Neurobehavioural changes and persistence of complaints in workers exposed to styrene in a polyester boat building plant: influence of exposure characteristics and microsomal epoxide hydrolase phenotype

M K Viaene, W Pauwels, H Veulemans, H A Roels, R Masschelein

Abstract

Objectives—To investigate neurobehavioural effects and the persistence of complaints in workers exposed to styrene relative to exposure characteristics and the enzyme microsomal epoxide hydrolase (mEH) activity.

Methods—A cross sectional study was performed in a retrospective cohort of workers of a polyester boat building plant 3 years after the main activity shut down in 1989. Workers still currently exposed to a much lower concentration of styrene in air than before ($n=27$) and formerly exposed workers ($n=90$) were compared with matched control workers ($n=64$). Currently and formerly exposed workers laminated 4700 and 3610 hours on average at mean exposure to styrene concentrations of 148 and 157 mg/m^3 respectively. A structured neurological anamnesis into former and present complaints, the NSC-60 questionnaire, and computer assisted neurobehavioural tests (NES) were administered. The mEH phenotype activity was measured in lymphocytes with a novel gas chromatography-mass spectrometry (GC-MS) method.

Results—For the period before 1989, currently and formerly exposed workers reported more complaints than control workers which related well with the mean exposure to airborne styrene concentration ($p=0.03$). Most complaints disappeared after the end of exposure, although the chest, equilibrium, and somatic category scores of NSC-60 and the number of workers reporting diminished sense of smell remained increased in formerly exposed workers ($p\leq 0.05$). Symbol-digit substitution and digit span forwards test results were worse in currently and formerly exposed workers ($p\leq 0.01$). In the combined group of currently and formerly exposed workers, the symbol-digit substitution and colour-word vigilance results related well to duration of exposure ($p<0.01$ and $p=0.03$) and mEH phenotype activity ($p=0.01$ and $p=0.05$), whereas the digit span forwards results only showed associations of borderline significance (duration of exposure ($p=0.08$) and mEH phenotype activity ($p=0.08$)).

Conclusion—Most subjective symptoms were reversible but some persisted after the end of exposure to styrene, whereas dysfunction of visuomotor performance and perceptual speed seemed to persist. Duration of exposure at lamination tasks and the interaction, duration of exposure $\times$ concentration of exposure, were found to be the best predictors of worsening visuomotor and perceptual speed performances. Activity of the mEH phenotype may play a modulating part in styrene neurotoxicity. The results suggested that less than 10 years of exposure to atmospheric styrene at an average concentration of 155 mg/m^3 may result in persistent neurotoxic effects.

(Occup Environ Med 2001;58:103–112)

Keywords: styrene; neurobehavioural performance; neurotoxicity; microsomal epoxide hydrolase

Cross sectional studies in workers exposed to styrene showed a dose dependent excess of subjective symptoms—for example, complaints of headache, dizziness, increased irritability, and concentration difficulties.^{1,2} Objective neurotoxic effects have also been found associated with occupational exposure to styrene, including diminished attention, worsened visuomotor performance, impaired memory functions,^{3,4} and slowing of peripheral nerve conduction velocities.^{5,6} These neurotoxic effects were consistently present in most of the studies dealing with current exposure to styrene, however, it is not well known whether they persist in the aftermath of a decrease or the end of exposure. Follow up studies in workers who had been exposed to mixtures of solvents reported the persistence of subclinical neurological, psychiatric, and neurobehavioural effects.⁷

Factors such as exposure intensity, duration of exposure, alcohol consumption, personality, and pre-morbid intelligence may be of some importance in the development of complaints and neurobehavioural deficits,⁸ but individual differences in the styrene metabolic pathway might also play a modulating part in the development of neurotoxic effects. It should be pointed out that in humans styrene is preferentially oxidised to styrene-7,8-epoxide, which in turn is metabolised to styrene glycol by a

Department of
Occupational
Medicine, Catholic
University of Leuven,
Leuven, Belgium
M K Viaene
R Masschelein

Laboratory of
Occupational
Toxicology and
Hygiene
W Pauwels
H Veulemans

Unité de Toxicologie
Industrielle et
Médecine du Travail,
Université Catholique
de Louvain, Burssels,
Belgium
H A Roels

Correspondence to:
Dr M K Viaene, Department
of Occupational Medicine,
University Hospital St
Rafaël, p/a Pas 285, 2440
Geel, Belgium
leenders.viaene@pandora.be

Accepted 5 October 2000

microsomal epoxide hydrolase (mEH) enzyme.⁹ In humans, mEH shows polymorphic genotypes at amino acid positions 113 and 139 that substantially alter mEH activity between the different isoenzymes.¹⁰ Studies of the enzyme activity of the mEH phenotype showed a twofold to 63-fold variation that may partly be due to methodological differences.^{11–14} Competitive inhibition of mEH has been reported to cause an excess of central nervous system toxicity¹⁵ suggesting that mEH with different activities may have important neurotoxicological consequences associated with exposure to styrene.

The present investigation is a retrospective cohort study in workers exposed to styrene from a polyester boat building plant who constituted one historical cohort that experienced the same exposure situation until the usual industrial activity stopped in 1989. Most of the workforce was dismissed. They formed the basis for the formerly exposed group of the present study. A limited number of workers continued to work in repair and maintenance of styrene polyester boats but their exposure to styrene dropped by a factor of about four at the time of the present study. Almost all of them constituted the currently exposed group.

The present epidemiological study was carried out in the winter of 1992–3. Its first aim was to compare on a group basis possible neurotoxic symptoms and neurobehavioural effects in formerly and currently exposed workers with a group of control workers who had never been occupationally exposed to organic solvents or other neurotoxic chemicals. The second aim was to assess in the combined exposed group (currently and formerly exposed workers) possible associations between neurotoxic effects and historical industrial hygiene data on exposure to airborne styrene,^{16–17} such as concentration, duration of exposure, or cumulative exposure. The third aim of the study was to investigate whether there may exist a link between mEH phenotype activity and neurobehavioural performance in workers exposed to styrene. The study also had the potential to investigate whether neurotoxic effects linked to styrene were persistent 3 years after the end of or a substantial decrease in moderate to high occupational exposure to styrene.

Subjects and methods

STUDY POPULATION

The production of polyester boats started early 1982. From 1984 to 1987, we carried out several cross sectional industrial hygiene studies in the styrene polyester boat building plant with a workforce that at that time consisted of 185 exposed male employees.^{16–17} In 1989, the usual production activities stopped for economic reasons and only repair and maintenance activities were continued with a reduced workforce ($n=31$) that could continue to work at the plant. Due to the changed industrial activities, the exposure to styrene dropped considerably to an average of 41 mg/m³. The other workers ($n=154$) were made redundant on the basis of age (workers more than 50 years old retired) or

family income (people who were economically less important to their family—for example, being married to a working spouse and having no children—were preferentially dismissed).

