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

Effects on the central nervous system: neurological examination, (quantitative) EEG, and evoked potentials

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

Part 3b

Effects on the central nervous system: neurological examination, (quantitative) EEG, and evoked potentials

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CONTENTS

SUMMARY

As a part of the Dutch study on occupational exposure to pesticides in the bulb-growing industry, two subgroups consisting of 76 bulb-growers and 34 controls were examined neurologically and neurophysiologically for disorders of the central nervous system. Some minor clinical abnormalities were found in both groups but these were probably not related to the exposure. The frequency and severity of the clinical abnormalities found were similar in both groups. The visual evoked potential studies revealed also no differences between the groups. The EEGs of the bulbgrowers contained more fast cerebral activity (beta-activity) than those of the reference group. This difference was also slight but could be the result of chronic exposure to pesticides. As such it should be considered as an adverse effect until proven otherwise.

Titel:	Effecten op het centrale zenuwstelsel van bestrijdingsmiddelen in de bloembollenteelt: klinisch neurologisch onderzoek, (quantitatief) EEG en evoked potentials.
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SAMENVATTING

Van de groep kwekers en controle personen die deelgenomen hebben aan het onderzoek "Gezondheid in verband met beroepsmatige blootstelling aan bestrijdingsmiddelen in de bloembollenteelt" werden 76 kwekers en 34 controle personen neurologisch en klinisch neurofysiologisch onderzocht op afwijkingen in de functie van het centrale zenuwstelsel.

Bij het neurologisch onderzoek werden bij in totaal 24 personen lichte afwijkingen gevonden. Er waren in deze geen verschillen tussen beide groepen.

Ook wat betreft het visual evoked potential onderzoek werden geen verschillen tussen de kwekers en de controle personen aangetoond.

Bij het EEG onderzoek werd bij de kwekers meer snelle cerebrale activiteit (beta-activiteit) aangetroffen dan bij niet-kwekers. Deze EEG stoornissen waren weinig ernstig van aard, doch dienen zolang het tegendeel niet bewezen is als ongewenst beschouwd te worden.

1 INTRODUCTION

In the report "Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture" (Brouwer et al., 1990) the results of a study of the effects of pesticides on workers in the bulb growing industry are summarized. Details of the study are given in reports on aspects of occupational hygiene, central nervous system, peripheral and autonomic nervous system, health assessment, the skin, and biological monitoring. Together with the report on the neuropsychological studies this report aims at providing data on the effects of pesticides on the central nervous system.

In flower bulb culture many toxic substances are used. Furthermore, in the course of time changes in the choice of substances have occurred. It is often impossible to delineate exactly what the exposure has been for an individual worker. In the last years, however, pesticides known under the names of zineb and maneb (ethylene-bis-dithiocarbamates) are preferred. The literature on effects on the central nervous system of chronic exposure to these substances is limited to one report (Ferraz et al., 1988). These authors mention rigidity and tremor as signs of cerebral disorder. Clinical neurophysiological aspects of such exposure are not known. The same holds true for metabolites of dithiocarbamates. On substances which are more or less chemically related to dithiocarbamates such as carbaryl, CS_2 and disulfiram some information is available. Acute intoxication with carbaryl, which is a cholinesterase inhibiting carbamate, induces a diffuse encephalopathy with transient electroencephalographic disturbances. Long term effects are not known (Dickoff et al., 1987). Workers chronically exposed to CS_2 are prone to tremor, visual disturbances, deafness and memory defects. The electroencephalogram (EEG) in these cases is slightly abnormal (Peters et al., 1988). Encephalopathy due to disulfiram is a well known clinical entity, probably as a result of the wide spread clinical use of this drug. Its prevalence is approximately 1-2% of the subjects who use the substance. Clinical symptoms are memory defects, disorientation, ataxia, hallucinations, and, in approximately 7% of the cases, epileptic fits. Diffuse EEG disturbances occur after acute intoxication; visual evoked potentials can be abnormal as well (Hotson and Langston, 1976; Miller, 1982).

The aim of the study was to evaluate the effects on the central nervous system of chronic exposure to pesticides used in the bulb growing industry. The neuropsychological aspects, which are an important part of such a study, are provided elsewhere (part 3a, Emmen et al.). The parameters used for the present report are derived from the clinical neurological examination, electroencephalography (EEG), and studies of the reaction of the central nervous system to visual stimuli (visual evoked potentials, VEPs).

