Organic solvent exposure, genes, and risk of neuropsychological impairment

F. DICK, S. SEMPLE, A. OSBORNE, A. SOUTAR, A. SEATON, J.W. CHERRIE, L.G. WALKER and N. HAITES

From the Departments of Environmental & Occupational Medicine, and Medicine & Therapeutics, University of Aberdeen, Aberdeen, Institute of Occupational Medicine, Edinburgh, and Institute of Rehabilitation, University of Hull, Hull, UK

Received 5 September 2001 and in revised form 8 February 2002

Summary

Background: Subtle cognitive and neurological impairments have been found in some workers exposed to organic solvents. Whether these effects occur at or below current legal limits for occupational exposure is controversial.

Aim: To determine whether occupational solvent exposure is associated with neuropsychological impairment and whether such risk is modified by polymorphisms in the genes for enzymes involved in detoxification.

Design: Retrospective case-control analysis.

Methods: We studied 78 former dockyard painters and 42 community controls. Individual respiratory and dermal exposures to solvents were estimated. Neuropsychological tests were administered, including paper and pencil tests, tests from the Neurobehavioural Evaluation System (NES2), together with a structured neurological examination and genotyping of polymorphic enzymes involved in detoxification: GSTM1, GSTT1, GSTP1, NAT1, NAT2, SOD1 and CYP1A1.

Results: While initial case-control analyses failed to identify any significant differences between symptomatic and asymptomatic painters, in regression analyses increasing solvent exposure was associated with increasing risk of cognitive impairment, after adjustment for IQ (or age, where appropriate), smoking and alcohol. There was also an association between exposure and reduction in grip strength. There was limited evidence of risk modification by some enzyme polymorphisms.

Discussion: This association between increasing intensity of solvent exposure and neuropsychological impairment may be important at current exposure levels in the UK.

Introduction

Organic solvents are widely used in industrialized countries, up to 25% of the adult population having some exposure. The Health and Safety Executive (HSE) estimate that 2 m workers are regularly exposed to these agents in the UK. Subtle cognitive and neurological impairments have been found in some, but not all, studies of exposed workers. Whether these effects occur at or below current legal limits for occupational exposure is controversial. Given the large numbers exposed to these agents, adverse effects may have public health significance.

Estimation of likely historical solvent exposure is an area that is central to this field of research. The use of measures such as ever/never exposed or job title classification may obscure differences between heavily exposed workers and their less heavily exposed colleagues. An American study of factory workers demonstrated this potential for misclassification when it found the ‘unexposed’
group in one factory had eight times the exposure of ‘exposed’ workers in other factories. The higher the quality of exposure estimation, the more reliable the results of any study are likely to be. Current exposure measurements are not necessarily an adequate reflection of past exposure, given the evidence that workplace exposures are dropping over time.

Genetic polymorphisms in the enzymes glutathione S-transferase theta 1 (GSTT1) and glutathione S-transferase mu 1 (GSTM 1) may modify the risk of developing Parkinson’s disease or neuropsychological deficits in solvent exposed workers. Candidate genes among other polymorphic enzymes involved in the biotransformation of foreign chemicals include glutathione S-transferase P 1 (GSTP1), N-acetyl transferase 1 (NAT1), N-acetyl transferase 2 (NAT2), superoxide dismutase (SOD1) and cytochrome P450 1A1 (CYP1A1). Several of these enzymes have previously been suggested to be risk modifiers for Parkinson’s disease or motor neurone disease: diseases that have, in turn, been linked to solvent exposure.

Our hypotheses were that symptomatic workers were at increased risk of neuropsychological impairment, that neuropsychological impairment was related to solvent exposure, and that this risk would be modified by polymorphisms in GSTM1, GSTT1, GSTP1, NAT1, NAT2, SOD1 or CYP1A1. We aimed to test this by a study of solvent-exposed workers in whom it was possible to make detailed estimates of exposure and to measure neuropsychological function.

Methods

Subjects

We carried out a nested case-control study on 120 subjects (42 community controls and 78 dockyard painters). All had previously taken part in a postal cross-sectional study using a neuropsychological questionnaire that comprised 16 questions derived from the Orebro Q-16 (Questions 4–19) and an additional six questions regarding neurological function (Questions 20–25). The Q-16 is a 16-item questionnaire developed in Sweden for use by occupational physicians as a screening tool to identify workers showing symptoms of solvent neurotoxicity.