Three years after the event in 1989, we retraced the 185 workers enrolled in our previous studies.^{16–17} Figure 1 shows how the population of the present retrospective study was recruited and found to be eligible on the basis of initial defined exclusion criteria (cranio-cerebral trauma with 10 minutes or more unconsciousness, alcoholism (five or more alcoholic drinks/day),¹⁸ epilepsy, major depression, or other major neurological disease). In total, 117 subjects of the original workforce participated in the present study (participation rate 63%) consisting of 90 formerly exposed workers and 27 workers currently exposed to low concentrations of styrene. A group of 111 control male subjects (electricians and assembly workers) from another plant were invited to cooperate in the study. Age, sex, and schooling level were matched as closely as possible across groups. Twenty seven refused to participate and 20 were excluded according to initial defined exclusion criteria (the same criteria as those applied to the exposed subjects as well as the absence of a history of occupational exposure to solvents). Eventually, 64 control workers (58%) were enrolled in the study. The neurobehavioural testing of these groups occurred from November 1992 to March 1993.

Unexpectedly, in February 1994 the plant with the workers still employed at low exposure planned to stop activities. A strike period of 1 year followed and eventually the plant shut down in February 1995. These events caused a delay in blood sampling for characterisation of the mEH phenotype. Blood was taken from September 1994 to June 1995—that is, about 6–15 months after the end of exposure in currently exposed workers and 5–6 years after the formerly exposed workers left the plant. In total, 56 workers (48%) from the combined group of currently and formerly exposed workers were willing to provide a blood sample.

CHARACTERISATION OF EXPOSURE TO STYRENE

Despite a well designed ventilation system, 4%–9% of the personal air samples exceeded the previous American Conference of Government Industrial Hygienists (ACGIH) threshold limit value time weighted average (TLV-TWA)¹⁹ of 213 mg/m³ and 4% of the peak exposures at the most exposed job sites exceeded the previous TLV-STEL (short time exposure limit)¹⁹ of 426 mg/m³ as shown in the earlier industrial hygiene studies from 1984 to 1987.^{16–17} Detailed individual records were kept for every worker as to the different tasks during his daily working hours (job characteristics, duration, and location of every individual task) allowing calculation of the exact total number of hours every individual worker was effectively exposed to styrene from 1982 to 1993 for currently and from 1982 to 1989 for formerly exposed workers. Also, instant measurements with colorimetric Dräger tubes were carried out daily by the industrial hygienist at locations where exposure to styrene was suspected to be

Figure 1 Recruitment of study population. Lost to examination: no address, moved abroad, dead. Exclusion because of confounding diseases (alcoholism, $n=6$; craniocerebral trauma, $n=5$; epilepsy, $n=1$; diabetes, $n=1$; severe depression, $n=3$) or current exposure to solvents in the formerly exposed group ($n=1$).

high (comparable with STEL measurements). These data were collected meticulously with reference to the time of the day, the location of the job site, and the work done (in total 4145 measurements). These Dräger tube data (y) were validated by comparison with peak exposure measurements (15 min personal air sampling, $n=80$) of the industrial hygiene studies (x). A good correlation was found between the two sets of data (linear regression: $y=1.066x$; $R^2=0.83$, $p<0.01$). On the basis of the daily Dräger measurements, an overall mean airborne exposure concentration was calculated for each person involved with lamination tasks that caused the bulk of the exposure to styrene. The site of each lamination job was characterised every year by a yearly average median exposure. A cumulative exposure index was then calculated for each worker through addition of the products obtained by multiplying the yearly average exposure at the site of each specific lamination job with the time (hours) yearly spent at that site. The overall mean concentration of exposure to styrene of each individual worker was obtained by dividing his cumulative exposure index by the total number of hours he was involved with lamination tasks from 1982 to 1989.²⁰ Because the workers also did other tasks with much less or nearly no exposure to styrene, a mean time

weighted exposure to styrene concentration was also calculated by dividing cumulative exposure by the total number of hours worked in the plant. At the time of the present study, biomonitoring data and full shift personal air sampling confirmed mean (SD, range) low exposure to styrene in currently exposed workers to be 41 (24, 11.5–120) mg/m^3 . Urinary mandelic and phenylglyoxylic acids showed good relations with styrene in the air suggesting that significant skin absorption of styrene was unlikely to occur (gloves were worn continuously).

SCREENING FOR NEUROTOXICITY

Before testing, the participants completed the NSC-60 questionnaire and a self administered questionnaire inquiring about lifestyle and medical history. The exposed and control workers were screened by one and the same interviewer with a structured interview about complaints at work (structured neurological anamnesis of complaints: SNAC). This questionnaire included complaints of headache, personality changes, concentration or memory difficulties, changed sensory functions in hand or foot, pain sensations, increased clumsiness, tremor, sleeping difficulties, diminished strength or endurance, diminished vision, hearing loss, tinnitus, altered smell, intolerance for alcohol, increased tiredness in the morning, increased tiredness in the evening, loss of equilibrium or dizziness, and "other complaints". The workers were asked if they ever had the complaint during their work as a laminator (exposure to styrene) or as an electrician or assembly worker (control). The answers were scored "0" if the complaint was absent and "1" if present. If the complaint was present, it was asked if it was still present at the time of the interview (1992–3). If the complaint was no longer present the answer was scored "0" (not persistent), if still present it was scored "1" (persistent).

The standardised and validated neurotoxicity symptom checklist-60 (NSC-60)²¹ was administered for screening complaints at the time of the study. The NSC-60 is a self administered questionnaire which has 10 categories of questions about personality, concentration, equilibrium, sensorimotor functioning, general somatic complaints, sleeping problems, mood, chest symptoms, fatigue, and a general category of neurotoxicity symptoms. The mean score obtained for each category is a value between 1 and 4, and is called the category score. The category scores were dichotomised to "0" (score ≤ 33 th percentile of the control group) and "1" (score >33 th percentile).

A computer assisted neurological test battery, the neurobehavioural evaluation system (NES, version 4.38), was used to administer neurobehavioural performance tests.²² The selected NES tests were hand-eye coordination, simple reaction time, symbol-digit substitution to assess visuomotor performance, associated learning, associated recall (number correct/associated learning $\times 100$), digit span forwards, and colour-word vigilance to test concentration and memory. The interviewer

was blinded to the intensity and duration of exposure of the workers.

ANALYSIS OF mEH PHENOTYPE ACTIVITY

Buffy coat was collected from two blood samples of 8 ml, each withdrawn by venepuncture with Vacutainer CPTTM cell preparation tubes (Becton-Dickinson USA) containing sodium citrate as anticoagulant. The mononuclear cells were separated and rinsed twice with phosphate buffered saline and then S-TKM buffer (0.25 M sucrose, 80 mM Tris, 25 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, pH 7.35) was added to obtain a 2 ml suspension which was sonicated on ice followed by microcentrifugation at 9000g at 4°C. Aliquots of the S9 fraction were taken for protein measurement by the BIORAD method,²³ and typical protein concentrations were between 1000 and 2000 µg/ml buffer solution. The S9 fraction solutions were stored at -80°C for further use.

