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Chromosome aberrations in tunnel workers exposed to acrylamide and N-methylolacrylamide

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Objectives The aim of this study was to examine chromosome aberrations in 25 tunnel workers exposed to acrylamide-containing grout in injection work.

Methods Blood samples were collected from 25 exposed and 25 unexposed tunnel workers matched for age, gender, and smoking habits. Whole blood was cultured for 50–53 hours according to conventional methods. Chromosome damage was scored in 200 metaphases per person on coded slides. The distribution of glutathione S-transferase (GST) genotypes (*M1* and *T1*) was examined for all the workers. Exposure assessment was performed with detailed interviews and questionnaires.

Results The chromosome examinations showed no statistically significant differences between the 25 exposed and 25 unexposed workers for cells with chromosome aberrations or for chromatid breaks, chromosome breaks, and chromosome gaps. The exposed workers had a significantly higher number of chromatid gaps (mean 10.6, SD 5.6) than the unexposed workers (mean 6.4, SD 4.4, P=0.004), but there was no exposure–response relationship. The limited stratum-specific numbers showed that the exposed workers with the *GSTM1-/GSTT1-* genotype had nonsignificantly higher frequencies of all the effect parameters than the unexposed workers; this finding indicates that individual susceptibility related to the detoxification of acrylamide and N-methylolacrylamide may have played a role in the observed effect.

Conclusions No increase in chromosome breaks or aberrations was observed for 25 workers exposed to acrylamide-containing grout during tunnel work. The increased frequency of chromatid gaps in the exposed workers may indicate a slight genotoxic effect related to exposure to acrylamide or N-methylolacrylamide.

Key terms grouting agent; glutathione S-transferase genotype, N-methylolacrylamide.

Monomeric acrylamide is neurotoxic and probably carcinogenic to humans. In addition, reproductive effects have been shown in animals (1, 2). Recently, there has been public concern about the potential health risks related to the dietary intake of acrylamide (3, 4). However, epidemiologic studies have primarily focused on occupational exposure, by examining neurotoxic effects (5–7) or cancer incidence related to acrylamide exposure (8).

Evidence that acrylamide induces chromosome effects was reported by Shiraishi in 1978 (9). Acrylamide has a clastogenic potential that affects both germ cells and somatic cells (10–13). It is primarily conjugated with gluthathione and is excreted as mercapturic acid (n-acetyl-cystein conjugate). A proportion is oxidized to glycidamide through the cytochrome P-450 system (CYP2E1) (14). Glycidamide is highly reactive, particularly against free thiol groups, and is excreted in conjugated forms. It is uncertain whether metabolic activation is required for acrylamide to exert its genotoxic effect, although several studies suggest a possible direct-acting mechanism for acrylamide (10, 11).

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, including one based on the less toxic N-methylolacrylamide (NMA). Exposure to the alternative NMA grout still, however, implies exposure to pure acrylamide (6). Thus occupational exposure to acrylamide-containing grouts may present a hazard to workers,

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through inhalation or, probably more importantly, through dermal exposure.

During 1995–1997, 340 000 kilograms of an NMA-based grouting agent, Rhoca-Gil (Sipro-Gel) was used for injection during the construction of a railway tunnel 14 kilometers in length. We have recently reported nervous system effects in 25 of the workers exposed to acrylamide and N-methylolacrylamide during this grouting work (7). The aim of the present study was to examine possible genotoxic effects in tunnel workers with relevant exposure to acrylamide-containing grout during a 2-year period. We have also examined chromosome aberrations related to polymorphism in the glutathione-S-transferase (GST) genotypes *M* and *T* in that the results may be of particular interest with respect to the biotransformation of acrylamide (11).

Study population and methods

The study base consisted of 73 tunnel workers who had been potentially exposed to NMA grout during tunnel work in a 2-year period from 1995 to 1997. Due to the media focus on possible health effects related to acrylamide exposure during the autumn of 1997, these 73 workers participated in a health examination at occupational health services during October 1997–January 1998. Among these workers, we identified the 25 persons with the highest exposure to acrylamide-containing grout. Those with known neurological disease or known alcohol or drug abuse were not eligible for the study. Twenty-five tunnel workers who had not been exposed to NMA grouts, matched for age (±3 years), gender, and smoking status were selected as controls. Two exposed workers who had stopped smoking just prior to the examination were matched with smoking unexposed workers. The distribution of age, lifestyle factors, years in construction work, and other relevant exposure factors are presented in table 1. The project was approved by the Regional Ethical Committee, and all the workers gave their written informed consent to participate in the study.

