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Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture

Neurobehavioral assessment of workers occupationally exposed to pesticides in the bulb growing industry

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Labour Inspectorate

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Part 3A

Neurobehavioral assessment of workers occupationally exposed to pesticides in the bulb growing industry

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Contents

Summary

The purpose of the present study was to evaluate the possible effects of occupational exposure to pesticides on central nervous system (CNS) function. A variety of neurobehavioral functions were assessed in a group of 129 workers in the bulb growing industry with at least ten years occupational exposure to pesticides and a group of 64 age and education matched non-exposed controls using the Neurobehavioral Evaluation System (NES). The NES consists of a battery of computerized neurobehavioral performance tests which are designed to evaluate changes in attention,learning and memory, motor performance and perception. In addition psychiatric and mood scale questionnaires were administered.

Statistical analyses showed no differences in the prevalence of psychiatric symptoms or subjective complaints between the two groups. Multivariate analyses of neurobehavioral functioning, however, revealed significant performance decrements in pesticide-exposed workers in tests related to attention ($p<0.05$) as well as those involving perceptual coding ($p<0.01$). Test scores of exposed workers on measures related to learning and memory as well as those related to motor performance were typically lower than controls but did not reach statistical significance. In order to examine the association between changes in test performance and the degree of pesticide exposure, a multiple linear regression model using a calculated lifetime pesticide exposure index was used. Results of these analyses indicated a significant relationship between the degree of exposure and test performance with greater exposure being significantly related to poorer performance on tests involving perceptual coding ($p<0.01$).

Taken together, these data indicate that occupational exposure to pesticides in the bulb growing industry is associated with subtle changes in neurobehavioral functioning. Given the large number of pesticides employed in the industry and the changing patterns of use over the last twenty years, it is not possible to identify which compounds may be specifically involved or whether these effects are due to agents in current usage or those which were used in the past. The long-term consequences of these effects for worker safety and health are unknown. Combined industrial hygiene and neurobehavioral studies aimed at monitoring exposures and neurobehavioral functioning in industrial workers on a longitudinal basis are recommended to address these issues. I

Introduction

The increased usage of pesticides such as chlorinated hydrocarbons, organophoshorous compounds and carbamates, together with knowledge of some of their adverse effects, has alerted the public to the need for regulation as well as for development of early warning indicators of such effects (WHO, 1987). Evidence from case reports and epidemiologic studies indicates that overexposure to a number of pesticides is primarily associated with disturbances in central nervous system (CNS) function (Hartman, 1988). In the light of the continuous exposure of approximately 7200 Dutch flower bulb workers (CBS, 1989), the contention that neurobehavioral sequelae may result from chronic low dose exposure was regarded as an important motive for including neurobehavioral testing in the present study.

There has been more (neurobehavioral) research on organophosphorous (OP) compounds than on any other type of pesticides, perhaps because of their intentional application in chemical warfare (Sharp et al., 1986). In case reports, chronic exposure to OP's has been reported to cause increased tendencies to depression and impairment in concentration and memory in agricultural workers and pilots of spraying aircraft (Gerson and Shaw, 1961; Dille and Smith, 1964). Tabershaw and Cooper (1966) examined individuals after three years of OP exposure. Continued visual disturbances, headaches and nervousness were the most common complaints. No control group was examined, however. Metcalf and Holmes (1969) interviewed OP exposed factory workers and reported disturbed memory and difficulty in maintaining alertness and appropriate focusing of attention. Interpretation of study results is limited by the lack of control data. Only a few cross-sectional epidemiologic studies using neurobehavioral tests have been carried out. Rodnitzky and Levin (1975) assessed a variety of neurobehavioral functions in OP exposed farmers and pest-control workers and a control group matched for age and education. No visuomotor or memory abnormalities were found, but pesticide-applicators scored significantly higher on the Taylor Manifest Anxiety Scale. Using subtests of the Halstead-Reitan battery, Korsak and Sato (1977) found that performance of exposed subjects was poorer on part B of the Trail Making Test and on the Bender Visual Gestalt test. Unfortunately, the authors did not include information about subject matching procedures. Savage et al. (1982, 1988) examined 100 matched-pairs of individuals with previous OP poisoning and nonexposed controls. Differences were found on tests of a wide variety of abilities, including intellectual functioning, academic skills, abstraction and flexibility of thinking and simple motor skills. Also, increased rates of emotional problems were observed. However, these results may be inflated by the fact that potential confounders (i.e. vocabulary and information scores as estimaters of premorbid intelligence, Mikkelsen, 1988; Triebig and Valentin, 1989) were treated as outcome measures, which turned out to be highly significant.

Instead of OP compounds and chlorinated hydrocarbons Dutch flower bulb farmers mainly use carbamates currently such as maneb and zineb (see for a detailed description of the pesticides used in the Dutch bulb growing industry, Brouwer et al. (1990, part 2)). Maneb and zineb belong to the class of ethylenebisdithiocarbamates (EBDC), for which evidence exist for their neurotoxicity (e.g. Miller, 1982). With respect to the EBDC compounds very few studies on exposed individuals have been reported in the literature. Israeli et al. (1983) described a maneb exposed patient who developed coma, seizures, and hemiparesis which spontaneously disappeared after a few days. Ferraz et al. (1988) noted a significantly higher prevalence of headache, fatigue, nervousness, memory complaints, rigidity and sleepiness in a group of rural workers occupationally exposed to maneb in comparison with a group of workers without fungicide exposure. No other controlled studies of chronic EBDC exposure in man appears to have been carried out. The metabolic relationship of EBDC compounds to the known neurotoxicant carbon disulfide (Wood, 1981) emphasizes the need for the investigation of the neurotoxic potential of these compounds in humans.

In summary, reports on pesticide exposed workers give some indication for impairment of a variety of behavioral variables. Affective symptoms include increased tendencies of depression, anxiety, and irritability. Cognitive symptoms can be characterized as impaired vigilance and reduced concentration, memory deficits, slowing of information processing and psychomotor speed. However, few controlled studies using neurobehavioral tests have been carried out and insufficient quantitative data on levels or duration of exposure have been available to elucidate dose-response relationships. Therefore, the objective of this study was to provide information on the following questions: 1) are there any differences in the neurobehavioral test performance and prevalence of subjective symptoms between pesticide exposed workers and controls? and 2) is there an indication of an exposure-effect relationship?

2

Methods

2.1 Subjects

Of the 922 eligible flower bulb growers and employees, 501 subjects (54%) agreed to participate in the health effect study. One hundred and sixty subjects (17% of 922 Ss.; 32% of 501 Ss.) met the selection criteria concerning gender (male), age (between 30 and 55 years), exposure to pesticides for a period of at least ten years and exposure involved in the application of pesticides in both crop-protection and bulb-disinfection. Of the 160 subjects 23 (14%) subjects were not invited for testing because of the following exclusion criteria: 1) a history of CNS disease or injury; 2) medication; 3) drug use and 4) self-reported alcohol consumption greater than 6 units per day. After testing eight more subjects were excluded from analysis because of the forementioned exclusion criteria (see Table 1). A total of 129 exposed subjects (14% of 922 Ss.; 26% of 501 Ss.; 81% of 160 Ss.) were incorporated in the statistical analyses.

Table 1 Reason for exclusion from analysis

Reason for exclusion	Controls	Exposed	Total
Number of subjects tested	73	137	210
Neurotoxic exposures not related to present employment	4	0	4
Medication	1	3	4
Diseases or injuries possibly related to CNS dysfunction	1	3	4
Insufficient motivation	2	0	2
Alcohol consumption greater than 6 units per day	0	2	2
Less than 30 years of age	1	0	1
Number of subjects excluded from analysis	9	8	17
Number of subjects in analysis	64	129	193

Of the 1122 potential male control subjects who lived in the same geographic region as the exposed workers, 556 (50%) originally agreed to participate in the study. Two hundred and twenty two subjects (20% of 1122 Ss.; 40% of 556 Ss.) employed in areas other than the agricultural sector or unemployed for less than one year met the selection criteria concerning gender, age and absence of nervous system disorders as well as the same exclusion criteria applied to the exposed group (e.g., alchohol use, medication etc.). Seventy three (33% of 220 Ss.) subjects were selected in such a way that the control and exposed group were matched for age and education. Preferentially subjects with physically demanding jobs were selected. Education level was evaluated using the Groninger Scale as described by Heslinga et al. (1983). After testing nine subjects were excluded from analysis because it became apparent that they did not fulfil the forementioned selection criteria (see Table 1). Thus, 64 control subjects (6% of 1122 Ss.; 12% of 556 Ss.; 29% of 220 Ss.) were available for analysis. For a detailed description of subject recruitment, the reader is referred to Brouwer (1990; part 1).