A novel mEH assay has been developed in which a labelled styrene epoxide was used as substrate. Briefly 5 µl of a 10⁻⁴ M styrene glycol solution in isotonic saline was added to 240 µl of the S9 fraction as internal standard. The reaction was initiated by addition of 5 µl [³H]styrene-7,8-oxide (10⁻⁴ M in acetone) and incubation for 30 minutes in an oil bath at 37°C. The incubation mixture was extracted with 300 µl ethyl acetate; 100 µl of the ethyl acetate layer was dried under an N₂ stream. The mixture was derivatised for 30 minutes at 110°C with BSTFA/acetonitrile (1/10), and analysed by GC-MS. The derivatised styrene glycol samples were injected by means of a solid injector (kept at 253°C) into an HP 5890 series-II gas chromatograph coupled with an HP 5970 quadrupole mass spectrometer. For chromatographic separation of the compounds of interest, a DB-5-ms fused silica capillary column (30 m×0.25 mm, 0.12 µm phase thickness, Alltech Associates, USA) was used with a source pressure of 0.006 Pa, the interface was kept at 270°C and the oven programmed at 7°C/minute from 100°C–140°C and at 30°C/minute from 140°C–300°C. The mass spectrometer operated in the electron impact mode with an ionisation energy of 70 eV. By referring to a calibration curve, the monitoring of ion-185 (trimethylsilylated deuterated styrene glycol) relative to ion-174 (undeuterated analogue, internal standard) allowed an accurate measurement of the mEH mediated formation of styrene glycol in the incubated samples. The mEH phenotype activity was assessed as the mean of triplicate incubations.

STATISTICAL ANALYSIS

The statistical analysis was performed with the statistical package for social sciences (SPSS) for Windows, version 7.0. When needed log normal transformation was applied (number of SNAC complaints, symbol-digit substitution, simple reaction time). Fisher's exact test (double sided) and odds ratios (ORs) were calculated for dichotomous outcome variables—for example, persistent SNAC complaints and NSC-60 categories. For comparisons between control workers, and currently and formerly

exposed workers one way analysis of variance (ANOVA) and Duncan's multiple range test were used for continuous outcome variables (number of complaints and NES test results). General ANOVA models were built allowing for age, alcohol consumption, years of welding, and the number of years at school as covariables for the NES results and additionally smoking and personality score for the questionnaire results. General ANOVA results are presented with ϵ^2 values (proportion of variance in the dependent variable explained by the difference across the groups). Multiple linear regression or multiple logistic regression analyses (dichotomous variables) were applied on the combined exposed group (currently and formerly exposed workers) to study dose-effect or response relations. The regression models were run with the forced entry method by introducing one of the exposure indices at the time (duration of exposure, mean concentration of exposure, interaction term between both, or cumulative exposure) together with the dependent variables and covariables. For the SNAC results and the NSC-60 category scores, age, alcohol consumption, the number of years at school, years of welding, smoking, and personality score were the covariables. In multiple regression models of the NES results, age, alcohol consumption, the number of years at school, and years of welding were included as independent covariables. Belonging to the current exposure group or not (1 or 0) was also included as a dummy variable in the regression analyses next to the exposure indices and the covariables to test separately the effect of being currently exposed. For the effect of mEH phenotype activity on the outcome variables, alcohol consumption and age were included as covariables. The level of significance was set at a p value ≤ 0.05 .

Results

CHARACTERISTICS OF THE STUDY GROUPS

The participating groups did not differ greatly for the covariables of interest (table 1). Despite efforts to match the groups according to schooling level, the currently and formerly exposed workers had fewer years of schooling and thus also a lower schooling level than the controls. It is interesting to note that in the three groups the scores for motivation of participation in the study were equally high (> 98%) and that their personality scores were similar. The currently exposed workers who laminated polyester from 1982 to 1992, on average were exposed for 4700 hours, whereas the formerly exposed workers laminated only from 1982 to 1989 (about 1100 hours less). The overall mean concentration of exposure to styrene during their lamination tasks from 1982 to 1989 amounted to 148 and 157 mg/m³ for currently and formerly exposed workers respectively, the combined exposed workers on average had 155 mg/m³ of exposure. Thus, before the 1989 event the exposure to styrene did not differ significantly between currently and formerly exposed workers. This was also shown by the time weighted exposure concentration.

Table 1 Characteristics (mean (SD, range)) of the exposed and control groups

	Currently exposed (n=27)	Formerly exposed (n=90)	Controls (n=64)
Age (y)	37.1 (7.9, 27–51)	38.1 (10.3, 26–64)	40.3 (11.3, 25–63)
Alcohol consumption (n drinks/week)	8.6 (8.6, 0–30)	8.7 (8.3, 0–35)	9.7 (10.2, 0–35)
Sleeping (n hours)	7.1 (0.8, 5–8)	7.1 (0.9, 5–9)	7.5 (0.8, 5–9)
Smoking (n cigarettes/day)	5.0 (7.5, 0–20)	6.9 (9.2, 0–30)	3.9 (7.2, 0–25)
Motivation (%)	98.3 (5.4, 75–100)	98.1 (6.8, 50–100)	99.3 (3.6, 80–100)
Personality score (NSC-60)*	1.50 (0.54, 1.00–3.00)	1.50 (0.44, 1.00–2.57)	1.53 (0.48, 1.00–3.29)
Years of schooling	11 (2, 7–14)	11 (2, 7–21)	13 (3, 7–24)
Schooling level:			
Primary	22%	13%	5%
First grade	63%	67%	61%
Second grade or above	15%	20%	34%
Duration of exposure (hours of lamination)†	4700 (1870, 530–7505)	3610 (2000, 165–8098)	—
Overall concentration of exposure to styrene while laminating (mg/m ³)‡	148 (14.1, 124–173)	157 (19.0, 99–189)	—
Time weighted exposure (mg/m ³)§	68 (31.1, 6–129)	70 (43.9, 1–174)	—

*Neurotoxicity symptom checklist-60²¹: score between 1 (never complains) and 4 (always complains).

†Referring to period 1982–92 for the currently exposed group and to period 1982–89 for formerly exposed group.

‡Mean calculated from the average exposure concentration of each person at lamination tasks for the period 1982–9 (for details, see section subjects and methods).

§Mean calculated from the individual cumulative exposure concentrations of styrene divided by the total numbers of working hours in the plant. Period 1982–9 for both exposed groups.