2 STUDY GROUPS

Of the group of bulb-growers and control persons who participated in the study on the effects of exposure to pesticides in the bulb growing industry 76 bulb-growers and 34 control persons participated in the study reported here (bulb-growers: age x 44.7 yrs, SD 6.8 yrs; controls: age x 41.8 yrs, SD 8.6 yrs; all males). As the participation in the clinical and neurophysiological part of the study was on voluntary basis, some bias might have been introduced. However, there were no demographic differences between the persons who underwent the clinical and neurophysiological examinations and those who did not. All participants in the study, those who were examined by us as well as those who were not, were medically screened by means of a questionnaire before entering in the study. Comparison of the answers provided by both groups revealed no differences. For the participants in the clinical and neurophysiological examinations the medical history was taken another time by one of us (A.W. de W. or E.J. J.).

3 METHODS

3.1 Clinical examination

All bulb-growers and controls were examined neurologically. The examination was performed by two neurologists; it focussed on visual acuity, function of eye muscles, nystagmus, disturbances of cranial nerves, dysarthria and aphasia, pareses, gait, coordination, and tremor.

3.2 Visual evoked potentials (VEPs)

The pattern reversal method was used for stimulation. The person under study was seated and looked at a screen on which a pattern of black and white checks in a checkerboard fashion was projected. He was asked to fixate on the centre of the screen. One eye was covered. After the examination of the first eye, this eye was covered and the procedure was repeated for the other one. One hundred stimuli, consisting of reversal of black and white in the checkerboard pattern, were given at a rate of one per second. The screen measured horizontally and vertically 28 degrees in the visual field; the size of the checks was one degree in the visual field. The contrast between black and white checks in the pattern was 97%. The evoked potentials were measured at the electrode Oz of the 10-20 system; the reference was placed on electrode position Cz. As control for the reliability of the measurements VEPs were also recorded at more laterally placed electrodes (O1 and O2). The filters of the system were set at 2 and 100 Hz (minus 3 dB) respectively. Automatic rejection of artefacts was done before averaging. The signals were amplified and averaged. Latencies and amplitudes of the potentials thus obtained, were measured using cursors. Finally, the VEPs of 75 bulb-growers and 32 controls could be evaluated. In the other 3 subjects technical failures precluded reliable recording. The group values of the most important VEP components (latencies of the components N70, P100 and N125), differences between left and right, and amplitudes of the P100 component were compared between bulb-growers and control subjects using the Student's t-test (two tailed; $\alpha = 0.05$).

3.3 Electroencephalography (EEG)

Visual interpretation of the EEG

The EEGs were read by two clinical neurophysiologists (A.W. de W. and E.J. J.). The records were evaluated without knowledge to which group the person under study belonged and were assessed in scores of rhythmic background activity, diffuse slow activity, reactivity of the background pattern, diffuse paroxysmal abnormalities, and focal abnormalities. These scores were summated to a sum score of the EEG (Total Visual Assessment, TVA), which varied between 1-36 (see table 1). This sum score had been validated before in studies of patients with uremia, Parkinson's and Alzheimer's disease (Rozeman, 1990; de Weerd et al., 1990). Discrepancies in assessment by both examiners were only on points of minor importance and occurred in 4 persons. In these cases a final score was given after discussion between the clinical neurophysiologists. The TVA scores for the group of bulb-growers were compared to those of the group of controls using the non-parametric Mann-Whitney U test (two tailed; $\alpha = 0.05$).

After analysis of the results of computer evaluation of the EEG (see below) a new post-hoc analysis was performed of the results of visual analysis of the EEG in respect to the occurrence of fast activity (beta-activity). The assessment of this parameter of the EEG was also performed independently by both clinical neurophysiologists.

Computer analysis of the EEG

Artefactfree samples of the EEG of at least 60 seconds were recorded with derivations C3-Cz, C4-Cz, T3-T5, T4-T6, P3-O1, P4-O2, F7-T3 and F8-T4 (international 10-20 system). The signals obtained were AD converted and transformed by fast Fourier analysis to a power spectrum. Computer analysis of the EEG could be performed in 62 bulb-growers and 28 controls.

Table 1 Assessment of the EEG

1) Frequency rhythmic background activity (fba)
   0. > 9.0 Hz.
   1. 8.1-9.0 Hz.
   2. 7.1-8.0 Hz.
   3. 6.1-7.0 Hz.
   4. 4.1-6.0 Hz.
   5. None.