2.2 Neuropsychological Testing

2.2.1 Automated Testing

Neurobehavioral performance was evaluated using the Neurobehavioral Evaluation System (NES) originally developed by Baker and Letz (1986) and adapted for use in Dutch populations (Emmen et al., 1988, 1989). The NES is a computerized test battery designed to assess the following basic dimensions of neurobehavioral functioning: 1) attention; 2) learning and memory; 3) motor performance and 4) perceptual coding (Hooisma et al., 1990). The tests and test parameters used are shown in Table 2. For a detailed description of the NES tests, see Appendix 1.

Table 2 Applied psychological tests taken from the NES

NES test	Functional domain	Test parameter
Simple Reaction Time Test	Attention	Latency (ms)
Continuous Performance Test	Attention	Latency (ms)
Color Word Vigilance Test	Attention	Latency (ms)
Associate Learning Test	Learning and Memory	Correct responses
Associate Recognition Test	Learning and Memory	Correct responses
Serial Digit Learning Test	Learning and Memory	Correct responses
Pattern Memory Test	Learning and Memory	Correct responses
Finger Tapping Test	Motor Performance	Number of taps
Hand-Eye Coordination Test	Motor Performance	Error (pixels)
Symbol-Digit Substitution Test	Perceptual Coding	Latency (s)
Pattern Comparison Test	Perceptual Coding	Latency (s)

2.3 Self-report and Rating Scales

2.3.1 Symptom Checklist (SCL-90)

In order to determine whether exposed subjects showed increased tendencies toward psychiatric disturbances, the Symptom Checklist (SCL-90), originally developed by Derogatis (1977), was administered to all participants. Subjects were asked to report symptoms experienced in the past week on a five-point rating scale ranging from not-at-all (1) to extremely (5). The SCL-90 which was translated and adapted for the Dutch situation by Arrindell and Ettema (1986) covers the following dimensions: 1) phobia; 2) fear; 3) depression; 4) somatic complaints; 5) obsessive behavior; 6) distrust; 7) hostility and 8) sleep disorders.

2.3.2 Mood States (MS)

Since changes in mood and affect are common features of overexposure to neurotoxicants, a frequently used mood inventory was given to all subjects. The Mood States questionnaire (MS) is a modification of the Profile of Mood States (POMS; McNair et al., 1971; Wald, 1984, 1990) and contains 32 adjectives that describe different mood states: 1) depression; 2) anger; 3) fatigue; 4) vigor; and 5) tension. Subjects were asked to indicate their mood states during the past week on a five-point rating scale ranging from not-at-all (1) to extremely (5). The MS was included as an automated subtest in the computer administered Neurobehavioral Evaluation System and is similar to that used previously in our laboratory for studies of occupational exposure to neurotoxicants (Emmen et al., 1988; Hooisma et al., 1990).

2.3.3 Neurotoxic Anxiety Scale (NAS)

In addition to the SCL-90 and the MS the Neurotoxic Anxiety Scale (NAS) was also administered to all participants. This brief scale was developed by Bowler and coworkers (1985) and consists of 28 true/false items, 23 of which were chosen from the Taylor Manifest Anxiety Scale and five items representing depression and psychomotor retardation. The anxiety items reflect the physical symptoms and affect disturbance found in patients with neurotoxic exposure and has been reported to discriminate between OP-exposed workers and matched controls (Bowler, 1988). The Neurotoxic Anxiety Scale was included as an automated questionnaire in the NES. For a summary of the number of items/trials used in each test/questionnaire, see Appendix 2.

2.4 Procedure

Prior to testing, subjects were informed about the general purpose of the project, i.e. the examination of possible health effects of pesticides. For the controls, travel expenses were reimbursed. The participants were instructed not to reveal their profession to the investigators. An interviewer was always present to instruct, observe and if necessary to assist subjects. Two subjects were tested at the same time in a large quiet room equipped with partitions which prevented visual contact between the subjects. During testing the subjects were monitored by means of a video camera. Time of testing was balanced over groups.

2.5 Apparatus

All neurobehavioral tests, as well as the MS and NAS questionnaires, were administered with an IBM-Personal Computer (XT) equipped with a color video monitor, push buttons mounted on a box and a joystick which permitted movement only in the vertical direction. A piece of plexiglas was used to cover the keyboard with a section cut out such that only the keys to be used were exposed.

2.6 Estimates of Exposure

Preliminary industrial hygiene studies were conducted in which a self-administered questionnaire containing items related to occupational pesticide exposure was used to calculate a lifetime pesticide exposure index for workers in the bulb growing farms (see Brouwer et al., 1990 part 2, for a full discussion). Method-specific exposure values were derived to estimate the contribution in external exposure of the most common application techniques (i.e. crop protection, bulb disinfection and preparation of the compound). Because of their widespread use, zineb and maneb were chosen as marker compounds: in crop protection and bulb disinfection the dithiocarbamates zineb and maneb account for 90% and 40% of the total weight of pesticides applied respectively. The average exposure index for the 129 exposed subjects available for analysis (see also table 3) was 333 (SD=338).

Although the exposure index was not aimed at quantifying the exposure to pesticides in the harvesting of flowers, during the course of gathering exposure information from the workers it became apparent that this potentially important factor had been omitted in determining this estimate of lifetime exposure: for approximately 62% of the workers (N=49), duties were not confined solely to bulb production but were extended to the harvesting of the flowers themselves. It is known that combined bulb growing and flower harvesting entails a different exposure pattern than bulb production alone. Given this uncertainty, analyses aimed at examining the relationship between neurobehavioral changes and level of exposure were performed for the group as a whole and then repeated omitting those workers with combined bulb and flower growing activities. For this latter group ('pure' bulb growers) the average exposure index was 402 (SD=365).

2.7 Statistical Analyses

In order to test the equality of group means of several variables simultaneously, combined z-scores were calculated and analysed using program 1V (one-way analysis of variance and covariance) of the BMDP software package (Dixon, 1988).

Analysis of covariance (ANCOVA) was also used for pairwise comparisons. Age, educational level, vocabulary score, and alcohol intake were incorporated as a priori covariates. Nonparametric data were analysed using the Mann-Whitney U test (MW; program 3D). Differences in the prevalence of symptoms between controls and exposed subjects were evaluated with contingency tables. The significance of differences was tested with the Chi Square statistic or the Fisher exact probability statistic for cells with less than five events (program 4F). To examine the association between performance on neurobehavioral tests and pesticide exposure, multiple linear regression (MLR) models incorporating the index for lifetime pesticide exposure and the a priori covariates was used (program 1R). In order to control for possible interactions between the exposure index and the covariates, additional MLR analyses were carried out on the residual scores, i.e. the predicted minus the observed scores of the exposed group with the predicted scores based on the test scores of the control group in relation to the set of a priori covariates. All tests with (two-tailed) p values less then 0.05 were considered as significant.

3

Results

3.1 Study Group Characteristics

Characteristics of the study groups are given in Table 3. The figures in this table are derived from answers given in the Personal and Occupational History Questionnaire (POHQ, see Appendix 2). Verbal ability test scores obtained on the Vocabulary Test of the Neurobehavioral Evaluation System are also presented.

Table 3 Characteristics of the study groups

	Controls	Exposed	P
Number of subjects			
- N	64	129	
Age			
- Mean years (SD)	42.8 (7.6)	44.1 (6.5)	NS
- Median years	42	44	
- Range	30-55	31-55	
Education			
- Mean years (SD)	10.6 (2.7)	10.1 (2.2)	NS
- Median years	10	10	
- Range	6-21	5-16	
- Mean level (SD)	4.2 (0.9)	4.1 (1.0)	NS
- Median level {*}	4	4	
- Range	2-7	1-7	
Verbal ability			
- Mean vocabulary score (SD)	12.6 (3.4)	12.4 (3.4)	NS
- Median vocabulary score	13	13	
- Range	2-18	2-18	
Alcoholic beverage consumption			
- Current alcohol users (%)	58 (90.6)	122 (94.6)	
- Mean drinks/week (SD)	12.8 (10.2)	10.3 (8.3)	NS
- Median drinks/week	10	8	
- Range0-400-36			
- Lifetime alcoholintake (SD)	304.0 (259.8)	250.6 (221.8)	NS
Neurotoxic exposure {**}			
- Exposure index; gram (SD)	0.0 (0.0)	333.1 (338.0)	
- Median exposure index	0	230.4	
- Range	0-0	0.5-2172.7	

P: level of significance NS = not significant; {*) level 4 = MAVO3/LTS; {**} see Brouwer et al. (1990, part 2)
Initial analyses were performed to determine whether the groups differed on possible confounding variables. Results indicated no differences (all p-values >0.20) between the groups on age, education, verbal ability or alcohol use.