When analysing the results, Chen et al. treated Questions 18–25 as indicating neurological dysfunction. The cohort for that postal study was drawn from all 1292 men who had worked in the paint shop at the dockyard for at least one year between 1950 and 1992. These painters were identified from personnel and payroll records of the dockyard. The 953 painters not known to be deceased at the end of 1995 and the same number of age-matched men, drawn from the lists of local General Practitioners, were invited to take part. There were 260 painters and 539 members of the local community who responded to the questionnaire (overall response rate 56.6%). That study found an increasing risk of neuropsychological symptoms with increasing duration of employment as a painter. Years of employment was treated as a surrogate measure of cumulative solvent exposure in that study. From this cohort, we defined cases as all men who had five or more positive answers to the Q-16 (n = 98) and controls as painters who reported two or fewer symptoms (n = 89) and a second group of community controls who also reported two or fewer symptoms (n = 143). The recruitment of Q16 subjects is shown in Figure 1. A second group of painters (n = 86) was identified with three or more positive answers to the eight neurological questions (Questions 18–25). They were treated as a second group of cases; their controls were painters with one or no positive answers to the eight neurological questions (n = 118), and similarly-defined community controls (n = 140). So far as possible, cases and controls were age-matched to within 5 years. There was substantial overlap between the two groups with most, but not all, Q16 cases also being N8 cases, and equally most painter controls being controls in both groups. All community controls recruited were controls in both groups. The dockyard painter cases and painter controls had had exposures to organic solvents and inorganic lead during the course of their work.

Neuropsychological tests

We used a battery of psychometric tests (Continuous Performance, Paired Associate Learning, Symbol Digit Substitution and Associate Recall), drawn from the computerized Neuro-behavioural Evaluation System (NES2), together with Trail Making A and B tests and the Benton Visual Retention Test (Form C, administration A). The Trail Making tests are measured in terms of time to completion (seconds) where increasing time equates to poorer performance. The Benton visual retention test generates two outcome measures: the number of correct reproductions (out of ten) and the number of errors made, where more than one error can be made in each incorrect reproduction. Pre-morbid ability (IQ) was estimated using a hold test, the National Adult Reading test (NART). We also carried out a structured neurological examination including grip strength dynamometry, nystagmus, tremor, two-point discrimination,
vibration perception, finger-nose approximation, and muscle wasting.

**Exposure estimates**

We obtained detailed work histories from all 78 dockyard painters and 42 community controls (four of whom had some experience as painters, but not in the dockyard). These interviews, together with data from dockyard solvent monitoring, paint manufacturers’ data, exposure reconstructions and painters’ job diaries, were used to produce estimates of solvent exposures. We identified some workers, who had been foremen or supervisors, as having especially good recall of working practices. These men we termed ‘experts’ and later re-interviewed them to clarify inconsistencies in other workers’ accounts. During this process, the exposure assessors were blind to the case/control status of subjects. We constructed an employment history database detailing each subject’s job in every quarter year. Employment was classified into one of 25 job codes depending on solvent use, location and nature of the described job. Our process of estimating personal exposures in each job code took account of changes over time in working practices and materials. We identified when important changes in processes and material formulation were introduced, so the 25 job codes were then sub-divided into 89 job-code eras. Each job code covered between one and nine eras. We then divided each job code into primary job tasks to produce a total of 127 job tasks. We found that each job code era had between one and eight tasks, but there was often considerable overlap between job tasks throughout the eras. Using reports from ‘expert’ interviews, we allocated an estimated fraction of the working day to each job task that made up a job code era. Finally, we reconstructed exposures using subjective modelling strategies for solvent absorbed via the inhalation route and the dermal route.\(^{16,17}\) Inhaled solvent levels were reconstructed using the previously validated methodology of Cherrie and Schneider.\(^{16}\) This model used assigned values for parameters that determine personal exposures. The exposure factors included the potential for the substance to become airborne; the methods by which the substance is handled; the use of local control measures to reduce exposure at source; and the amount of substance released from passive or fugitive sources. The personal exposure level is then estimated from this derived concentration, using parameters related to the time fraction the source is active, protection offered by any respiratory equipment worn and the degree of ventilation.

---

Figure 1. Flow chart showing recruitment of Q16 subjects.
relative to room volume. Values on a logarithmic scale are assigned to the model parameters depending on the assessor’s judgement and the exposure calculated using a simple multiplicative procedure. The exposure assessment is developed on an arbitrary scale, where an intrinsic emission of unity is chosen to be the substance’s occupational exposure limit or some other appropriate target concentration.

Where the job was identified as consisting of one or more tasks involving dermal exposure to solvents, a further reconstruction was done to determine how much solvent was likely to be absorbed. A combined exposure and uptake model was developed that allowed the dermal exposure to be expressed in terms equivalent to an airborne exposure level. Details of this are provided elsewhere. In summary, a deterministic model was expressed as a fraction of the UK occupational exposure limit (OEL) for the solvent or solvent mixture. For example, an AAI of 0.5 is indicative of an average solvent exposure of half of the UK OEL. A similar process was carried out to estimate exposures to lead.