2) Diffuse slow activity (dsa)
   0. None.
   1. Intermittent theta.
   2. Intermittent theta + sporadic delta.
   3. Continuous theta + intermitt. delta.
   4. Continuous theta + delta.
   5. Continuous delta.

3) Reactivity of the rhythmic background activity (rba)
   0. Normal reactivity.
   1. Diminished on eye opening.
   2. Absent on eye opening.
   3. No reaction to somatosensory stimulation.
   4. No reaction to auditory stimulation.
   5. Absence of all reactivity.

4) Paroxysmal activity (par)
   0. None.
   3. Paroxysmal slow activity.
   5. F.I.R.D.A.

5) Effect of rhythmic photic stimulation (rfs)
   0. Normal driving.
   1. Diminished driving.
   2. Suppression only.
   4. On-responses only.
   5. No effect of photic stimulation.

6) Focal disturbances (foc)
   0. No focal disturbances.
   1. Mild disturbances unilateral.
   2. Mild disturbances bilateral.
   3. Severe unilateral and mild contralateral.
   4. Severe bilateral.
   5. Multifocal.

7) Sharp wave activity (swa)
   0. None.
   2. Sporadic sharp waves.
   3. Frequent sharp waves.
   4. Triphasic waves.
   5. J.C.* complexes or PLEDS.

Total Visual Assessment (TVA): (sum 1-7) + 1.

* Jakob-Creutzfeldt complexes

Technical failures and/or abundant artefacts precluded evaluation in the other ones.

The spectra were analysed in two ways:

a) Measurement of the mean frequency of the total spectrum. The mean frequencies of the spectra of the bulb-growers were compared to those of the controls using the Student's t-test (two tailed; $\alpha = 0.05$).

b) Neurometrics analysis. From the EEG spectra the relative power of the main frequency bands (beta, alpha, theta, and delta) was calculated as a percentage of the total power in all derivations. These relative power values were transformed in order to get a Gaussian distribution. After verification of this procedure the values were corrected for age using age regression lines obtained previously (Jonkman et al., 1985; John et al., 1987). Finally, the 32 monovariate power values (8 derivations times 4 frequency bands) were normalized using Z-transformations. These Z-transformed values of bulb-growers were compared with those obtained in the group of normal references (Student's t-test, two tailed for the alpha and theta power values and one tailed for the delta and beta values; $\alpha = 0.05$).

4 RESULTS

4.1 Clinical examination

Sixty-one of the 76 bulb-growers and 25 of the 34 controls participating in the study were completely normal at neurological examination. In the other persons abnormalities (mainly in visual acuity and coordination of hand and eye movements) were found (see table 2).

Table 2 Clinical examinations

	Exposed N = 76	Controls N = 34
Normal	61	25
Abnormal	15	9*
visual acuity	5	5
nystagmus	1	2
ataxia	3	1
tremor	2	-
various	4	1

* chi-square test: n.s.

The signs mentioned under the heading 'various' were slight paresis of foot muscles after lumbar disc surgery, a congenital ptosis of one of the eye lids, congenital facial asymmetry (in two subjects), and paresis of one of the eye muscles after eye surgery. Abnormal visual acuity occurred in 5 participants in each group. These visual abnormalities were probably not correlated to exposure, as one half could be attributed to amblyopia ex anopsia, existing since childhood and the other half to presbyopia.
All signs were slight and did not give rise to handicaps in normal daily life.

4.2 VEPs

The VEPs in bulb-growers and controls were normal. Group comparisons revealed no differences (see table 3).

Table 3 Visual evoked potentials

	Exposed N = 75	Controls N = 33	Significance* of difference : p
Latencies $\frac{(right+left)}{2}$			
N70	75.2 (5.6)**	75.1 (6.1)	0.95
P100	100.7 (4.0)	100.0 (4.3)	0.43
N125	133.7 (9.7)	133.5 (8.4)	0.97
P100 (right-left)	-0.3 (3.7)	-1.3 (3.3)	0.19
Amplitude $\frac{(right+left)}{2}$			
N70-P100	9.1 (3.7)	8.2 (2.9)	0.20

* two-tailed Student's t-test
** mean (SD) values

4.3 EEG

Visual interpretation

In the group of 76 bulb-growers the mean total score of the visually interpreted EEG (TVA) was 1.85. In the 34 controls the group mean TVA was 2.14. This difference was without statistical significance.