3.2 Neuropsychological Test Performance

Examination of the statistical properties of the different performance measures including distribution characteristics, skewness and kurtosis indicated that transformations were required for some performance variables in order to use parametric statistical techniques. Data from the Simple Reaction Time Test, Color Word Vigilance Test, Symbol-Digit Substitution Test and Pattern Comparison Test were transformed to logarithmic values. Scores of the Associate Learning Test were transformed by taking the square root of the original measurement.

Means and standard deviations for controls and exposed subjects for the different psychological performance variables, as well as the results of the statistical analyses are shown in Table 4. A comparison of the groups using analysis of covariance (ANCOVA) revealed significant differences in test performance between the exposed and control groups on two functional domains i.e. Attention ($F(1,184)=6.57$, $p=0.011$) and Perceptual Coding ($F(1,187)=9.34$, $p=0.003$). Subsequent analyses showed that the exposed workers performed at a significantly lower level than controls on the Simple Reaction Time Test ($F(1,187)=5.09$, $p=0.025$) and the Symbol-Digit Substitution Test ($F(1,187)=13.29$, $p<0.001$). Neither analysis on combined z-scores nor analysis on single tests could demonstrate any difference between exposed workers and controls on tasks representing the Learning and Memory factor or Motor Performance.

Figure 1 (see page 12) presents the mean performance differences between the two groups on each of the variables analyzed in the different performance tests and questionnaires for subjective symptoms. The differences are expressed as standardized mean score differences (z-values) which were calculated as follows: $Z=(Mc-Me)/SDp$ where Mc is the mean value of the performance variable in the control group; Me is the mean value of the same variable in the exposed group and SDp is the standard deviation for the variable pooled over groups. For the sake of simplicity, the resultant z-value was multiplied by +1 or -1 such that a bar directed to the right in the figure indicates a better performance of the control group compared to the exposed group, while a bar to the left means a better performance of the exposed group.

Table 4 Means and standard deviations for controls (n=64) and exposed subjects (n=129) on the neuropsychological tests and the results of the statistical analyses

Neuropsychological tests (NES)	Controls Mean	Controls SD	Exposed Mean	Exposed SD	ANCOVA Me-Mc	ANCOVA P	MLR MEi	MLR P
Attention								
- Combined z-score	0.23	0.77	-0.09	0.77	-0.32	*	-0.07	NS
Simple Reaction Time Test								
- Latency (ms)	234.26	33.82	246.89	36.46	11.54	*	8.68	NS
Continuous Performance Test								
- Latency (ms)	380.24	41.37	390.57	38.91	9.92	NS	4.25	NS
Color Word Vigilance Test								
- Latency (ms)	618.41	66.04	635.62	66.07	17.23	NS	8.50	NS
Learning and Memory								
- Combined z-score	0.07	0.68	-0.02	0.74	-0.01	NS	-0.02	NS
Associate Learning Test								
- Correct responses	14.27	5.12	14.33	5.37	0.46	NS	0.07	NS
Associate Recognition Test								
- Correct responses	5.25	2.14	5.25	2.44	0.14	NS	0.02	NS
Serial Digit Learning Test								
- Correct responses	39.31	7.06	37.42	7.57	-1.35	NS	-0.02	NS
Pattern Memory Test								
- Correct responses	20.63	3.56	20.13	3.97	-0.05	NS	-0.29	NS
Motor Performance								
- Combined z-score	0.15	0.84	-0.08	0.79	-0.17	NS	-0.01	NS
Finger Tapping Test								
- Number of taps	62.28	11.53	59.86	9.98	-1.75	NS	-0.72	NS
Hand-Eye Coordination Test								
- Mean absolute error (ln)	1.51	0.28	1.58	0.31	0.06	NS	0.05	NS
Perceptual Coding								
- Combined z-score	0.31	0.86	-0.15	0.88	-0.37	**	-0.22	**
Symbol-Digit Substitution Test								
- Latency (s)	2.52	0.44	2.78	0.46	0.21	***	0.10	**
Pattern Comparison Test								
- Latency (s)	4.16	0.93	4.45	0.96	0.23	NS	0.18	**

P: level of significance * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant ($p \leq 0.05$)
Me-Mc: difference in adjusted means between exposed and control group
MEi: difference in adjusted means between exposed and control group for an average exposure index

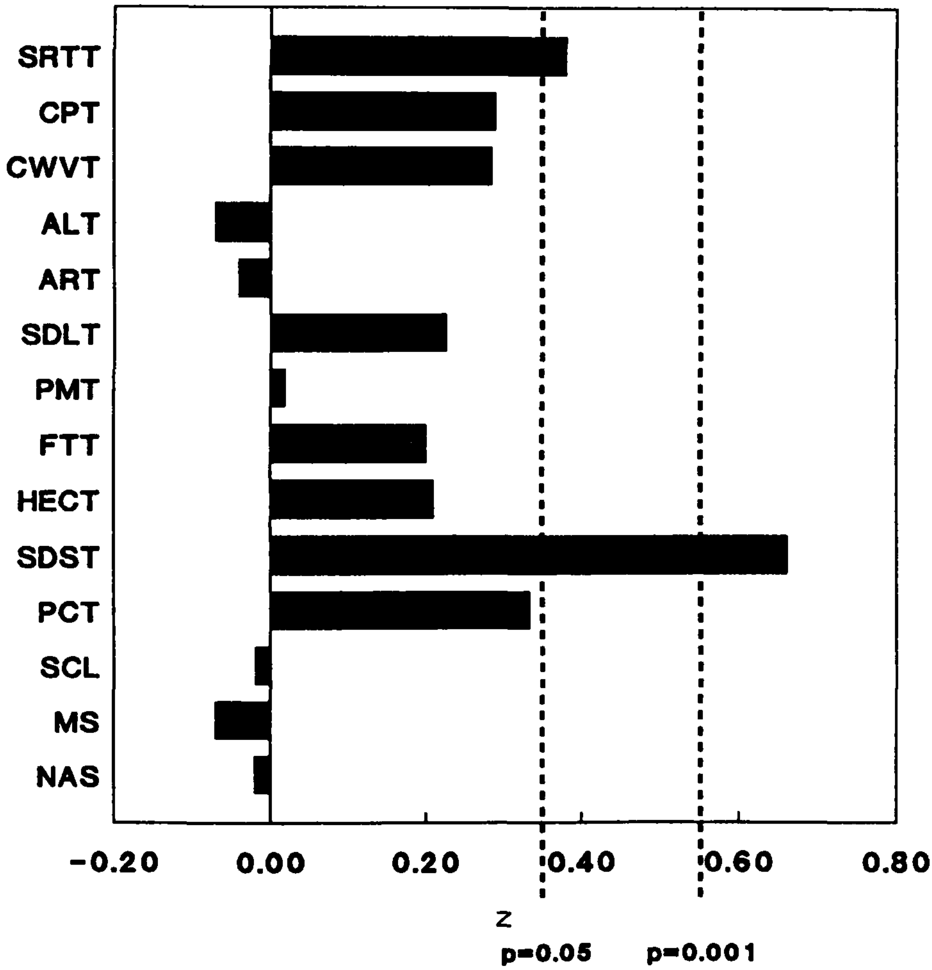

Figure 1 Differences in performance between pesticide-exposed workers and controls on different psychological tests and questionnaires expressed as differences of standardized mean scores (z-values). In the diagram a bar directed to the right indicates a better performance of the control group compared to exposed group, while a bar to the left indicates a better performance of the exposed group

SRTT: Simple Reaction Time Test
CPT: Continuous Performance Test
CWVT: Color Word Vigilance Test
ALT: Associate Learning Test
SDLT: Serial Digit Learning Test
PMT: Pattern Memory Test
FTT: Finger Tapping Test
HECT: Hand-Eye Coordination Test
SDST: Symbol-Digit Substitution Test
PCT: Pattern Comparison Test
SCL: Symtom Checklist
NAS: Neurotoxic Anxiety Scale

Multiple Linear Regression (MLR) analysis (see also Table 4, page 13) revealed a significant association between the combined z-scores for the domain of Perceptual Coding and the exposure index: decreasing z-scores were associated with increasing exposure levels (Perceptual Coding z-score = 0.66521 - 0.03850 age + 0.08670 education level + 0.06700 vocabulary score + 0.00007 alcohol-index - 0.00067 exposure index, r=0.55). Subsequent MLR analyses on single tests showed both for the Symbol-Digit Substitution Test (SDST) and the Pattern Comparison Test (PCT) a significant association between latency and exposure index. Increasing latencies were related with increasing exposure levels (logSDST = 0.39359 + 0.00275 age - 0.00810 education level - 0.00530 vocabulary score - 0.00002 alcohol-index + 0.00005 exposure index, r=0.52; logPCT = 0.53334 + 0.00388 age - 0.00800 education level - 0.00500 vocabulary score + 0.00002 alcohol-index + 0.00005 exposure index, r=0.52). No other exposure related changes in neurobehavioral test performance were found.