Genetics

DNA extracted from 5 ml venous blood was used to genotype the enzymes GSTM1, GSTT1, GSTP1, NAT1, NAT2, SOD1, and CYP1A1 using previously published methods. Briefly, GSTM1 and GSTT1 were amplified in a multiplex polymerase chain reaction (PCR). As determination of the null genotype is based on the presence or absence of a PCR product, GSTM4 was co-amplified as an internal control. Polymorphisms at codons 105 and 114 of the GSTP1 gene were investigated by PCR amplification of the two regions of interest followed by digestion with the enzymes BsmA I or Cac 81. NAT1 genotype was determined by PCR amplification of the entire coding region along with an additional 278 base pairs (bp) of the 3’ untranslated region, resulting in a product of 1158 bp. This product was used in nested PCR/RFLP (RFLP, restriction fragment length polymorphism) analysis to identify the polymorphisms: C1095A, T1088A, C560A and a 9 bp deletion at nucleotide 1095. A second polymorphism at codon 114 of the NAT2 gene was determined by PCR amplification of the entire coding region along with an additional 278 base pairs (bp) of the 3’ untranslated region, resulting in a product of 1158 bp. This product was used in nested PCR/RFLP (RFLP, restriction fragment length polymorphism) analysis to identify the polymorphisms: C1095A, T1088A, C560A and a 9 bp deletion at nucleotide 1095. Genotyping of NAT2 was done by PCR amplification of the NAT2 gene, resulting in a product of 895 bp, followed by RFLP analysis to identify six common polymorphisms in Caucasian populations: A803G, G857A, G590A, C481T, T341C, and C282T. This allowed identification of fast and slow acetylator alleles. SOD1 was genotyped by PCR amplification of the regions of interest followed by RFLP analysis using the enzymes: Taq1, Sau3A, MboII, or BstNI to identify four common polymorphisms in the Scottish population: Gly21Ly, Gly93Arg, Glu100Gly and Ile113Thr. Three polymorphisms in the CYP1A1 gene were investigated at nucleotides 6235, 4889, and 4887 by PCR/RFLP analysis using the enzymes Mspl, BsrDI and Bsal. This allowed identification of the alleles M1, M2 and M4.

Statistical analyses

Statistical analyses used SPSS v.9.0. The relationships between test results and AAI were investigated using multiple linear regression or logistic regression, as appropriate. We adjusted for smoking and alcohol consumption in all cases. We adjusted for age for the neurological tests and Trail Making A and B tests. We adjusted for IQ in the case of all
other cognitive tests. Trail Making A, Trail Making B, Continuous performance test, Symbol digit substitution and Benton visual retention test error scores were log-transformed using natural logarithms before analysis. AAI was treated as a categorical variable (AAI < 1 vs. AAI ≥ 1) for the analyses shown in Table 2. The odds ratios in Table 2 are therefore measures of the estimated probability of impairment among those with AAI ≥ 1, versus those with AAI < 1.

Logistic regression analysis was also used to investigate the relationships between neurological deficits and AAI (AAI < 1 vs. AAI ≥ 1), controlling for each of the gene types in turn and for age, alcohol and smoking in each case. For GSTM1 and GSTT1, individuals were classified as zero if they were null genotype for that gene and one if they were the positive genotype. For NAT1 and NAT2, individuals were classified as zero if they had the slow acetylator phenotype and one if they had the fast acetylator phenotype (including fast/fast phenotype and fast/slow phenotype). For GSTP1, we tested 105I/114A (wild type) versus all other genotypes, and for CYPIA1 *1/1 (wild type) versus all other genotypes. Because of co-linearity between age and cumulative exposure, we used the average annual intensity of exposure metric, AAI, for the analyses.