Chromosome examination

Chromosome studies were performed for 25 exposed and 25 unexposed workers. Since it was impossible to collect blood samples from the exposed worker and matched unexposed worker on the same day, 31 reference blood samples were collected from seven staff members at the National Institute of Occupational Health to control for possible postal and culture factors.

Samples of 10 ml of heparinized blood from two exposed or unexposed workers, together with one reference sample, were collected on the same day and sent by mail for delivery to the Telemark Hospital the next day. Four whole-blood lymphocyte cultures per sample were established. They were cultured for 50–53 hours and processed as described previously (15). Slides were coded and stained in Giemsa, and chromosome damage was scored in 200 metaphases per person. The chromosome results are reported as the number of cells with aberrations (excluding gaps) and the number of chromatid breaks, chromosome breaks, chromatid gaps, and chromosome gaps, all as group means per 200 cells. A gap was defined as an unstained region of less width than the width of two chromatids (16). The results for the 31 reference samples (footnote to table 2), gave no

Table 1. Background factors and other exposure factors reported by the exposed group (N=25) and the control group (N=25).

Group	Age			Construction work (years)		Current smokers			Alcohol >5 l/year (%)	X-ray exposure last month (%)	Common cold last week (%)	Allergy (%)	Current medication (%)	Previous work with solvents (%)	Previous lead exposure (%)
	Mean	SD	Range	Mean	SD	N	%	Cigarettes/day							
Exposed	43.4	8.6	31–62	19.5	7.6	12	48	13.4	32	12	8	8	12	48	8
Unexposed	44.2	9.1	25–60	22.0	10.7	14	56	15.6	20	16	4	8	36	68	20

Table 2. Chromosome aberrations in 25 exposed and 25 unexposed workers.

Group	N	Cells with aberrations [a]		Chromatid breaks [a]		Chromosome breaks [a]		Chromatid gaps		Chromosome gaps	
		Mean/200 cells	SD	Mean/200 cells	SD	Mean/200 cells	SD	Mean/200 cells	SD	Mean/200 cells	SD
Exposed	25	2.9	1.7	1.4	1.1	1.2	1.1	10.6[b]	5.6	1.5	1.5
Unexposed [c]	25	2.4	1.6	0.9	1.2	0.9	0.9	6.4	4.4	1.4	1.7

[a] Excluding gaps.
[b] Mann-Whitney's nonparametric test (2-tailed) between the exposed and unexposed workers (P=0.04).
[c] The comparable quality control values for the reference samples were 2.7 (SD 1.4), 1.2 (SD 1.1), 1.3 (SD 1.1), 8.6 (SD 4.7) and 1.6 (SD 1.2), respectively.

indications of any methodological problems related to postal or culture factors.

Glutathione S-transferase genotypes

All 25 of the exposed workers and 23 of the unexposed workers were genotyped for the deletion polymorphism in *GSTM1* and *GSTT1*. DNA (deoxyribonucleic acid) was extracted from 100 µl of blood, and the polymorphic regions were amplified by polymerase chain reaction (PCR) using previously described methods (17). The PCR products were analyzed by capillary electrophoresis using internal size standards. Genotypes consisting of homozygote deletions were indicated as *GSTM1*- and *GSTT1*-. All other genotypes were indicated as *GSTM1*+ and *GSTT1*+.

Grouting agent and exposure conditions

The grout was based on two solutions. Solution 1 consisted of 26–31% *N*-methylolacrylamide, 2.5–5.4% acrylamide, 0.02–0.03% methylene-bis-acrylamide, 12–17% methylic diesters (catalyst), 0.9% formaldehyde, and water. Solution 2 contained sodium silicate, sodium persulfate, and water. Due to the low temperature in the tunnel, however, polymerization could be considerably delayed, and exposure to the mixed, but not yet polymerized, product could occur. All 25 of the exposed workers had taken active part in the injection of grouting agents for an average of 586 (range 243–724) days. The median value was 639 days. Ten of the twelve workers exposed above the median value were exposed for 724 days, while seven of the thirteen workers exposed below the median were exposed for less than 500 days. Twenty of the twenty-five exposed workers reported direct skin contact with acrylamide grout, while all of the exposed workers stated that they had skin contact with tunnel water. Further details of the exposure conditions in the tunnel have been presented elsewhere (7, 18).