In line with forementioned results, MLR analyses only revealed significant associations between the resiudal scores and exposure index for tests of Perceptual Coding. In figure 2 the residuals of the combined z-scores for the Perceptual Coding factor (RPCz) are plotted against the exposure index.

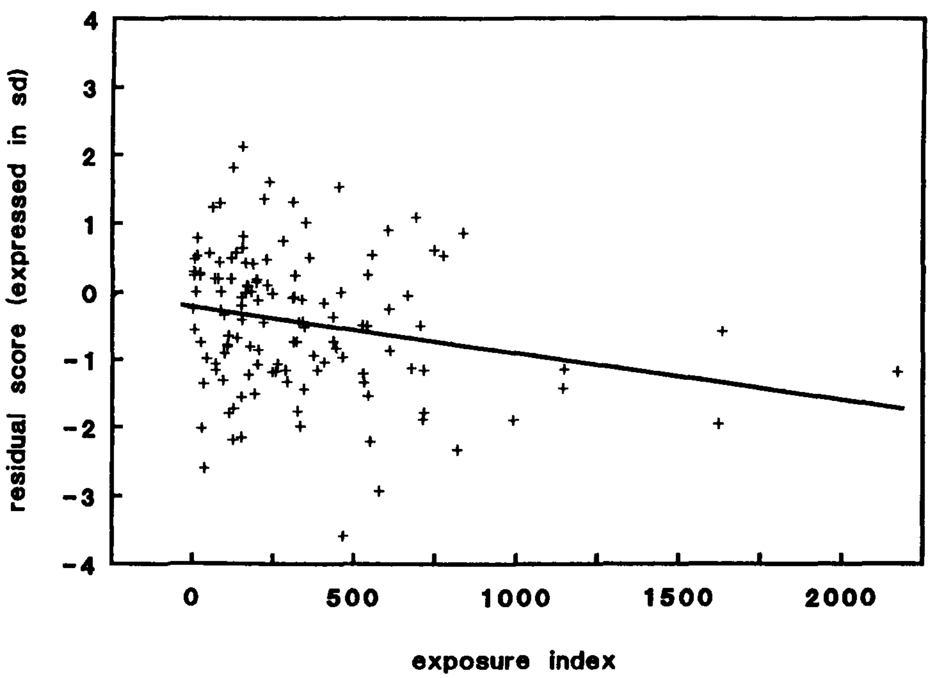

Figure 2 Residual scores of the exposed workers on the domain of perceptual coding versus the exposure index. Residuals are expressed as standard deviations

Residuals are expressed as standard deviations (RPCz = - 0.21393 - 0.00069 exposure index, r=0.23). The equations for the Symbol-Digit Substitution Test (SDST) and Pattern Comparison Test (PCT) are respectively: SDST = - 0.30709 - 0.00051 exposure index (r=0.17) and PCT = 0.02493 - 0.00069 exposure index (r=0.22).

Analyses in the subgroup of workers not involved in the cultivation of flowers revealed significant differences between controls and exposed subjects (see Appendix 4) on the domains of Attention (F(1,106)=8.88; p=0.004) and Perceptual Coding (F(1,107)=4.80; p=0.031). Exposed subjects performed at a lower level than the controls. Subsequent analyses revealed that the exposed group had significantly longer latencies than the control group on the Continuous Performance Test ($F(1,109)=4.18$; $p=0.043$), Color Word Vigilance Test ($F(1,107)=6.86$; $p=0.010$) and Symbol-Digit Substitution Test ($F(1,107)=4.80$; $p=0.031$). Adverse exposure related changes in test performance were found only for the domain of Perceptual Coding (Perceptual Coding z-score = 0.72388 - 0.03920 age + 0.0420 education level + 0.08390 vocabulary score - 0.00006 alcohol-index - 0.00079 exposure index, $r=0.59$) and tests belonging to this domain. Increased latencies on the Symbol-Digit Substitution Test (SDST) and Pattern Comparison Test (PCT) were associated with increasing levels of exposure (logSDST = 0.41490 + 0.00244 age - 0.00800 education level - 0.00650 vocabulary score - 0.00001 alcohol-index + 0.00007 exposure index, $r=0.56$; logPCT = 0.50307 + 0.00440 age - 0.00120 education level - 0.00650 vocabulary score + 0.00002 alcohol-index + 0.00006 exposure index, $r=0.52$). No significant associations were found between the resiudal scores and the exposure index.

3.3 Self-report and Rating Scales Results

Means, standard deviations and results of the statistical analyses for the Symptom Checklist, Mood States and Neurotoxic Anxiety Scale are given in Table 5. No significant differences were found between control and exposed subjects using the Mann-Whitney U test (for a detailed description of the results on the Neurotoxic Anxiety Scale the reader is referred to Appendix 3). Summary scores for the self-report / rating-scales were not associated with exposure levels. Similar results were found for comparisons between controls and exposed subjects not engaged in the cultivation of flowers (see Appendix 5).

Table 5 Means and standard deviations for controls (n=64) and exposed subjects (n=129) on the questionnaires for subjective symptoms and the results of the statistical analyses

Self-report and rating scales (SCL-90; MS; NAS)	Controls Mean	Controls SD	Exposed Mean	Exposed SD	Mann-W Me-Mc	Mann-W P	MLR MEi	MLR P
Affective symptomatology/subjective complaints								
Symptom Checklist								
- Phobia		12.16	3.57	12.01	3.56	-0.15	NS	
- Fear		7.59	1.78	7.62	1.75	0.03	NS	
- Depression	19.17	5.58	18.92	5.62	-0.25	NS		
- Somatic complaints	15.16	4.29	15.12	3.69	-0.04	NS		
- Obsessive behavior	11.76	3.75	11.86	3.33	0.10	NS		
- Distrust	22.29	7.04	22.50	6.64	0.21	NS		
- Hostility	7.03	2.12	6.74	1.37	-0.29	NS		
- Sleep disorders	4.06	2.02	4.13	2.01	0.07	NS		
- Rest items	10.14	1.73	10.24	2.70	0.10	NS		
- SCL-90 total score	109.37	27.10	109.14	23.48	-0.23	NS	-0.16	NS
Mood States								
- Depression	1.19	0.51	1.14	0.29	-0.05	NS		
- Anger		1.34	0.47	1.36	0.45	0.02	NS	
- Fatigue	1.41	0.50	1.31	0.48	-0.10	NS		
- Vigor		3.26	0.84	3.34	0.79	0.08	NS	
- Tension	1.43	0.55	1.43	0.49	0.00	NS		
- MS total score	1.42	0.42	1.38	0.34	-0.04	NS	-0.11	NS
Neurotoxic Anxiety Scale								
- NAS total score	5.52	4.04	5.51	3.53	-0.01	NS	-0.06	NS

P: level of significance * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant ($p \geq 0.05$)
Me-Mc: difference in means between exposed and control group
MEi: difference in means between exposed and control group for an average exposure index

4

Discussions and conclusions

Previous work in our laboratory examining the factor structure of the NES battery of tests indicated that the NES tests could be grouped into four functional domains: Attention, Memory and Learning, Motor Performance, and Perceptual Coding (Hooisma et al., 1990). Results from the present study indicate that workers in the bulb growing industry with at least ten years of occupational exposure to pesticides show significant decrements in NES test performance in tests related to the factors of Attention and Perceptual Coding.

The tests falling under the factor Attention include the Simple Reaction Time Test, the Continuous Performance Test and the Color Word Vigilance Test. Performance of pesticide-exposed workers in the present study was poorer on all three attention tests when analyzed for each test individually (p ranging from 0.025 for the Simple Reaction Time Test to a value of 0.12 for the other two tests). Further, a composite analysis based on the combined z-scores of these tests yielded a significant overall effect on the Attention factor of $p = 0.011$.