The available characteristics for the workers exposed to styrene who did not participate had a similar age distribution (mean (SD, range) 39.0 (10.6, 26–65)), but their educational level was lower than in participating currently and formerly exposed workers (primary 34%, first grade 58%, second grade or higher 6%). The mean duration of exposure (3230 h) and overall mean exposure concentration (146 mg/m³) were not different from those of participating exposed workers.

QUESTIONNAIRE RESULTS

The results for each single complaint question of the SNAC questionnaire are summarised in table 2. During the moderate to high exposure to styrene, the combined exposed group had a significantly higher frequency of complaints such as irritability, fatigue in the evening, headache, common colds, intolerance for alcohol, diminished endurance, memory, and smell than did the controls. Despite not having been exposed for 3 years at the time of the SNAC questionnaire, 10 of the formerly exposed subjects still complained of a persistently diminished sense of smell ($p<0.01$). In the currently exposed workers, who experienced lowered

exposure to styrene condition after 1989 (mainly due to a new type of boat introduced at the beginning of 1992), some complaints were still overreported—such as fatigue in the evening, common colds, and sleeping difficulties.

By comparison with control workers, of which 29 workers (45%) ever experienced some complaint during work, significantly more formerly exposed workers (79%) experienced some kind of complaint during lamination tasks versus only 52% in currently exposed workers (table 3). Although both currently and formerly exposed workers had worked under the same exposure conditions until 1989, there was a significantly higher proportion of currently exposed workers (48%) who never experienced complaints during lamination than formerly exposed workers (21%) (Fisher's exact test, $p<0.01$; OR 3.5; 95% confidence interval (95% CI) 1.4–8.6). The number of complaints per person during exposure was twice as high in currently exposed and four times as high in formerly exposed workers compared with controls (one way ANOVA, $p<0.01$). The excess of complaints in the exposed groups remained unchanged after

Table 2 Structured neurological anamnesis of complaints (SNAC): results (n (%)) on single complaint questions

Complaint	Ever experienced complaints during work (n)				Still experiencing complaints at the time of anamnesis (n)		
	Moderate to high styrene exposure 1982–9						
	Current exposure (n=27)	Former exposure (n=90)	Current+former exposure (n=117)	Controls CG (n=64)	Low current exposure (n=27)	Exposure ended 3 years ago (n=90)	Controls (n=64)
Irritability	4 (15)	24 (27)	28 (24)**	4 (6)	3 (11)	3 (3)	4 (6)
Fatigue in the evening	12 (44)	47 (52)	59 (50)**	9 (14)	8 (30)*	5 (6)	8 (13)
Headache	0 (0)‡	13 (14)	13 (11)**	0 (0)	0 (0)	4 (3)	0 (0)
Common colds	5 (19)	13 (14)	18 (15)**	1 (2)	5 (19)**	4 (3)	0 (0)
Sleeping difficulties	7 (26)	14 (16)	21 (18)+	5 (8)	6 (22)*	2 (2)	4 (6)
Alcohol intolerance	1 (4)	15 (17)	16 (14)**	0 (0)	1 (4)	3 (3)	0 (0)
Diminished endurance	1 (4)	12 (13)	13 (11)**	0 (0)	1 (4)	5 (6)+	0 (0)
Diminished memory	0 (0)	12 (13)	12 (10)*	1 (2)	0 (0)	3 (3)	0 (0)
Imbalance	1 (4)	7 (8)	8 (7)+	0 (0)	1 (4)	1 (1)	0 (0)
Diminished smell	2 (7)	14 (16)	16 (14)**	0 (0)	2 (7)+	10 (11)**	0 (0)
Morning fatigue	0 (0)	4 (4)	4 (3)	2 (3)	0 (0)	3 (3)	2 (3)
Anorexia	0 (0)	6 (7)	6 (5)	2 (3)	0 (0)	0 (0)	1 (2)
Pyrosis	2 (7)	12 (13)	14 (12)	6 (9)	1 (7)	4 (3)	3 (5)
Other complaints†	4 (15)‡	34 (38)	38 (32)	10 (16)	4 (15)	9 (10)	4 (6)

Fisher's exact test: +0.05< p ≤0.1, * p ≤0.05, ** p ≤0.01.

†Skin problems, lumbago, local overuse syndromes—for example, tendinitis or carpal tunnel, stress, distortions, or fractures.

‡1982–9: significantly lower in current than former exposure, p ≤0.05.

Table 3 Structured neurological anamnesis of complaints (SNAC): sum of all complaint questions

Study group†	Ever experienced a complaint during work		Still experiencing the complaints at the time of the anamnesis	
Currently exposed (n=27):				
People with complaints (n (%))	14 (52)		11 (41)	
OR (95% CI) Fisher's exact test	1.30 (0.53 to 5.58)		1.5 (0.37 to 13.05)	
Formerly exposed (n=90):				
People with complaints (n (%))	71 (79)**		28 (31)	
OR (95% CI) Fisher's exact test	4.5 (2.3 to 9.0)		0.99 (0 to 1.99)	
Controls (n=64):				
People with complaints (n (%))	29 (45)		20 (31)	
	Unadjusted results‡	Adjusted results‡	Unadjusted results‡	Adjusted results‡
Complaints per person (mean (SD, range)):				
One way ANOVA, p value	<0.01		0.07	
Currently exposed	1.4 (1.7, 0–5)§	–0.3 (1.6, –2.3 to 3.3)	1.2 (1.6, 0 to 5)¶	0.6 (1.6, –0.9 to 4.4)¶
Formerly exposed	2.5 (2.1, 0–8)¶	0.8 (2.1, –2.7 to 6.3)¶	0.6 (1.1, 0 to 8)	–0.0 (1.2, –0.8 to 6.5)
Controls	0.6 (0.8, 0–3)	–0.9 (1.0, –2.8 to 2.2)	0.4 (0.7, 0 to 3)	0.2 (0.7, –0.9 to 2.5)

**p<0.01.

†At the time of anamnesis (1992–3) after shut down of original production activities in 1989: the currently exposed group had 3 years of low exposure to styrene, and the formerly exposed group had ended exposure 3 years previously. Until 1989, both groups had the same moderate to high exposure to styrene.

‡Unadjusted results=raw data; adjusted results are the raw data adjusted for age, schooling, alcohol use, years of welding, smoking, and personality score.

§p<0.05 v controls; ¶p<0.05 v the two other groups, Duncan's multiple range test.

allowing for covariables in the model—such as age, schooling, alcohol consumption, years of welding, smoking, and personality score (ANCOVA: exposure group, $\epsilon^2=0.18$, $p<0.01$; none of the covariables reached significance). For the period after the 1989 event, low exposure for currently exposed workers and 3 years after the end of exposure for formerly exposed workers, the proportion of formerly exposed workers still reporting complaints markedly decreased from 79% to 31%. The mean number of complaints per person in formerly exposed workers drastically decreased as well and did not differ from that in controls, whereas currently exposed workers still reported more complaints than the two other groups (one way ANOVA, $p=0.07$). Currently exposed workers still showed an excess number of complaints after allowing for the covariables in the model (ANCOVA: exposure group, $\epsilon^2=0.03$, $p=0.07$; none of the covariables reached significance).