Exposure assessment

Measurements of acrylamide and N-methylolacrylamide in tunnel water. Due to the technical and environmental problems of water leakage into the tunnel, the digging work came to a halt on 25 August 1997. Before this date, only a few sporadic measurements of acrylamide had been made in the drainage water from the tunnel. The highest concentrations were measured on 25 August, with an acrylamide concentration of 9654 µg/l and a NMA level of 16 600 µg/l (7). During September, the average concentration of acrylamide was 100–110 µg/l, while during October 1997–April 1998 most of the measurements were below 50 µg/l. However, in January 1998, during the injection of other grouting agents, several drill holes with unpolymerized grout were discovered. Very high concentrations of acrylamide were measured in the water dripping from these holes in the tunnel wall (up to 90 600 µg/l).

Qualitative exposure information. Using the information obtained from the questionnaires, we estimated the accumulated time for tunnel work for each worker ["tunnel time" in months] and the accumulated time for injection work ["injection time" in days] during the period September 1995–August 1997. Using information about the amount of grout used in the different periods, we also developed a time-weighted "exposure-time index" based on the amount of grout used in the different periods (7).

Statistical methods. Mann-Whitney's nonparametric ranking test was used for comparison between the groups in the chromosome studies. In order to create a crude exposure contrast, we divided the 25 exposed workers into a long-term exposure group (above the median of 639 days of injection, N=12) and a short-term exposure group (below 639 days of injection, N=13). A stepwise linear regression was also applied. The level of statistical significance was set two-tailed at $P<0.05$. The Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA) was applied using a personal computer.

Results

Comparisons between all the exposed and unexposed workers are shown in table 2. There were no striking differences between the groups. Only the number of chromatid gaps differed significantly between the matched exposed and unexposed workers. In order to look for a possible skewed distribution of aberrations between the groups, we compared the number of persons with ≥3 aberrations. A slightly higher number of exposed persons was found with the number of cells with aberrations above the cut point, 14 exposed versus 9 unexposed.

The distribution of the chromosome results according to the length of exposure and smoking habits is shown in table 3. There were no differences between the long-term (≥639 days) and short-term (<639 days) exposure groups. For the chromatid gaps there was a nonsignificant difference between the workers exposed long-term and their matched controls, and a significant difference was found between the workers exposed short-term and their matched controls. Comparing the exposed smokers and nonsmokers with their respective controls did not reveal any significant differences except for chromatid gaps.

Table 3. Chromosome aberrations for the exposed and unexposed workers stratified for time of exposure [cut point of 639 days of injection work (median)] and smoking status.

	N	Cells with aberrations [a]		Chromatid breaks [a]		Chromosome breaks [a]		Chromatid gaps		Chromosome gaps	
		Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD
Exposure time											
≥639 days											
Exposed workers	12	3.0	1.5	1.5	1.1	0.9	1.0	11.3 [b]	7.0	0.9	1.4
Unexposed workers	12	2.7	1.7	1.2	0.9	1.0	1.0	7.0	5.6	1.5	2.0
<639 days											
Exposed workers	13	2.8	1.9	1.2	1.2	1.4	1.2	9.9 [b]	4.1	2.0	1.5
Unexposed workers	13	2.2	1.6	0.7	1.3	0.8	0.8	5.8	3.1	1.4	1.5
Smokers											
Exposed	12	2.5	1.7	1.2	1.1	0.8	0.9	10.2 [b]	6.5	1.3	1.2
Unexposed	14	2.6	1.6	0.8	1.0	0.9	0.9	6.8	4.0	1.7	2.2
Nonsmokers											
Exposed [c]	13	3.3	1.7	1.5	1.1	1.5	1.2	10.9 [b]	4.8	1.6	1.8
Unexposed	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 nonparametric test (2-tailed) between the exposed and unexposed workers for ≥639 days (P=0.1) and <639 days (P=0.01), smokers (P=0.19), and nonsmokers (P=0.01).
[c] Two workers stopped smoking just prior to the examination.

Table 4. Chromosome aberrations in the exposed and unexposed workers grouped according to genotype GSTM1 ±.

Genotype group	N	Cells with aberrations [a]		Chromatid breaks [a]		Chromosome breaks [a]		Chromatid gaps		Chromosome gaps	
		Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD
GSTM1-											
Exposed workers	13	2.7	1.5	1.5	1.1	1.0	0.9	12.2[b]	6.9	1.6	1.9
Unexposed workers	15	2.3	1.6	1.0	1.2	0.7	0.7	6.5	6.5	1.2	1.2
GSTM1+											
Exposed workers	12	3.2	1.9	1.3	1.2	1.3	1.3	8.8[c]	2.9	1.3	1.1
Unexposed workers	8	3.1	1.6	1.0	1.2	1.5	1.1	6.3	5.5	2.0	2.8

[a] Excluding gaps.
[b] P=0.02 (2-tailed).
[c] P=0.08 (2-tailed).