Tests loading on the factor Perceptual Coding include the Symbol-Digit Substitution Test and the Pattern Comparison Test. Similarly, the performance of pesticide-exposed workers was also lower with respect to these two tests, with a p value of 0.08 obtained for differences in performance on the Pattern Comparison Test and a value of <0.001 for the Symbol-Digit Substitution Test. Again, the composite analysis based on combined z-scores of these tests yielded a significant overall effect ($p=0.003$).

With respect to individual tests related to the factor Motor Performance (i.e., the Hand-Eye Coordination Test and the Finger Tapping Test) and those related to the factor Learning and Memory (i.e., Pattern Memory Test. Serial Digit Learning Test, Associate Learning Test, Associate Recognition Test) test scores of exposed workers were, in general, lower than controls. However, these differences did not reach statistical significance. Moreover, the composite analysis based on the combined z-scores for each of these factors also failed to reach significance: The p value for tests grouped under the Motor Performance factor was 0.171 and that for the tests falling under the domain of Learning and Memory was >0.5. Finally, no differences in affective symptomatology or subjective complaints between the two groups were observed.

A point of considerable importance in the interpretation of the results of a cross-sectional occupational study such as this one is the question of the comparability between the control and experimental groups. It is known, for example, that differences in age and education level between exposed and non-exposed groups can produce marked differences in neurobehavioral test performance irrespective of exposure (e.g. Gade et al., 1988). Because of this fact, considerable effort was made in the present study to exclude, on an a priori basis, possible sources of bias. The two groups were matched on the basis of gender, age, education level and alcohol consumption and persons with a history of medical illness expected to affect performance were excluded. In addition, the groups were also similar with respect to employment status with persons in both groups either employed or unemployed for less than one year. Thus, it is unlikely that differences found in the present study can easily be explained on the basis of these potential sources of confounding. Despite these efforts to ensure comparability between the two groups, however, it is not impossible that hidden sources of bias could remain undetected. Self-knowledge of exposure status has been suggested by some authors to promote anxiety in exposed groups which could account for poorer performance (Savage, 1982; Johnson, 1987; Maizlish et al., 1987). However, a failure to find differences between the groups on anxiety-related questionnaire items makes it unlikely that such a factor was operating in the present study.

A possible source of bias which could not be controlled, however, was the so-called "healthy worker effect". The healthy worker effect refers to a bias which may arise if workers with a possible exposure-related disease leave their profession (e.g. Mikkelsen, 1988) and thus become unavailable for testing. Such a bias, however, operates to dilute. rather than exaggerate, the effects seen since the most severely affected individuals in the exposed group have already left their original profession. Indeed, it has been suggested that the healthy worker effect may partially account for the relatively small magnitude of performance decrements typically noted in cross-sectional occupational studies.

In addition to the group effects seen in the present study, evidence was also obtained for a relationship between the degree of exposure and the degree of behavioral effects. Specifically, with respect to tests related to the functional domain of perceptual coding, significant exposure-effect relationships were found. In contrast, tests of attention showed no relationship to the exposure index. Whether this finding reflects a genuine difference in the association to the degree of exposure or whether it can be attributed to differences in the statistical properties of the tests and/or "noise" in the exposure index is uncertain. As pointed out by Brouwer et al. (1990, part 2), estimates of exposure are, at best, imprecise and even when a fair degree of precision has been achieved, exposure estimates are still only crude estimates of absorbed dose and target organ exposure. Moreover, the assumption of a linear relation between the performance variable and the exposure-index is not necessarily the most appropriate model, although it is typically the most common model used, and probably the most reasonable especially in the range of moderate exposure (Fidler et al., 1987). Given the complexity of the system under investigation and the amount of uncertainty in the statistical model, it is not surprising that the amount of variance explained by the (combined) independent variables in the regression models (0.30 as the highest r-square value for the Perceptual Coding factor) was relatively small. Other occupational exposure studies (Iregren, 1982; Fidler et al., 1987) have yielded similar values.

Since the workers in the present study have a present and past history of multiple exposures, it is impossible to identify specific causal agents or agent interactions. Further, it is uncertain as to whether the performance decrements noted in the present study group reflect the effects of ongoing exposures or whether they reflect overexposure at a specific time point in the past. For example, the majority of workers in this agricultural sector have been previously exposed to organic mercury compounds before the use of organomercury compounds was banned in the Netherlands. It is unlikely, however, that the effects found in the present study are acute in nature. Efforts were made to avoid the measurement of possible acute effects by conducting the testing during the lowest exposure period of the year (November- March). Moreover, the low prevalence and similar distribution of subjective complaints between the groups with respect to symptoms experienced in the week before testing argues against the role of acute effects of recent overexposure. Finally, some evidence for a relationship between ongoing exposures and accumulating performance decrements was found by examining the relationship between behavioral effects and the exposure index calculated over the last five years. The results of this analysis indicated that for tests of Perceptual Coding, associations between performance and the exposure-index were still significant but at a lower level ($p<0.05$).

With respect to the nature of the effects seen in the present study, the findings of reduced performance on tests related to the factors Attention and Perceptual Coding in exposed workers are in keeping with the results of a number of other occupational exposure studies. Slowing on simple reaction time tasks, for example, is one of the most consistent effects found in occupational studies of workers exposed to organic solvents (Gamberale, 1985). Further, deficits on tests related to perceptual coding also appear to be a relatively common finding following neurotoxic exposures. For example, Maizlish and coworkers (1987) studied the neurobehavioral effects of short-term, low-level exposure to diazinon among pest control workers and found a slower Symbol-Digit pairing speed (4%-9%) among the applicators compared with control subjects. Also, Savage et al. (1982, 1988) detected deficits on the non-automated version of the SDST among OP-poisoned workers.

Although the mechanisms underlying the deficits seen in the present study are unknown, slowing in response speed on simple reaction time tasks and on tasks requiring digit-symbol pairing are some of the most commonly reported general neuropsychological findings in patients with nervous system disorders (Lezak, 1983). However, slowing in the performance on these specific tests is not necessarily pathological in nature: Normal aging also produces slowing on these types of tasks as does acute exposure to psychoactive agents. Moreover, in neurological patient groups with cognitive disorders, the degree of reported impairment is typically of a much larger magnitude. For example, performance on the SDST of a group of patients who are undergoing dialysis because of renal failure, a disease with known effects on CNS function, is at a much lower level. In this group a 25%-40% decrease in response speed has been observed (Rozeman, 1990). In the present study, response speed was approximately 5% slower on the Simple Reaction Time Test and approximately 8% slower on the Symbol-Digit Substitution Test (SDST) in the exposed group. Thus, it is questionable whether the differences seen in the present study should be considered to be of clinical significance. They do, however, indicate that work-related exposures in this group, either ongoing or in the past, have been sufficient to produce effects on the nervous system.

In conclusion, the results of the present study provide evidence that occupational exposure to pesticides is accompanied by subtle decrements in neuropsychological performance. Although these effects were relatively small, they do indicate that the level of exposure in this agricultural sector over the last ten years has not been sufficiently low to avoid effects on neuropsychological functioning. Whether or not the severity of these effects can be expected to increase over time is unknown. It may be, for example, that a history of long-term pesticides exposure may exacerbate the normal degenerative processes involved in aging. Conclusions in terms of health impairment will require longitudinal studies in which workers are followed and changes in CNS function are related to individual changes in measured levels of exposure.

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References

Acker, A. (1982) A computerized approach to psychologic screening. International Journal of Man-Machine Studies, 18, 361-369.

Arrindell, W.A. and Ettema, J.H.M. (1986) SCL-90. Handleiding bij een multidimensionele psychopathologie-indicator. Lisse (The Netherlands): Swets & Zeitliner BV.

Baker, E.L. and Letz, R. (1986) Neurobehavioral testing in monitoring hazardous workplace exposures. Journal of Occupational Medicine, 28, 987-990.

Bowler, R., Becker, C. and Harrison, R. (1985) Workers exposed to organophosphate poison and their controls. Unpublished manuscript. Department of Medicine, University of California, USA.

Bowler, R. (1988) Neuropsychological sequelae of pesticide exposure. Unpublished manuscript. Department of Medicine, University of California, USA.

Brouwer, D.H., Brouwer, E.J. and Hemmen, J.J. van (1990) Effects of occupational exposure to pesticides in the dutch flower bulb culture. Part 2, Estimation of long-term exposure to pesticides. Department of Occupational Toxicology, TNO Medical Biological Laboratory, Rijswijk, The Netherlands.

