal changes.

- A slight reduction of mean sensory nerve conduction and mean sensory amplitude in the sural nerve was observed after 16 months, which may indicate a protracted axonal effect
- Examinations of visual evoked reponse and electroretinography 16 months post exposure indicated slight effects on the visual system with significant reduction in the cone photoreceptor function of the retina, responsible for colour vision and sharpness of vision.
- There were no clear differences in the neurophysiological measurements related to polyporphism in the Glutathion-S-transferase genotypes M and T.
- Chromosome studies showed that exposure status and genotype GSTT1- came out as significant for the number of chromatid gaps.

Thus, the results indicate slight effects on the peripheral nervous system in tunnel workers related to exposure to N-methylolacrylamide and acrylamide during grouting operations. The effects seemed largely to have been reversible, with normalisation 16 months post exposure. A possible slight effect on the visual system, and also a possible slight genotoxic effect require further attention. To date, these findings are without any known clinical relevance. Subjects lacking GST-M1 and GST-T1 seemed to have the highest number of chromatid gaps, indicating that individual susceptibility related to detoxification of acrylamide and N-metylolacrylamide may have played a role in the observed effect. However, in this study of so many outcomes, random associations due to multiple comparisons may have occurred, and these may have complicated the interpretation of possible effects.

Based on these results, we recommend that exposure to NMA-based grouting agents should be avoided. At present, this is an unnecessary statement, as the use of NMA-based grout was banned in Norway in 2000, owing to the combined experiences from Hallandsåsen and Romeriksporten. Tunnel workers performing grouting work in the future, however, will also need close follow-up, as several of the substitutes for NMA-based grouts also may have adverse health effects.

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