We also stratified the workers according to genotype (*GSTM1* and *GSTT1*). Among the workers with *GSTM1*-, a statistically significant increase in chromatid gaps was observed for the exposed workers when they were compared with the unexposed workers (12.2 versus 6.5, P=0.02), but not for the corresponding groups among the workers with *GSTM1*+ (8.8 versus 6.3, P=0.08) (table 4). When the groups were further stratified for smoking, the observed difference in chromatid gaps among the *GSTM1*- exposed and unexposed workers was observed both among the nonsmokers (11.6 versus 4.6, P=0.02) and the smokers (13.0 versus 8.7, P=0.45) (data not shown). There was a corresponding pattern when the *GSTT1* genotype was stratified for, the highest number of chromatid gaps occurring in the exposed workers with *GSTT1*- in a comparison with the corresponding controls (16.3 versus 9.6, P=0.19) (data not shown). We did not stratify the workers according to smoking habits for genotype *GSTT1*, as the number of persons was too small.

The same trend was observed when the workers were grouped according to both *GSTT1* and *GSTM1*, the biggest absolute difference in the number of chromatid gaps occurring between the exposed workers with *GSTM1-/GSTT1*- and their corresponding controls (16.3 versus 8.7) (table 5). A corresponding nonsignificant trend was also observed for cells with chromosome aberrations (3.8 versus 1.3), chromatid breaks (1.8 versus 0.3), chromosome breaks (1.8 versus 0.7), and chromosome gaps (2.3 versus 1.7).

Using the number of chromatid gaps as the dependent variable, we performed a stepwise linear regression analysis with the parameters exposed/controls, smoker/nonsmoker, use of medication (yes/no), *GSTM1+/GSTM1-*, *GSTT1+/GSTT1*- in the model. The exposure status and genotype *GSTT1* were significant for the

Table 5. Chromosome aberrations in the exposed and unexposed workers grouped according to the combination of genotypes GSTM1 ± and GSTT1 ±.[a]

Genotype group	N	Cells with aberrations [b]		Chromatid breaks [b]		Chromosome breaks [b]		Chromatid gaps		Chromosome gaps	
		Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD	Mean/ 200 cells	SD
GSTM1- GSTT1-											
Exposed workers	4	3.8	1.3	1.8	1.3	1.8	1.0	16.3	9.2	2.3	2.6
Unexposed workers	3	1.3	0.8	0.3	0.6	0.7	1.2	8.7	4.2	1.7	1.2
GSTM1- GSTT1+											
Exposed workers	9	2.2	1.4	1.3	1.0	0.7	0.7	10.4 [c]	5.3	1.3	1.5
Unexposed workers	12	2.5	1.7	1.2	1.3	0.7	0.7	6.0	4.3	1.1	0.7
GSTM1+ GSTT1+											
Exposed workers	12	3.2	1.9	1.3	1.2	1.3	1.3	8.8 [c]	2.9	1.3	1.1
Unexposed workers	6	3.2	1.3	0.8	1.0	1.7	1.0	4.7	3.4	2.7	3.0

[a] No exposed workers and two unexposed workers had the genotype combination GSTM1+ and GSTT1-.
[b] Excluding gaps.
[c] Mann-Whitney's nonparametric test (2-tailed) between the exposed and unexposed workers: exposed workers with GSTM1- GSTT1+ (P=0.07) and exposed workers with GSTM1+ GSTT1+ (P=0.02).

number of chromatid gaps only with P=0.002 and P=0.004, respectively. In a separate analysis, using *GSTT1* in combination with the injection time and exposure-time index, respectively, as the continuous variables for the exposed group, only *GSTT1*- proved to be significant for the number of chromatid gaps (P=0.02).

Discussion

A direct measure of damage to cells after occupational exposure to genotoxic agents can be obtained with chromosome studies. An association between a high amount of chromosome damage in lymphocytes and cancer has been shown (19, 20), indicating the relevance of these measures as predictors for carcinogenic risk. Lymphocyte populations in humans seem to have a longer life-span than previously considered (21, 22), which gives the possibility of scoring the effects for recent, but not prevailing, exposure. In addition, the metabolite glycidamide is a relatively stable epoxide with a long half-time in vivo, and some studies have suggested that this metabolite is the active chromosome-damaging agent after acrylamide exposure (11). Although the acrylamide injection work was stopped before the blood samples were taken, the workers were still exposed to small amounts of unpolymerized grout from the NMA-based grouting agent, as high concentrations of acrylamide were measured in tunnel water after the grouting agent was abandoned. These arguments were the background for studying chromosome aberrations in this group of acrylamide-exposed tunnel workers.