Brouwer, E.J. (1990) Effects of occupational exposure to pesticides in the dutch flower bulb culture. Part 1, General study design. Toxicology Laboratory, University of Leiden, The Netherlands.

Centraal Bureau voor Statistiek (1989) Hoofdafdeling Landbouwstatistieken, Landbouwtelling 1987. Voorburg, The Netherlands.

Derogatis, L.R. (1977) SCL-90: Administration, scoring and procedures manual-I for the revised version. Clinical Psychometrics Research Unit, Johns Hopkins University School of Medicine, Baltimore, USA.

Dille, J.R. and Smith, P.W. (1964) Central nervous system effects of chronic exposure to organophosphate insecticides. Aerospace Medicine, 35, 475-478.

Dixon, W.J. (Ed.) (1988) BMDP Statistical software. Berkeley (USA): University of California Press.

Emmen, H.H., Hoogendijk, E.M.G., Hooisma, J., Orlebeke, J.F. and Uijtdehaage, S.H.J. (1988) Adaptation of two standardized international test batteries for use in the Netherlands for detection of exposure to neurotoxic compounds. Internal report 1988-18. TNO Medical Biological Laboratory, Rijswijk, The Netherlands.

Emmen, H.H., Hooisma, J., Orlebeke, J.F., Uijtdehaage, S.H.J. and Letz, R. (1989) Reliability of the Neurobehavioral Evaluation System (NES) and WHO core tests. In: Abstracts of the third international symposium on neurobehavioral methods in occupational and environmental health. Washington, USA.

Ferraz, H.B., Bertolucci, P.H.F., Pereira, J.S., Lima, J.G.C. and Andrade, L.A.F. (1988) Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology, 38, 550-553.

Fidler, A.T., Baker, E.L. and Letz, R. (1987) Neurobehavioural effects of occupational exposure to organic solvents among construction painters. British Journal of Industrial Medicine, 44, 292-308.

Gade, A., Mortensen, E.L. and Bruhn, P. (1988) "Chronic painter's syndrome". A reanalysis of psychological test data in a group of diagnosed cases, based on comparisons with matched controls. Acta Neurologica Scandinavica, 77, 293-306.

Gamberale, F. (1985) Use of behavioral performance tests in the assessment of solvent toxicity. Scandinavian Journal of Work Environment and Health, suppl. 1, 65-74.

Gershon, S. and Shaw, F.H. (1961) Psychiatric sequelae of chronic exposure to organophosphate insecticides. Lancet, 1, 1371-1374.

Hartman, D.E. (Ed.) (1988) Neuropsychological Toxicology. New York: Pergamon Press, USA.

Heslinga, H. et al. (1983) Het koderen van het opleidingsnivo. Internal report. Department of Psychology, University of Groningen, The Netherlands.

Hooisma, J. Emmen, H.H. and Jonkman, E.J. (1990) Subjective symptoms and neurobehavioural functioning in young and older Dutch painters. In: Abstracts of the 23rd international congress on occupational health, 183. Montreal, Canada.

Hooisma, J. Emmen, H.H., Kulig, B.M., Muijser, H., Poortvliet, D. and Letz, R. Factor analysis of tests from the Neurobehavioral Evaluation System and the World Health Organization Neurobehavioral Core Test Battery. In: B.L. Johnson (Ed.) (1990) Proceedings of the third international symposium on neurobehavioral methods in occupational and environmental health. Boca Raton (USA): CRC Press Inc.

Iregren, A. (1982) Effects of psychological test performance of workers exposed to a single solvent (toluene): A comparison with effects of exposure to a mixture of organic solvents. Neurobehavioral Toxicology and Teratology, 4, 695-701.

Iregren, A, Akerstedt, T., Anshelm Olsen, B. and Gamberale F. (1986) Experimental exposure to toluene in combination with alcohol intake: Psychophysiological effects. Scandinavian Journal of Work Environment and Health, 12, 128-136.

Israeli, R., Sculsky, M. and Tiberin, P. (1983) Acute central nervous system changes due to intoxication by Manzidan (a combined dithiocarbamate of maneb and zineb). Archives of Toxicology, Supplement, 6, 238-243.

Johnson, B.L. (Ed.) (1987) Prevention of neurotoxic illness in working populations. Chichester (USA): John Wiley & Sons.

Kolb, B. and Whishaw, I.Q. (1980) Fundamentals of human neuropsychology. New York (USA): W.H. Freeman and Company.

Korsak, R.J. and Sato, M.M. (1977) Effects of chronic organophosphate pesticide exposure on the central nervous system. Clinical Toxicology, 11, 83-95.

Lezak, M.D. (1983) Neuropsychological assessment. New York (USA): Oxford University Press.

Luteijn, F. and Ploeg, F.A.E. van der (1983) Handleiding GIT. Lisse (The Netherlands): Swets & Zeitlinger BV.

Maizlish, N., Schenker, M., Weisskopf, C., Seiber, J. and Samuels, S. (1987) A behavioral evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. American Journal of Industrial Medicine, 12, 153-172.

McNair, D.M., Lorr, M. and Droppelman, L.F. (1971) EITS Manual for the Profile of Mood States. San Diego, California: Educational and Industrial Testing Service. In: B.L. Johnson (Ed.) (1987) Prevention of neurotoxic illness in working populations. Chichester (USA): John Wiley & Sons.

Metcalf, D.R. and Holmes, J.H. (1969) EEG, psychological and neurological alterations in humans with organophosphate exposure. Annals of the New York Academy of Sciences, 160, 357-365.

Miller, D.B. (1982) Neurotoxicity of the pesticidal carbamates. Neurobehavioral Toxicology and Teratology, 4, 779-787.

Mikkelsen, S., Jorgensen, M., Browne, E. and Gyldensted, C. (1988) Mixed solvent exposure and organic brain damage. Acta Neurologica Scandinavica, Supplement, 118, 78.

Rodnitzky, R.L. Levin, H.S. and Mick, D.L. (1975) Occupational exposure to organophosphate pesticides. Archives of Environmental Health, 30, 98-103.

Rozeman, C.A.M. (1990) Terminale nierinsufficiëntie en het zenuwstelsel. Thesis, Free University Amsterdam, The Netherlands.

Savage, E.P., Keefe, T.J., Mounce, L.M., Lewis, J.A., Heaton, R.K. and Parks, L.H. (1982) Chronic neurological sequelae of acute organophosphate pesticide poisoning: An epidemiologic study. Fort Collins: Colorado Epidemiologic Pesticide Studies Center. In: R.E. Tarter et al. (Eds.) (1988). Medical Neuropsychology, pp. 252-254. New York (USA): Plenum Press.

Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A., Heaton, R.K. and Burcar, P.J. (1988) Chronic neurological sequelae of acute organophosphate pesticide poisoning. Archives of Environmental Health, 43, 38-45.

Sharp, D.S., Eskenazi, B., Harrison, R., Callas, P. and Smith, A.H. (1986) Delayed health hazards of pesticide exposure. Annual Reviews of Public Health, 7, 441-471.

Smith, A. (1968) The Symbol Digit Modalities Test: A neuropsychologic test for economic screening of learning and other cerebral disorders. Learning Disorders, 3, 83-91.

Tabershaw, I.R. and Cooper, W.C. (1966) Sequelae of acute organic phosphate poisoning. Journal of Occupational Medicine, 8, 5-20.

Triebig, G. and Valentin, H. (1989) Current aspects of solvent neurotoxicity: Experiences in occupational medicine in the Federal Republic of Germany. In: World Health Organization. Occupational health in the chemical industry. Copenhagen, Denmark.

Wald, F.D.M. (1984) De verkorte POMS. Internal report. Department of Psychology, University of Amsterdam, The Netherlands.

Wald, F.D.M. and Mellenbergh, G.J. (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). Nederlands Tijdschrift voor de Psychologie, 45, 86-90.

Warrington, E.K. and James, M. (1967) Disorders of visual perceptions in patients with localized cerebral lesions. Neuropsychologica, 5, 253-266.

Wechsler, D. (1945) A standardized memory scale for clinical use. Journal of Psychology, 19, 87-95.

Wechsler, D. (1955) Wechsler Adult Intelligence Scale Manual. New York (USA): Psychological Corporation.

Wood, R.W. (1981) Neurobehavioral toxicity of carbon disulfide. Neurobehavioral Toxicology and Teratology, 3, 397-405.

World Health Organization. (1987) Organophosphorous pesticides: an epidemiological study, 22. Copenhagen, Denmark.