Results

Overall, 120 subjects took part in this study over 15 months through 1998 and early 1999. Our initial case-control analyses failed to demonstrate any evidence of increased neurological or cognitive impairment among Q16 cases when compared to Q16 painter controls. Similarly, there were no statistically significant differences in neurological function between painter cases and painter controls as defined by the eight neurological questions. Analysis by Q16 status was restricted to the dockyard painter cases and controls, as the community controls were found to have significantly higher IQ. The mean AAI for all painters, including the four community controls who had worked as painters but not in the dockyard, (n = 82) was 0.82 times the OEL (range 0.02–2.2, SD 0.49). The mean duration of employment in solvent-exposed work for these painters was 22.9 years. The mean AAI for all non-painters (n = 38) was 0.03 times the OEL (range 0–0.41, SD 0.07). The mean duration of employment in solvent-exposed work for these non-painters was 8.4 years. On analysing the entire cohort (n = 120), adjusting for the effects of smoking (cigarettes/day), alcohol (units/week) and pre-morbid IQ (as estimated by NART), we found evidence of an association between increasing intensity of solvent exposure and poorer performance on a range of cognitive tests (Table 1), including those measuring visual memory, verbal memory and planning. The negative association between intensity of solvent exposure and mean grip strength, after adjusting for smoking, alcohol and age, was statistically significant (p = 0.002, Table 2). This represents a reduction in grip strength of 4.4 kgf, (∼10% reduction), per unit rise in AAI.

The distribution of the polymorphisms among the painters and community controls was not significantly different. One community control’s DNA failed to amplify for GSTP1 and CYPIA1.

Table 1 Results from the regression analysis for cognitive impairment (n = 120)

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
</table>
| Multiple regression analysis for exposure to solvents (AAI) adjusted for age, alcohol and smoking
| Trail Making A (s)     | 0.18  | 0.06  | 0.003 |
| Trail Making B (s)     | 0.29  | 0.07  | <0.001|
| Continuous Performance Test (milliseconds) | 0.05 | 0.03 | 0.079 |
| Paired Associate Learning Test (number correct) | −0.03 | 0.26 | 0.898 |
| Symbol Digit Substitution (mean response latency: 0.01 s) | 0.14 | 0.05 | 0.006 |
| Associate Recall Test (number correct) | −0.75 | 0.29 | 0.011 |
| Benton Visual Retention Test (number correct) | −0.62 | 0.30 | 0.043 |
| Benton Visual Retention Test (number of errors) | 0.23 | 0.11 | 0.032 |

†In these tests, a positive coefficient indicates deteriorating performance.
*Trail Making A, Trail Making B, Continuous performance test, Symbol digit substitution and Benton visual retention test error score were log-transformed before analysis.
No mutations were identified in SOD1. The results of genotyping for the other enzymes were fitted into the regression models individually. Owing to the small sample size, it was not possible to test for interactions between different enzymes. We found limited evidence for risk modification by several of the polymorphisms studied, and those results which are significant or which approach significance are reported in Table 3. NAT2 fast acetylator phenotype showed a non-significant association with coarse tremor, and a statistically significant association with nystagmus. There was a suggestion of an association between GSTM1 positive genotype and two-point discrimination.

### Discussion

Although chronic solvent intoxication is recognized in a number of European countries, in the UK it is not widely diagnosed by clinicians, nor is it scheduled for Industrial Injuries Benefits. Nevertheless, the association of chronic intoxication with neurological disease such as parkinsonism, and with impairment of cognitive function, is quite well-established epidemiologically.\(^{27,28}\) In 1992, we published in this journal a short series of case studies of patients with organic neurological disease, three of whom had been heavily exposed to solvents in a dockyard.\(^{29}\) This led us to an epidemiological study of all painters in this yard that showed evidence of both excess neuropsychological symptoms and personality change in relation to duration of exposure.\(^{12,30}\) This cohort formed the basis of the present more detailed study.

It was not practicable to study all painters using our detailed investigative methods, and our initial intention was to study all those who had prior evidence of increased symptoms as cases, contrasting them with fellow workers without symptoms and community controls without symptoms. If a high Q16 score was associated with neuropsychological impairment then a biased association with painting might arise, hence our use of an internal
undoubtedly, a low response rate made for a selection bias, and this may be the reason that we found no differences between painter cases and painter controls in terms of exposures or neuropsychological impairment. But this absence of differences between the original groups allowed us to carry out regression analysis across the whole sample, in relation to our very detailed estimates of exposure to a range of solvents. During this study we detected in several individuals a pattern of coarse tremor, cognitive deficit, impaired colour vision and reduction in vibration sense, sufficient to be labelled a syndrome. In this paper, we report the results of the epidemiological study and the various associations between exposure, genetic polymorphisms and neuro-psychological impairment.