For the NSC-60 questionnaire, significantly more formerly exposed workers exceeded the cut off scores of several categories: chest (formerly exposed 36 (40%) v control workers 14 (22%), $p=0.02$); equilibrium (formerly exposed 28 (31%) v control workers 10 (16%), $p=0.04$); and somatic complaints (formerly exposed 66 (73%) v control workers 34 (53%), $p<0.01$). In currently exposed workers only the equilibrium category score reached borderline significance (currently exposed nine (33%) v control workers 10 (16%), $p=0.09$). The proportions of workers that exceeded the cut off scores for the chest and somatic complaints categories were similar in currently and formerly exposed workers, nine (33%) v 36 (40%) and 18 (67%) v 66 (73%) respectively.

NES TEST RESULTS

In general, currently and formerly exposed workers performed worse than control workers (table 4). One way ANOVA and Duncan's multiple range test showed that compared with control workers currently and formerly exposed workers had significantly worse test results for symbol-digit substitution ($p=0.01$)

and digit span forwards ($p<0.01$). Hand-eye coordination was significantly ($p<0.01$) worse only in formerly exposed workers compared with control workers. After allowing for covariables (age, alcohol consumption, number of years at school, and years of welding) the group effect for symbol-digit substitution, digit span forwards, and hand-eye coordination remained unchanged (ANCOVA: $\epsilon^2=0.05$, $p=0.01$; $\epsilon^2=0.09$, $p<0.01$; $\epsilon^2=0.05$, $p<0.01$, respectively). Age was a significant covariable for the three neurobehavioural tests (symbol-digit substitution, $\epsilon^2=0.32$; digit span forwards, $\epsilon^2=0.06$; hand-eye coordination, $\epsilon^2=0.06$; all $p<0.01$) and, also, the years of schooling explained a significant part of the symbol-digit substitution variance ($\epsilon^2=0.05$, $p<0.01$). Simple reaction time, colour-word vigilance, associated learning, and associated recall did not differ significantly between the groups.

ACTIVITY OF THE mEH PHENOTYPE

The assay for the activity of mEH phenotype had a coefficient of variation of 13% for three repeated measurements in the same person. Enzyme assay blanks with incubation of heat denatured S9-fraction showed a non-enzymatic conversion of 3%–4% of the substrate into [$^3\text{H}_s$]styrene glycol. The mEH phenotype activity was normally distributed in the studied population and ranged from 0.54 to 2.68 pmol/mg protein/min (mean (SD) 1.57 (0.49)) which represented a fivefold variation in mEH phenotype activity between people.

DOSE-EFFECT OR DOSE-RESPONSE RELATIONS

In multiple linear regression, the number of complaints per person in currently and formerly exposed workers during the period of high exposure (thus before the 1989 event) was significantly related to the individual overall mean concentration of exposure to styrene (regression coefficient $\beta=0.25$, $p=0.03$), but not to duration of exposure ($\beta=0.09$, $p=0.34$) or cumulative exposure ($\beta=0.08$, $p=0.44$). The interaction term concentration of exposure x duration of exposure did not reach significance ($\beta=0.13$, $p=0.19$). None of the cov-

Table 4 Neurobehavioural examination system (NES) results

NES tests*	One way ANOVA†‡ p value	Current exposure (n=27)		Former exposure (n=90)		Controls (n=64)		Dose-effect relation††	
		Unadjusted results**		Adjusted results**		Unadjusted results**		Duration of exposure	
		Unadjusted results**	Adjusted results**	Unadjusted results**	Adjusted results**	Unadjusted results**	Adjusted results**	β	p Value
Symbol-digit substitution§ (s/digit)	0.01	2.6 (1.2,† 2.1–3.6)	1.0 (1.2,† 0.8–1.5)	2.5 (1.2,† 1.7–4.9)	0.9 (1.1, 0.8–1.2)	2.4 (1.2, 2.0–4.0)	0.9 (1.1, 0.8–1.2)	0.21	<0.01
Hand-eye coordination¶ (root mean squared error)	<0.01	1.8 (0.3, 1.3 to 2.7)	–0.0 (0.3, –0.56 to 0.73)	1.9 (0.3,† 1.3 to 2.7)	–0.1 (0.2, –0.4 to 0.8)	1.7 (0.2, 1.3 to 2.6)	–0.1 (0.2, –0.4 to 0.8)	0.18	>0.10
Digit span forwards¶ (mean number)	<0.01	5.4 (0.9,† 3.7 to 7.0)	–0.33 (0.9,† –2.26 to 1.07)	5.6 (0.9,† 3.9 to 8.0)	0.3 (0.9, –1.8 to 2.0)	6.2 (1.0, 4.0 to 7.9)	0.3 (0.9, –1.8 to 2.0)	–0.16	0.08
Simple reaction time§ (ms)	>0.10	204 (1.2, 151 to 324)	1.1 (1.2, 0.8 to 1.6)	197 (1.2, 118 to 381)	1.0 (1.2, 0.7 to 1.4)	192 (1.6, 132 to 292)	1.0 (1.2, 0.7 to 1.4)	0.08	>0.10
Colour-word vigilance¶ (ms)	>0.01	553 (67, 444 to 666)	–1.9 (65.6, –122 to 08)	558 (62.4, 445 to 749)	–3.5 (66.7, –98 to 140)	558 (74, 449 to 742)	–3.5 (66.7, –98 to 140)	0.21	0.03
Associate learning¶ (number correct)	>0.10	6.4 (2.3, 3 to 9)	–0.1 (2.2, –4.0 to 3.3)	6.3 (2.1, 2 to 9)	0.1 (2.0, –5.1 to 3.5)	6.9 (2.1, 2 to 9)	0.1 (2.0, –5.1 to 3.5)	–0.18	0.05
Associate recall (%)¶	>0.10	102.4 (31.4, 40 to 200)	9.0 (29.1, –45 to 103)	89.8 (25.3, 17 to 150)	–1.3 (27.5, –77 to 90)	90.8 (27.9, 20 to 180)	–1.3 (27.5, –77 to 90)	0.04	>0.10

*Neurobehavioural examination system. For details, see subjects and methods.

†p<0.05 v other two groups, ANOVA one way Duncan's multiple range test.

‡Log normal distribution: geometric mean (geometric SD, range).

¶Mean (SD, range).

**Unadjusted results are raw data; adjusted results are the raw data adjusted for age, schooling, years of welding, and alcohol use.