Appendices

Appendix 1

Description of NES tests

Simple Reaction Time Test: In the Simple Reaction Time Test, the subject is asked to press a button as quickly as possible when a red square (2.5x2.5 cm) appears on the screen. The inter-trial interval (2.5-5.0 sec) is varied randomly to reduce effects of stimulus adaptation. The maximum response interval is set at 1 second. Individual reaction time latencies are recorded. The first 16 trials serve as practice; performance (mean reaction time) is measured over the next 80 trials. A minimum of 40 correct trials (i.e. 100_RT_1000 ms) is required.

Continuous Performance Test: The Continuous Performance Test measures sustained visual attention by having the subject press a button upon seeing a large letter S when it is projected onto the video display. Five different letters (A,C,E,S,T) flash briefly on the screen (for about 50 ms) at a rate of one per second for five minutes. Each stimulus is selected randomly, except that it is rejected if it is the same stimulus as the last, i.e. no two stimuli in a row are the same. Individual response latencies for each critical trial (S) are recorded as well as the number of non-response (NR) and false-positive (FP) errors. The first 12 critical trials serve as practice, performance (mean reaction time) is measured over the next 48 trials. A minimum of 24 correct trials (i.e. 100_RT_1000 ms) is required.

Color Word Vigilance Test: The Color Word Vigilance Test was developed by Iregren et al. (1986). The test consists of the presentation of colored stimuli (words) for 1.5 seconds with 2.2 seconds between stimuli. The words 'groen' (green), 'geel' (yellow), 'blauw' (blue) and 'rood' (red) are presented in each of the four colors. The test includes 208 stimului in 13 identical blocks with four targets (i.e. word in the right color) in each block. The task of the subject is to press a button as fast as possible in case of congruency between word and color. Individual response latencies for each critical trial are recorded as well as the number of non-response (NR) and false-positive (FP) errors. The first 12 critical trials serve as practice, performance (mean reaction time) is measured over the next 40 trials. A minimum of 20 correct trials (i.e. 100_RT_1500 ms) is required.

Associate Learning Test: The Associate Learning Test is similar to the paired-associate learning test used in the Wechsler Memory Scale (Wechsler, 1945). Nine pairs of names and occupations are subsequently presented on the visual display screen. Pairs are displayed for two seconds with one second between stimulus pairs. After all the pairs are presented one name is prompted and all occupation alternatives are presented. Upon answering the subject receives feedback. Three trials are given with the order that the paired names and occupations are presented, the order for which the names are prompted, and the order that the answer alternatives are presented changed on each trial. The score is the total number of correct answers in three trials.

Associate Recognition Test: The Associate Recognition Test is a single recall trial of the (nine) names and (nine) occupations used in the Associate Learning Test. One name is prompted and all occupation alternatives are presented. The subject is asked to match name and occupation correctly. No feedback is given. The score is the number of correct responses.

Serial Digit Learning Test: The Serial Digit Learning Test (also called digit supraspan) consists of presentation of the same sequence of eight digits for several (2-8) trials. Digits are displayed for 600 ms with 600 ms between stimulus digits. The subject is asked to remember, and enter into the computer, as many digits as possible in the correct order after having been presented all eight digits. Testing continues until the subject responds correctly two trials in a row or when the maximum number of eight trials is reached. The score is 64 minus the number of incorrect responses.

Pattern Memory Test: The Pattern Comparison Test is similar to a computer-administered test given by Acker (1982). Stimulus patterns are similar to those used in a memory test devised by Warrington and James (1967). A single 10 by 10 character array with array elements randomly selected to be either a blank or a filled square is presented for a period of five seconds. After a retention interval of 2 seconds, three similar arrays are presented together side by side on the screen. One of these is the original array and the subject must choose which of the arrays is the same as the first array presented alone. The test includes 29 trials and 1 practice trail. The response latency for each item as well as the number of correct responses are recorded. The score is the number of correct responses.

Finger Tapping Test: The subject is asked to press a button as many times as possible within a 10 second period. Two trials with the left hand pressing one button are adminstered first, followed by two right-hand trials, and two trials with both hands alternately tapping two buttons. Visual feedback for each button press is given. The score is the mean of: - the maximum number of taps with the preferred hand, - the maximum number of taps with the non-preferred hand, and - the maximum number of taps with both hands alternating.

Hand-Eye Coordination Test: In the Hand-Eye Coordination Test the subject is asked to use a joy-stick to trace over a sine wave pattern on the video display. A cursor moves horizontally at a constant velocity, while the subject controls the vertical motion of the cursor with the joystick. Deviations from the line are recorded and constitute a measure of coordination ability. The Hand-Eye Coordination Test includes 3 trials and 1 practice trial. The vertical distance (pixels) of the cursor from the set line (300 data points) is sampled for each trial. The score is the natural logaritm of the mean absolute error (MAE) of the best two trials.

Symbol-Digit Substitution Test: The Symbol-Digit Substitution Test is similar to the Symbol Digit Modalities Test (Smith, 1968) which is a modification of the Digit-Symbol Substitution Test from the Wechsler Adult Intelligence Scale (Wechsler, 1955). Nine symbols and nine digits are paired at the top of the screen and the subject has to press the digit keys corresponding to a test set of the nine symbols scrambled. Five sets on nine symbol-digit pairs are presented in succession with the first as a practice set. Assignment of symbols is random, subject to the restriction that a symbol may not appear in the stimulus column directly below where it appears in the key. Pairing of symbols and digits over trials is varied. Subjects are asked to respond as fast as possible and to make no mistakes. The latency required to complete each item in each of the sets of symbols and the number of digits incorrectly matched with the test symbols are recorded. The score is the mean time per correct item on the best two trials. A minimum of seven correct items per trial is required.

Pattern Comparison Test: The Pattern Comparison Test is similar to a computer-administered test given by Acker (1982). Stimulus patterns are similar to those used in a memory test devised by Warrington and James (1967). Three 10 by 10 character arrays are presented side by side. Array elements are randomly selected to be either a blank or a filled square. Two of the figures are identical and the third is similar, but with a number of array elements (4-6) changed. The test includes 29 trials and 1 practice trial. The response latency for each item as well as the number of correct responses are recorded. The task of the subject is to point out the irregular pattern as fast as possible. The score is the mean reaction time per correct response. A minimum of 20 correct items is required.

Mood Scale: The Mood Scale is similar to that of the Profile of Mood States self-administered questionnaire (McNair et al., 1971) in which subjects rate themselves with respect to their feelings over the previous week. Subjects are asked to indicate their mood states on a five-point scale ranging from 'not at all' to 'extremely'. Our adaptation is limited to a 32-item inventory (Wald, 1984, 1990). The mood adjectives are displayed, one by one, on a screen. A five-dimensional mood profile (depression, anger, fatigue, vigor, and tension) is obtained by combining ratings on the individual test items.

Vocabulary Test: The Vocabulary Test is used to evaluate verbal ability. It is adapted from the vocabulary sub-test of the Groninger Intelligence Scale (GIT; Luteijn and Van der Ploeg, 1983). The subject is asked to select the synonym from a set of five words. The first 3 items serve as practice, performance is measured over the next 20 items.

Appendix 2

Number of items/trials for the tests, self-report and rating scales used

Testing sequence		Abbreviation	Number of items/ trials analyzed
01	Personal and Occupational History Questionnaire (a)(*)	POHQ	48 items
02	Symptom Checklist (*)	SCL-90	90 items
03	Finger Tapping Test	FTT	3x2 10s trials
04	Pretest Questionnaire (b)	PRQ	8 items
05	Mood Scales	MS	32 items
06	Simple Reaction Time Test	SRTT	80 stimuli
07	Associate Learning Test	ALT	3x9 items
08	Vocabulary Test	VT	20 items
09	Serial Digit Learning Test	SDLT	max. 8 trials of 8 digits
10	Symbol-Digit Substitution Test	SDST	2 best of 4 trials
11	Continuous Performance Test	CPT	48 target stimuli
12	Pattern Comparison Test	PCT	29 items
13	Pattern Memory Test	PMT	29 items
14	Hand-Eye Coordination Test	HECT	2 best of 4 trials
15	Associate Recognition Test	ART	9 items
16	Color Word Vigilance Test	CWVT	40 target stimuli
17	Neurotoxic Anxiety Scale	NAS	28 items
18	Posttest Questionnaire (c)	POQ	11 items

(*) Tests of "paper-and-pencil type"; all other tests are automated

(a) Personal and Occupational History Questionnaire: In order to identify persons whose behavioral test performance may have been affected by organic disease, trauma, prescribed or recreational drug use, subjects were asked to complete a self-administered personal and occupational history questionnaire (POHQ) which covered: 1) demographic and physical information; 2) medical history; 3) history of exposure to neurotoxic substances; 4) consumption of drugs, alcohol and tobacco and 5) subjective symptoms of complaints and symptoms related to CNS dysfunction (for further information on this questionnaire the reader is referred to Brouwer (1990, part 1).