We repeated our analyses in a sub-group (n = 82) after all exclusions for competing causes of neuropsychological impairment. The trend for worsening performance on psychometric testing with increasing solvent exposure persisted, although Benton visual retention test score was now no longer significant. This suggests that these associations are not due to confounding by other diseases. The 38 subjects excluded in this second analysis suffered from diabetes (1 case, 2 painter controls and 2 community controls), head injury, defined as loss of consciousness for >1 h, (4 cases, 1 painter control, 2 community controls and 1 painter with a Q-16 score of 3, 4 or 5), solvent exposure within the preceding 16 h (2 cases and 2 painter controls), previously diagnosed neurological disease, including neurofibromatosis (1 painter control), spinal muscular atrophy (1 case), multiple sclerosis (1 case), grand mal epilepsy (1 painter control), and brain abscess secondary to mastoiditis (1 painter control), alcohol consumption that day (1 painter case and 1 community control), substance misuse (1 case), use of anti-depressants or benzodiazepines (7 cases, 1 painter control and 5 community controls). Some individuals excluded from the second analysis may have been suffering from solvent-related illness. These exclusions would tend to obscure differences rather than amplify them. The small sample size may make extrapolation of our results less secure, but this does not invalidate the associations described within the group.

Analysis using average annual intensity of exposure as the independent variable showed that there was evidence of cognitive impairment in the heavily exposed men. While we cannot be sure that this relationship is causal, of the three paper and pencil tests and four computerized tests employed, six of the eight outcome measures showed statistically significant relationships with exposure (AAI), and all associations were in the direction of impairment. The evidence for neurological impairment and, in particular, peripheral neuropathy is less convincing. The impairment in mean grip strength in association with increasing intensity of exposure was statistically significant. Reduced grip strength has been described previously in solvent exposed micro-electronic workers. Our failure to demonstrate evidence of an effect on vibration perception may be an artefact of the study design. Vibration perception was categorized as abnormal if there was any delay (greater than a count of 3) in reporting loss of the perception of vibration when a vibrating tuning fork was stopped. This low threshold for abnormality may have obscured a true effect.

Our analysis of the genetic factors was based on the hypothesis that the risk of neuropsychological impairment would be modified by polymorphisms in GSTM1, GSTT1, GSTP1, NAT1, NAT2, SOD1 or CYP1A1. The genetic studies did not identify many significant associations with impairment. Although this may be due to an absence of effect, it may be that the sample size was simply too small. We undertook multiple comparisons for these polymorphisms, and cannot rule out the possibility that any significant results are chance associations. We consider that any positive findings should be regarded as simply suggestive of areas for further investigation. There was a protective effect for GSTM1 positive genotype on two-point discrimination for both index fingers, though this was not significant for the right finger. This accords with published data associating GSTM1 null genotype with a diagnosis of Chronic Toxic Encephalopathy. Only a proportion of the men in this study would meet that criterion, and this may explain our failure to confirm an association between cognitive impairment and GSTM1 genotype. There was a suggestion that the GSTT1-positive genotype may be protective against coarse tremor. A previous study has suggested a link between GSTT1 null genotype and increased risk of Parkinson’s disease. NAT2 fast acetylator phenotype showed a non-significant increased odds ratio for coarse tremor and a significantly increased odds ratio for nystagmus, contrasting with a previous study that found an association between slow acetylator status and familial Parkinson’s disease.

There are many problems in carrying out studies such as these. The time-consuming nature of the investigations and the demands they place on participants mean that some selection bias is almost unavoidable and very powerful studies are difficult to achieve. Confounding factors include age and impairment of IQ, alcohol and tobacco habit, and
co-exposures to heavy metals such as lead. Usually estimation of exposures is very inexact and sometimes, as in our original study, is no better than years worked. Multiple candidate gene polymorphisms mean that very large studies are necessary to find convincing associations. We acknowledge a likely selection bias, but have obtained very detailed estimates of exposure to a range of solvents in all our subjects. We have been able to take account of the above confounders, including making estimates of heavy metal exposures (which we believe to have been at levels unlikely to have an adverse effect). Our results are at least consistent both internally and with the published literature, and go some way to supporting our previous clinical impressions of a neuro-psychological syndrome associated with heavy solvent exposure. They also indicate that such exposure, at or above the current occupational standards, is the dominant determinant of these subjects’ impairment and that genetic factors, though possibly relevant, are clearly less important.

Taken with our clinical experience, this study suggests that there is real cause for concern about the risks of neuro-psychological damage among workers exposed to organic solvents in the UK, even at concentrations around the occupational exposure level. We believe that lack of recognition of these syndromes by physicians, perhaps related to a failure to take careful occupational histories, has led to them being missed and thus to a relative complacency among workers and their employers. Only when doctors start diagnosing chronic solvent intoxication will people start to treat these chemicals with the respect they deserve.

Acknowledgements

We thank the British Occupational Health Research Foundation, the Health and Safety Executive and Grampian University Hospitals Research Endowment Trust for support for this study. FD was the recipient of a travelling scholarship from the William Ramsay Henderson Trust. We gratefully acknowledge the support of these bodies.

References