††Multiple linear regression analysis. Regression coefficients (β) and p values for the predictor variables, duration of exposure, cumulative exposure, and the interaction term duration×concentration of exposure are presented. Overall mean concentration of exposure: not significant predictor (β<0.14, p>0.17).

variables (age, years at school, years of welding, personality score, smoking, alcohol use) was significantly associated with the number of complaints (all p>0.2). Neither exposure indices, nor any of the covariables explained the persistence of complaints at the time of the interview in formerly exposed workers (multiple logistic regression, p≥0.38). Allowing for the overall mean concentration of exposure and the covariables in the model, the dummy variable “being currently exposed or not (1 or 0)” showed a significant association with the number of complaints during exposure. Being currently exposed gave significantly less complaints than being formerly exposed (β=–0.15, p=0.03). In multiple logistic regression, there was no relation between the NSC-60 category scores and overall mean concentration of exposure to styrene, duration of exposure, interaction term between both, or cumulative exposure (p≥0.10). Among the covariables only the personality score was related to all NSC-60 category scores (p≤0.04) and alcohol consumption had a negative influence on the mood, equilibrium, and sensorimotor category scores (p≤0.04).

In multiple linear regression, symbol-digit substitution in currently and formerly exposed workers was found to slow significantly with increasing duration of exposure (β=0.21, p<0.01, fig 2). Furthermore, there was a trend of diminished digit span forwards (β=–0.16, p=0.08) with increasing numbers of hours of exposure. Although, no group differences were found, increasing duration of exposure was significantly related to worsening of colour-word vigilance (β=0.21, p=0.03) and associated learning (β=–0.18, p=0.05). The overall mean concentration of exposure did not enter the models. Table 4 shows that by comparison with duration of exposure, cumulative exposure or the interaction term, duration of exposure×concentration of exposure, did not produce better p values when entered in the multiple linear regression models. It is, however, interesting to note that symbol-digit substitution and colour-word vigilance (perceptual speed) were quite well associated with the interaction term (β=0.18, p=0.02 and β=0.21, p=0.03, respectively). Among the covariables, a borderline significance (p≤0.08) was found for age in explaining part of the variance of the neurobehavioural test results, except for associated recall. Similar results were found for the educational level and symbol-digit substitution, digit span forwards, and associated learning. The personal monitoring data at the time of the study did not explain a significant part of the variance of the neurobehavioural results of currently exposed workers (p>0.10). Allowing for the exposure indices and the covariables in the model, the dummy variable “belonging to currently exposed or not (1/0)” did not correlate significantly in any regression analysis.

The symbol-digit substitution test in formerly exposed workers showed a delay of 0.19 s/digit after 3600 hours of lamination tasks. Currently exposed workers laminated 1100 hours more, and their symbol-digit substitution time increased by 0.22 s/digit compared

Figure 2 Linear regression model in the exposed study population (+, currently exposed and o, formerly exposed) ($n=117$) between the duration of exposure and symbol-digit substitution test results after adjustment for age, educational level, alcohol consumption, and years of welding (residual log (symbol-digit substitution)).

Figure 3 Linear regression model in the mEH phenotype study group ($n=56$) between the mEH phenotype activity and symbol-digit substitution test results after adjustment for age, alcohol use, educational level, and duration of exposure (residual log (symbol-digit substitution)).

with controls. The estimated value for the upper limit of normal for symbol-digit substitution time is 3.3 s/digit (mean of the controls +2SDs), which is similar to normal data reported by Dutch investigators (3.2 s/digit).²⁴ This suggests that under the given exposure conditions, the evolution from a normal mean (2.5 s/digit) to reach pathological values of the symbol-digit substitution time will be reached on average after 15 160 hours of lamination. This is equivalent to a full time exposure of 8.6 years. A similar calculation based on the relation between symbol-digit substitution time and the interaction term, duration of exposure $\times$ concentration of exposure, showed that a pathological value for symbol-digit substitution time would be reached at an interaction product of 2 419 220 $\text{mg/m}^3 \times \text{hour}$ or 1375 $\text{mg/m}^3 \times \text{year}$ (taking 220 working days a year and 8 hours work a day). This means 6.5 years of full time lamination tasks at the previous styrene TLV-TWA value of 213 mg/m^3 or 9.2 full time working years at a mean exposure to styrene concentration of 155 mg/m^3 . Although the safety measures and the ventilation system in the plant were very well designed and resulted in low to moderate concentrations of exposure to styrene, it should be pointed out that up to 4% of the air measurements were above the previous ACGIH TLV-STEL and up to 9% of the air measurements were above the previous TLV-TWA depending on location and characteristics of the lamination task.^{16 17}

In multiple regression analysis, no relation was found between the number of complaints per person and the mEH phenotype activity ($\beta=0.17$, $p=0.25$), nor with the persistence of complaints ($p=0.59$). There was a significant relation between the mEH phenotype activity and symbol-digit substitution test results ($\beta=0.34$, $p=0.01$, fig 3) or colour-word vigilance ($\beta=0.28$, $p=0.05$), and a borderline significant relation with digit span forwards ($\beta=-0.24$, $p=0.08$). The test results worsened with increasing activity of the mEH phenotype.

Discussion

As far as we know, only one short term follow up study about occupational exposure to styrene has dealt with the question of revers-

ibility of neurobehavioural effects.² Although difficult to carry out, the ideal study design in this context is a longitudinal follow up after the end of exposure in a relatively large cohort of workers. Economic and trade union linked instabilities of the late 1980s forced us to change our investigation into a design aiming at collecting retrospectively neurological information in a cohort of workers exposed to styrene in which 83% lost their job while the rest continued to work in the same plant at job sites gradually decreasing in exposure to styrene. In those field conditions, the maximum that could be done to answer the crucial question of whether neurotoxic complaints were persistent in this workforce was to organise a structured neurological anamnesis of complaints in such a way, that reliable responses could be obtained from the participants 3 years after the 1989 event in the plant. We are confident that a potential bias did not operate in the recruitment and selection procedure of the subjects in the present study. Sixty three per cent of the original cohort of workers exposed to styrene participated in the study. For non-participant workers, all basic group and exposure characteristics were available and were similar to the study group, except for a lower educational level. This could have lowered the robustness of the study, as it has been suggested that low intellectual capacity may predispose to the development of neurotoxic effects of organic solvents.⁸ It can be argued that the division of the original workforce into currently and formerly exposed workers could have introduced a bias due to the procedure by which workers were made redundant. However, no medical or productivity criteria were used in this procedure. The criterion of age (>50 years) is not likely to have introduced a bias as there was no relation between age and duration of exposure in this study population. Older workers were exposed to lower air concentrations of styrene and they had spent less hours on lamination tasks. Similarly, years of welding, years or level of education, and alcohol use were not associated with concentration or duration of exposure.