Fast EEG activity (beta-activity) occurred more often in bulb-growers (20%) than in the controls (3%) (chi-square test: $p < 0.02$).

Computer analysis of the EEG

In the controls the group mean frequencies of the power spectra were in all EEG derivations lower than those in the group of bulb-growers. However, for none of the derivations these differences were significant. Table 4 provides details.

Table 4 EEG; mean frequency of power spectrum (Herz)

	Exposed N = 62	Controls N = 28	Significance* of difference : p
Derivation**			
C3-Cz	10.7 (1.6)***	10.1 (1.2)	0.10
C4-Cz	10.5 (1.6)	9.8 (1.2)	0.07
T3-T5	10.3 (1.2)	10.1 (1.2)	0.42
T4-T6	10.2 (1.2)	9.9 (1.2)	0.35
P3-O1	10.2 (1.0)	9.9 (1.0)	0.15
P4-O2	10.3 (1.0)	10.0 (1.1)	0.14
F7-T3	10.1 (1.6)	9.9 (1.5)	0.64
F8-T4	10.0 (1.3)	9.6 (1.5)	0.21

* two tailed Student's t-test

** C = central; P = parietal; T = temporal; O = occipital; F = fronto lateral; even number = right sided; odd number = left sided; in accordance with the international 10-20 system

*** mean (SD) values

The significance of differences at neurometrics analysis of the mono-variate EEG parameters are given in table 5.

Table 5 EEG; neurometrics analysis of relative power values

Comparison between exposed persons (N = 62) and controls (N = 28)

					p values*				
power band	derivation**	C3-Cz	C4-Cz	T3-T5	T4-T6	P3-O1	P4-O2	Fz-T3	F8-T4
delta		0.38	0.44	0.16	0.24	0.29	0.39	0.28	0.47
theta		0.62	0.57	0.93	0.63	0.80	0.79	0.95	0.89
alpha		0.42	0.60	0.33	0.22	0.18	0.24	0.28	0.26
beta		0.11	0.06	0.37	0.27	0.07	0.03	0.38	0.21

* Student's t-test, see text

** See table 4

In the right parietal and occipital regions (P4-O2) more beta-activity occurred when compared to the reference group. Trends in a similar direction were found in the right central (C4-Cz) and left parietal and occipital (P3-O1) regions.
The control group did not differ from another reference group (N = 66, all males) previously studied in our laboratory using the same methods of EEG analysis.

4.4 Correlation to the exposition index

Exposition indices were known of all bulb-growers (for the definition of this index see the report by E. Brouwer et al.). Using multiple linear regression techniques with the exposition index as dependent variable and TVA, and number of abnormal beta power values in the individual neurometrics analysis as tested variables, no correlations were found (probabilities 0.73 and 0.59 respectively).

5 DISCUSSION AND CONCLUSION

The results of the study can be summarized as follows:

- Some bulb-growers as well as some of the controls showed slight disturbances at clinical examination. There were no differences between both groups in respect to occurrence and severity of the abnormalities.
- The VEPs were normal in the bulb-growers and in the controls; there were no differences in group mean absolute values of the various parameters.
- The overall visual assessment of the EEG (TVA values) revealed no differences between both groups. The group mean values were low and within normal limits. For comparison: mean TVA values in groups of patients suffering from mild Parkinson's disease and patients with Alzheimer's disease were 7.4 and 14.8 respectively.
- Visual assessment as well as analysis of relative power values of the EEG showed that bulb-growers had more fast EEG activity than the controls. These findings were endorsed by trends found in the analysis of the mean frequencies in the power spectra. Similar changes in the EEG are found in patients using various medications, for example hypnotics, in particular benzodiazepines, and cardiovascular preparations, such as propranolol, but also in workers exposed to organophosphates (Duffy et al., 1979).
- The clinical significance of these - probably aspecific - changes found in the EEGs of bulb-growers is not yet clear. From the results of the present study a correlation between the EEG abnormalities found in the bulb-growers and the exposure to pesticides cannot be proven.

Fast EEG activity occurred more frequent in bulb-growers, but the lack of dose-effect relations attenuated the value of this finding. Neurophysiological examination of bulb-growers shortly after exposition to high doses of pesticides and follow-up of the participants in the present study might be of importance to study more closely the correlation between exposure to pesticides and abundant beta activity in the EEG. However, until further notice the disturbances in the EEG should be considered as signs of abnormal brain function, possibly related to the exposure to pesticides.

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