(b,c) Pretest and Posttest Questionnaire: The Pretest Questionnaire (PRQ) included items on present physical and mental state as well as on use or consumption of medication, alcohol, caffeinated beverages, or tobacco in the past 24 hours. The Posttest Questionnaire (POQ) included items on present physical and mental state as well as on subjective evaluation of test performance. The PRQ was administered immediately before neuropsychological testing and the POQ immediately after neuropsychological testing. Both the PRQ and the POQ were used to identify subjects whose behavioral test performance may have been affected by recent intake of medications or drugs, or lack of motivation.

Appendix 3

Prevalence of symptoms on the Neurotoxic Anxiety Scale (NAS) (translated questions)

	Controls (N=64)		Exposed (N=122)		
NAS-questions	%	#	%	#	P
01 I have diarrhea once a month or more	3.1	(2)	3.3	(4)	NS
02 I am troubled by attacks of nausea and vomitting	0.0	(0)	1.6	(2)	NS
03 I have nightmares every few nights	6.2	(4)	2.5	(3)	NS
04 I find it hard to keep my mind on a task or job	14.1	(9)	12.3	(15)	NS
05 My sleep is fitful and disturbed	14.1	(9)	16.4	(20)	NS
06 I wish I could be as happy as others seem to be	9.4	(6)	13.1	(16)	NS
07 I have a great deal of stomach trouble	10.9	(7)	12.3	(15)	NS
08 I certainly feel useless at times	6.2	(4)	4.1	(5)	NS
09 I do not tire quickly	48.4	(31)	58.2	(71)	NS
10 I have very few headaches	81.3	(52)	73.8	(90)	NS
11 I frequently find myself worrying about something	32.8	(21)	48.4	(59)	*
12 I have periods of such great restlessness that I cannot sit long in a chair	39.1	(25)	38.5	(47)	NS
13 I believe that I am no more nervous than most others	89.1	(57)	87.7	(107)	NS
14 I sweat easily even on cool days	26.6	(17)	15.6	(19)	NS
15 I have very few fears compared to my friends	39.1	(25)	39.3	(48)	NS
16 I work under a great deal of tension	35.9	(23)	18.0	(22)	*
17 I am more sensitive than most other people	21.9	(14)	16.4	(20)	NS
18 I cannot keep my mind on one thing	17.2	(11)	18.9	(23)	NS
19 I feel anxiety about something or someone almost all the time	4.7	(3)	4.1	(5)	NS
20 I am usually calm and not easily upset	82.8	(53)	81.1	(99)	NS
21 It makes me nervous to have to wait	34.4	(22)	45.1	(55)	NS
22 I have had periods in which I lost sleep over worry	42.2	(27)	45.9	(56)	NS
23 I am a high strung person	6.2	(4)	9.0	(11)	NS
24 I don't seem to care what happens to me	9.4	(6)	7.4	(9)	NS
25 I am happy most of the time	93.8	(60)	91.0	(111)	NS
26 I am afraid of losing my mind	17.2	(11)	18.0	(22)	NS
27 I have difficulty in starting to do things	23.4	(15)	13.1	(16)	NS
28 I enjoy many different kinds of play and recreation	89.1	(57)	77.0	(94)	NS

P: level of significance * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant ($p\geq0.05$)

%: percentage of subjects answering with 'true'

#: number of subjects answering with 'true'

Distribution of number of complaints on NAS	Controls (N=64)		Exposed (N=122)	
	%	#	%	#
0 - 3	28.1	(18)	32.8	(40)
4 - 6	46.0	(30)	34.4	(42)
7 - 9	10.9	(7)	21.3	(26)
10 - 12	7.8	(5)	7.4	(9)
13 - 28	6.3	(4)	4.1	(5)

Chi Square statistic: 4.93 (not significant)

Appendix 4

Means and standard deviations for controls (n=64) and exposed subjects without flower-activities (n=49) on the neuropsychological tests and the results on the statistical analyses

Neuropsychological tests (NES)	Controls Mean	Controls SD	Exposed Mean	Exposed SD	ANCOVA Me-Mc	ANCOVA P	MLR MEi	MLR P
Attention								
- Combined z-score	0.23	0.82	-0.24	0.81	-0.47	**	-0.12	NS
Simple Reaction Time Test								
- Latency (ms)	234.26	33.82	246.68	38.05	9.32	NS	2.84	NS
Continuous Performance Test								
- Latency (ms)	380.24	41.37	397.00	41.35	16.82	*	6.77	NS
Color Word Vigilance Test								
- Latency (ms)	618.41	66.04	655.36	72.19	36.04	*	15.61	NS
Learning and Memory								
- Combined z-score	0.08	0.64	-0.14	0.65	-0.10	NS	-0.08	NS
Associate Learning Test								
- Correct responses	14.27	5.12	13.82	5.01	0.35	NS	0.42	NS
Associate Recognition Test								
- Correct responses	5.25	2.14	4.96	2.28	-0.08	NS	-0.10	NS
Serial Digit Learning Test								
- Correct responses	39.31	7.06	36.94	7.90	-1.85	NS	-1.17	NS
Pattern Memory Test								
- Correct responses	20.63	3.56	19.41	4.05	-0.65	NS	-0.80	NS
Motor Performance								
- Combined z-score	0.15	0.73	-0.20	0.73	-0.23	NS	-0.09	NS
Finger Tapping Test								
- Number of taps	62.28	11.53	59.16	9.94	-1.68	NS	-1.60	NS
Hand-Eye Coordination Test								
- Mean absolute error	1.51	0.28	1.64	0.28	0.10	NS	0.01	NS
Perceptual Coding								
- Combined z-score	0.31	0.83	-0.22	0.84	-0.35	*	-0.33	**
Symbol-Digit Substitution Test								
- Latency (s)	2.52	0.44	2.84	0.49	0.24	**	0.16	**
Pattern Comparison Test								
- Latency (s)	4.16	0.93	4.48	1.05	0.14	NS	0.14	**

P: level of significance * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant ($p\geq0.05$)
Me-Mc: difference in adjusted means between exposed and control groupMEi: difference in adjusted means between exposed and control group for an average exposure index

Appendix 5

Means and standard deviations for controls (n=64) and exposed subjects without flower-activities (n=49) on the questionnaires for subjective symptoms and the results of the statistical analyses

Self-report and rating scales	Controls		Exposed		Mann-W		MLR	
(SCL-90; MS; NAS)	Mean	SD	Mean	SD	Me-Mc	P	MEi	P
Affective symptomatology/subjective complaints								
Symptom Checklist								
- Phobia		12.16	3.57	12.47	4.83	0.31	NS	
- Fear		7.59	1.78	7.51	1.68	-0.08	NS	
- Depression	19.17	5.58	19.64	7.00	0.47	NS		
- Somatic complaints	15.16	4.29	15.70	4.59	0.54	NS		
- Obsessive behavior	11.76	3.75	12.32	4.11	0.56	NS		
- Distrust	22.29	7.04	22.79	6.27	0.50	NS		
- Hostility	7.03	2.12	7.00	1.74	-0.03	NS		
- Sleep disorders	4.06	2.02	4.47	2.14	0.41	NS		
- Rest items	10.14	1.73	10.11	1.91	-0.03	NS		
- SCL-90 total score	109.37	27.10	111.98	27.16	2.61	NS	0.42	NS
Mood States								
- Depression	1.19	0.51	1.19	0.34	-0.00	NS		
- Anger		1.34	0.47	1.40	0.47	0.06	NS	
- Fatigue	1.41	0.50	1.33	0.56	-0.08	NS		
- Vigor		3.26	0.84	3.31	0.88	0.05	NS	
- Tension	1.43	0.55	1.49	0.60	0.06	NS		
- MS total score	1.42	0.42	1.42	0.43	-0.00	NS	-0.17	NS
Neurotoxic Anxiety Scale								
- NAS total score	5.52	4.04	6.33	4.23	0.81	NS	0.30	NS

P: level of significance * p<0.05; ** p<0.01; *** p<0.001; NS = not significant (p≥0.05)
Me-Mc: difference in means between exposed and control group
MEi: difference in means between exposed and control group for an average exposure index