In line with complaints described in other studies,^{1 2} the present study also showed a significantly higher prevalence of complaints

(irritability, fatigue, headache, alcohol intolerance, concentration difficulties, imbalance, and diminished sense of smell) when workers were exposed to styrene compared with control workers. The ambient air concentration of styrene during lamination tasks was the most important factor explaining the number of SNAC complaints at work. Interestingly, 3 years after the end of exposure (mean TWA 70 mg/m³ before the 1989 event), formerly exposed workers still reported an excess of atypical SNAC and NSC-60 (chest, equilibrium, somatic) complaints. These included epigastric discomfort, headache, difficulties in swallowing, heart palpitations, dyspnoea, nausea, tinnitus, diminished sense of smell, imbalance, chest oppression, feelings of drunkenness, and increased sensitivity to cold.²¹ In long term but less exposed workers (mean TWA 36 mg/m³), Edling *et al*² reported that most complaints were reversible after the end of exposure to styrene. In the present study, none of the predictors such as concentration of exposure, duration of exposure, activity of the mEH phenotype, or any other covariable could explain the persistence of complaints at the time of the interview. Unlike those of formerly exposed workers, the NSC-60 and SNAC results of currently exposed workers did not equally reach significance, most probably because of a smaller group size and because of a significantly greater proportion of currently exposed workers that never reported complaints although their exposures did not differ from those formerly exposed. This confirms the view that long term exposed workers may experience less subjective complaints than workers who left exposure earlier. Alternatively, the excess of complaints in formerly exposed workers might be seen as reflecting a revenge attitude of the dismissed workers. Although this could explain part of the variance, it is likely to be less plausible as the number of complaints at the time the workers were exposed was well associated with the concentration of exposure. It should also be pointed out that at the time of the study all the formerly exposed workers had been hired by other companies and were thus no longer unemployed. Also, formerly exposed workers had the same results on the hold questions of the NSC-60 (personality category) as currently exposed and control workers. This is a strong indication that formerly exposed workers had no tendency to tick high scores on the questionnaire items. An interviewer bias for the SNAC results seems unlikely, not only because of the blinding of the interviewer, but also because a comparison between the results of the SNAC questionnaire and the self administered NSC-60 questionnaire proved the consistency of the results.²⁰ Despite lower TWA exposure at the time of the study (40 mg/m³) a slightly higher total number of complaints per person remained in currently exposed workers (diminished sense of smell, increased fatigue in the evening, imbalance, and sleeping difficulties at night) but a dose-effect relation could not be found. This excess of complaints in currently exposed workers could very well be a mixture of effects, due to the current day to day exposure,

and chronic effects, due to the integrated dose. This has also been suggested by others.²⁵

The neurobehavioural test findings are in line with most other styrene studies reporting effects on attention demanding tasks, visuomotor performance, learning, and memory.^{3 4 26} In the present study, hand-eye coordination (visuomotor accuracy), digit span forwards (short term memory, attention), and symbol-digit substitution (visuomotor performance) test results were significantly different between formerly or currently exposed workers and controls. Unlike many who consider reaction time tests to be the most sensitive tests, the lack of effect on simple reaction time in the workers currently exposed to very low concentrations of styrene is consistent with findings of Edling *et al*² and supports the view that slowing of reaction time reflects acute narcotic effects of higher concentrations of exposure.^{27 28}

Symbol-digit substitution time showed significant relations with the duration of exposure and the interaction term, duration of exposure × exposure concentration, whereas digit span forwards showed a trend to worsen as exposure time increased. Although no group differences could be found, reaction times of colour-word vigilance (perceptual speed) and associated learning tests worsened significantly with increasing duration of exposure in the exposed group. None of these variables was related to the overall mean concentration of exposure. The results suggested that less than 10 years of exposure to a mean atmospheric styrene concentration of 155 mg/m³ (STEL measurements) or 70 mg/m³ (TWA measurements) and the concomitant occurrence of up to 9% and up to 4% of these measurements being above the previous TLV-TWA and TLV-STEL values, resulted in the development of dose-dependent effects and the persistence of some complaints and neurobehavioural changes. This would indicate that sporadic peak exposures may play a part in the development of chronic or persistent neurobehavioural effects. This is in line with publications on organic encephalopathies due to exposure to solvents,²⁹ and strengthens the recent lowering of the TLV-TWA and TLV-STEL concentrations by ACGIH from 213 and 416 mg/m³ to 85 and 170 mg/m³, respectively.³⁰

This study also showed a fivefold variation of the activity of the mEH phenotype between people, a variation in line with some of the activity ranges previously reported, ninefold,¹¹ fivefold,¹² and 1.6-fold.¹³ There was no association between activity of the mEH phenotype and age, alcohol use, overall mean concentration of exposure, or duration of exposure (all $p > 0.10$). Activity of the mEH phenotype did not seem to influence either the number of complaints during exposure or the persistence of the complaints. By contrast, visuomotor performance (symbol-digit substitution) and perceptual speed (colour-word vigilance) were worse in fast styrene metabolisers, but only if the duration of exposure was included in the multiple regression analysis. In other words, although exposure to styrene is still the primary factor determining the development of neu-

robehavioural effects, the occurrence of these effects may be modulated by the mEH phenotype. A similar pattern was found for the role of glutathione S-transferase M1 null genotype in the development of organic psychosyndrome, in which the effect became only significant in highly exposed workers.³¹ The slowing of visuomotor speed (increase of symbol-digit substitution time) with increasing activity of the mEH phenotype (fig 3) was surprising and might have different explanations. Firstly, it could be that styrene-7,8-oxide is not the most toxic metabolite of styrene in humans, but another toxic metabolite further down the metabolic chain could be. Mutti *et al*^{32, 33} suggested that the ultimate metabolite, phenylglyoxylic acid, could be the active neurotoxicant. A second explanation might be that glutathione S-transferase activity in humans is more important for styrene detoxification in non-hepatic tissues than is commonly thought. On the other hand, the lack of glutathione S-transferase M1 could possibly lead to an aspecific induction of other metabolic pathways, for instance activity of the mEH phenotype,³⁴ which may indirectly contribute to a positive relation between activity of the mEH phenotype and neurotoxic effects.

Conclusions

Some subjective symptoms or complaints and neurobehavioural effects in workers exposed to styrene were most likely persistent, even at TWA exposure concentrations which were thought to be safe in the 1980s. The concentration of styrene in the air during lamination tasks seemed to be the most important factor in the development of complaints, although most of these complaints disappeared after exposure ended. Also, the duration of exposure at lamination tasks seemed to be the best predictor of toxic effects on visuomotor performance and perceptual speed, which could still be detected in workers 3 years after the exposure to styrene had ended. The detoxification metabolism of styrene-7,8-oxide may also influence the development of these dysfunctions, although no clear conclusion about the underlying mechanism can be drawn from the present study. The results suggested that less than 10 years of exposure to a mean concentration of atmospheric styrene of 155 mg/m³ may result in persistent neurotoxic effects.