Of the selected outcome parameters used in our study, only cells with aberrations including both chromatid breaks and chromosome breaks have previously been shown to be of any relevance to possible health effects (19, 20). Recently the same predictivity has been shown for chromatid breaks and chromosome breaks separately (23). We did not find any consistent significant differences between the matched exposed and unexposed workers for these three effect parameters in relation to exposure time and smoking status. Only three of the workers had clearly elevated adduct levels (7). They did not have higher levels of chromosome aberrations than the remainder of the exposed workers. Using the crude, semi-quantitative exposure information gave no further indications of any exposure–response relationship.

The only consistent significant finding in our study was the observed increase in chromatid gaps among the exposed workers. This finding was independent of a crude, dichotomized variable for exposure length and could not be explained by smoking status. As both acrylamide and glycidamide have the ability to form adducts with DNA, they can be expressed as chromatid gaps or breaks after one cell cycle in culture. A gap is formed when a section of a chromatid becomes attenuated so that there appears to be a discontinuity where the two adjacent regions are still connected, but the DNA thread is so fine that it seems invisible (24). Although chromatid gaps may be indicative of damage to DNA as a result of exposure, as was also shown by Lander et al (25), we have no documentation for a connection between this parameter and possible health effects. However, it has recently been recommended that such analyses should be included in studies of the genotoxic effects of environmental agents (26) in particular since the definition of gaps may vary between studies (16, 27, 28). In our study a gap was defined to be the width of two chromatids (16), while other studies have scored gaps as the width of one chromatid. Our

study may therefore include gap lesions that some other laboratories would score as breaks.

Glutathione may play an important role in the detoxification of acrylamide (29), at least in the nervous system (30). In Norway, about 50% of the population lacks *GSTM1* activity and 15% lacks *GSTT1* activity due to genetic deletion polymorphism. These people may, in theory, have an increased risk for injuries caused by acrylamide. Since many GST genotypes are potentially involved in the detoxification of acrylamide and glycidamide, only a weak effect could be expected if one of them was lacking. When the workers were grouped according to genotype, the numbers decreased drastically, and it was difficult to demonstrate any statistically significant differences between the groups. However, for the exposed workers with *GSTM1-/GSTT1-*, a trend toward higher frequencies for the all the effect parameters in the exposed group was indicated when compared with the unexposed group with the same genotype (table 5). It is also of interest that *GSTT1*, which, in a kinetic study in our laboratory, has been shown to have the highest affinity for acrylamide (31), was the only genotype that proved to be significant for the number of chromatid gaps in the stepwise linear regression analysis. However, the kinetic parameters of different GST genotypes are not sufficient for evaluating their importance in vivo since the relative expression level in the target cells, which is an important determinant, is still an unknown factor. The interpretation of the limited data of our study, which were analyzed in relation to *GST* genotypes, should therefore be made with care. The most consistent findings related to the *GSTM1* and *GSTT1* genotypes concerned the number of chromatid gaps (table 4 and 5). These data may indicate that people with intact genes (active enzymes) are less prone to such chromosome aberrations when exposed to acrylamide.

With regard to comparability, both the exposed and unexposed groups were experienced construction workers who were expected to have similar exposures and lifestyles. Corresponding age and smoking habits were secured by a matching procedure. There were, however, some differences in the reported exposure to other factors between the groups, mainly with higher reported frequencies among the unexposed workers. The observed difference in the number of chromatid gaps between the groups was not related to alcohol consumption. More unexposed than exposed persons used medication regularly, but, for both groups, the people using medication had lower frequencies of all types of chromosome aberrations than the people not using medication.

In 25 tunnel workers exposed to acrylamide and NMA during grouting work during a 2-year period, chromosome studies showed no increase in chromosome breaks or aberrations. However, this exposure was associated with an increased number of chromatid gaps. Workers lacking *GSTM1* and *GSTT1* had the highest number of chromatid gaps; this finding indicates that individual susceptibility related to the detoxification of acrylamide and N-methylolacrylamide may have played a role in the observed effect. The results from this small study need to be confirmed by other studies.

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