We are particularly indebted to the participants in the study, the plant management and the occupational hygienist for their collaboration and technical assistance. We gratefully thank Etienne Phillips for his help in supervising tests and data entry. This project was supported by the research programme of the Fund for Scientific Research (Flanders) NFWO, Project No 3.0175.96.

- Flodin U, Ekberg K, Andersson L. Neuropsychiatric effects of low exposure to styrene. *Br J Ind Med* 1989;46:805-8.
- Edling C, Anundi H, Johanson G, *et al*. Increase in neuropsychiatric symptoms after occupational exposure to low levels of styrene. *Br J Ind Med* 1993;50:843-50.
- Letz R, Mahoney FC, Hershman DL, *et al*. Neurobehavioral effects of acute styrene exposure in fibreglass boat builders. *Neurotoxicol Teratol* 1990;12:665-8.
- Mutti A, Mazzucchi A, Rustichelli P, *et al*. Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsychological functions. *Am J Ind Med* 1984;5:275-86.
- Yuasa J, Kishi R, Eguchi T, *et al*. Study of urinary mandelic acid concentration and peripheral nerve conduction among styrene workers. *Am J Ind Med* 1996;30:41-7.
- Cherry N, Gautrin D. Neurotoxic effects of styrene: further evidence. *Br J Ind Med* 1990;47:29-37.
- Dick RB. Neurobehavioral assessment of occupationally relevant solvents and chemicals in humans. In: Chang LW, Dyer RS, eds. *Handbook of neurotoxicology*. New York: Marcel Dekker, 1995:217-323.
- Bleeker ML, Bolla KI, Agnew J, *et al*. Dose-related subclinical neurobehavioral effects of chronic exposure to low levels of organic solvents. *Am J Ind Med* 1991;19:715-28.
- International Agency for Research on Cancer. *Some industrial chemicals. IARC monographs on the evaluation of carcinogenic risks to humans*. Vol 60. Geneva: World Health Organisation, 1994.
- Hassett C, Aicher L, Sidhu JS, *et al*. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994;3:421-8.
- Glatt HR, Halfer-Wirkus H, Herborn J, *et al*. Interindividual variations in epoxide detoxifying enzymes. In: Müller JH, Weber W, eds. *Familial cancer*. Basel: Karger, 1985:242-7.
- Nakajima T, Elovaaara E, Gonzalez FP, *et al*. Characterization of human cytochrome P450 isozymes responsible for styrene metabolism. In: *Abstract book of the International Symposium on Health Hazards of Butadiene and Styrene*. Helsinki: Finnish Institute of Occupational Health, 1993:23.
- Kroetz DL, Kerr BM, McFarland LV, *et al*. Measurement of in vivo microsomal epoxide hydrolase activity in white subjects. *Clin Pharmacol Ther* 1993;53:306-15.
- Omicinski CJ, Aicher L, Holubkov R, *et al*. Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. *Pharmacogenetics* 1993;3:150-8.
- Beiswanger CM, Mandella RD, Greasle TR, *et al*. Synergistic neurotoxic effects of styrene oxide and acrylamide: glutathione-independent necrosis of cerebellar granule cells. *Toxicol Appl Pharmacol* 1993;118:233-44.
- Veulemans H, Daems J, Masschelein R. (Polyester ship building. Air monitoring and biomonitoring.) *Cahiers Arbeidsgeneeskunde* 1986;22:75-83. (In Dutch.)
- Daems J, Masschelein R, Veulemans H. (Health care in a fibreglass boat building factory.) *Cahiers Arbeidsgeneeskunde* 1986;23:289-295. (In Dutch.)
- British Special Committee of the Royal College of Psychiatrists. *Alcohol, our favorite drug*. London: Tavistock Publications, 1986.
- American Conference of Governmental Industrial Hygienists. *Threshold limit values for chemical substances, physical agents and biological exposure indices*. Cincinnati: ACGIH, Technical Affairs Office, 1992.
- Viaene MK. (Acute and chronic effects of styrene exposure and their persistence. Results of a cross-sectional study of workers working in polyester ship-building industry) [PhD thesis]. Leuven, Belgium: Department of Occupational Health, Katholieke Universiteit Leuven, 1998. (In Dutch.)
- Hooisma J, Emmen HH. (*Defining the normal data of the neurotoxicity symptom checklist-60 (NSC-60)*). Amsterdam: Publicatie Stichting Arbouw, 1992. (In Dutch.)
- Letz R. Use of computerized test batteries for quantifying neurobehavioral outcomes. *Environ Health Perspect* 1991;90:195-8.
- Bradford MM. A rapid and sensitive method for quantification of µg quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- van der Laan G, van Dun RECS, Roos Y, *et al*. *Organic psychosyndrome caused by solvents? A protocol for diagnosis*. Amsterdam: Nederlands Centrum voor Beroepsziekten. Coronel Laboratorium, Universiteit Amsterdam, 1995. (In Dutch.)
- Gregersen P. Neurotoxic effects of organic solvents in exposed workers: two controlled follow up studies after 5.5 and 10.6 years. *Am J Ind Med* 1988;14:681-701.
- Anger WK. Worksite behavioral research: results, sensitive methods, test batteries and transition from laboratory data to human health. *Neurotoxicology* 1990;11:629-720.
- Schoenhuber R, Gentilini M. Influence of occupational styrene exposure on memory and attention. *Neurotoxicol Teratol* 1989;11:585-6.
- Mackay CJ, Kelman GR. Choice reaction time in workers exposed to styrene vapour. *Hum Toxicol* 1986;5:85-9.
- Houck P, Nebel D, Milham jr S. Organic solvent encephalopathy: an old hazard revisited. *Am J Ind Med* 1992;22:109-15.
- American Conference of Governmental Industrial Hygienists. *Threshold limit values for chemical substances, physical agents and biological exposure indices*. Cincinnati: ACGIH, Technical Affairs Office, 1997.
- Söderkvist P, Ahmadi A, Åkerbäck A, *et al*. Glutathione S-transferase M1 null genotype as a risk modifier for solvent-induced chronic toxic encephalopathy. *Scand J Work Environ Health* 1996;22:360-3.
- Mutti A, Falzoi M, Romanelli A, *et al*. Regional alterations of brain catecholamines by styrene exposure in rabbits. *Arch Toxicol* 1984;55:173-7.
- Mutti A, Falzoi M, Romanelli A, *et al*. Brain dopamine as a target for solvent toxicity: effects of some monocyclic aromatic hydrocarbons. *Toxicology* 1988;49:77-82.
- Petruzzelli S, Camus AM, Carrozzi L, *et al*. Long-lasting effects of tobacco smoking on pulmonary drug metabolizing enzymes: a case-control study on lung cancer patients. *Cancer Res* 1988;48:4695-700.