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

General study design
Results, conclusions and recommendations

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Labour Inspectorate

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Part I

General study design
Results, conclusions and recommendations

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CONTENTS

1 INTRODUCTION

1.1 History

In the Dutch flower bulb culture a large number of pesticides is used for different applications: weed control, crop protection, disinfection of barns (stock protection) and soil, and bulb disinfection. In 1982 some employees of Dutch flower bulb farms contacted their trade union about a few cases in which exposure to pesticides was assumed to be the cause of illness. The Food and Allied Workers Union of the Federation of the Dutch Labour Unions asked the Science Shop of the University of Leiden whether working with pesticides might be the cause of health impairment in these workers. The Faculty of Medicine at the University of Leiden was approached and a Steering Committee was nominated. In 1984 the Toxicology Laboratory of the University Hospital of Leiden carried out a preliminary study: an inventory was made of the type of pesticides used in the flower bulb culture, of existing methods for monitoring the uptake of pesticides, and of methods for monitoring health effects of pesticides (Koemeester, 1984). As a result, a cross-sectional study was proposed to investigate uptake of possible genotoxic and neurotoxic pesticides in relation to the health status of workers exposed to pesticides in the flower bulb culture (Kerklaan *et al.*, 1985).

1.2 Objective of the study

Although a considerable amount of animal research has been performed for the registration of pesticides, little is known about health effects of long-term exposure of workers to relatively low doses of pesticides. It is well known that organophosphorous pesticides affect the nervous system, both after acute and long-term exposure (Jusic *et al.*, 1980; Baron *et al.*, 1985). The preliminary study of Koemeester (1984) indicated that effects of pesticides used in the Dutch flower bulb culture on the nervous system might be expected. Only few methods were available by then for the biological monitoring of pesticides in order to relate internal exposure to health effects.

The main objectives of the present study were therefore defined as:

1. to investigate the type and extent of exposure to pesticides in the flower bulb culture;
    - 1a. to develop methods for biological monitoring in order to relate the internal exposure to different exposure situations;
    - 1b. to estimate long-term past exposure to pesticides;
2. to investigate possible neurotoxic effects of long-term exposure in pesticide workers in the flower bulb culture in relation to the extent of exposure (health effect study).

In addition, it was decided to make an inventory of skin disorders as well as possible effects on the liver and the kidney.

1.3 Flower bulb culture in the "Bulb Region"

Flower bulb farms in The Netherlands are concentrated in the provinces of North and South Holland (the western part of the country). The "Bulb Region" in South Holland, between Leiden and Haarlem, is the area where the flower bulb culture originally started. In comparison to those in the Northern Region, the farms in the Bulb Region are relatively small: 80% of the farms is smaller than 5 hectares. The average number of workers per farm is about two.

The type of pesticides being used depends to some extent on the type of bulbs cultured. In the Northern area, where bulbs of lilies and irisses are cultured, insecticides are applied more often, whereas in the Bulb Region fumigants for soil disinfection are predominantly used.

Pesticides are applied at different times in the whole process: starting with soil fumigation, and followed by disinfection of the bulbs before being planted, weed control, crop protection and, after harvesting the bulbs, disinfection of the barns (stock protection) where they are stored (figure 1). For soil fumigation, dichloropropene is by far the most important pesticide; for crop protection this is true for zineb and maneb; captan and zineb/maneb-containing pesticides are of major importance for bulb disinfection (Liem and de Groot, 1984).

With respect to the techniques used for crop protection and bulb disinfection, the Bulb Region is also quite different from the Northern Region. In the Bulb Region traditional methods like spraying with a knapsack or a spraying bicycle and dipping of baskets in 200 liter dipping baths for bulb disinfection are still in use on many farms. Therefore, the farms in the Bulb Region are not fully representative of pesticide exposure in the flower bulb culture in The Netherlands. It is expected that exposure in the region under study is relatively high in comparison with other regions.

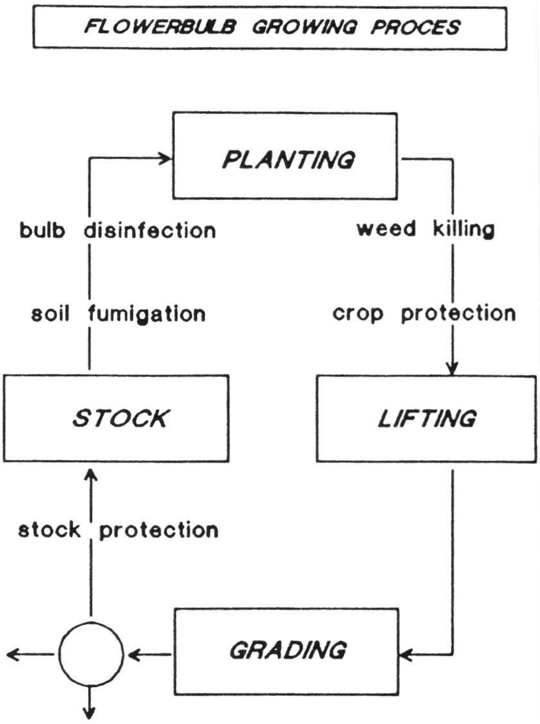

Figure 1 Schematic overview of the bulb growing process

2 STUDY DESIGN, RESULTS AND DISCUSSION

An occupational hygiene study (section 2.1) was performed to investigate the type and extent of exposure to pesticides on flower bulb farms and to identify a group of workers who have a relatively high exposure to pesticides.
To relate the external exposure (determined by type of work) to the uptake of pesticides, methods for biological monitoring were developed for 1,3-dichloropropene, pirimicarb, zineb, maneb and captan (section 2.2). Dichloropropene, used for soil fumigation, is mainly applied by contractors (commercial applicators). Therefore, a study was performed separately in a group of fourteen contractors. This investigation included environmental monitoring, biological monitoring and a study to estimate effects of subchronic exposure to DCP on liver and renal condition and on glutathione conjugation in blood.
A health effect study was performed in an exposed and a control group using a cross-sectional design with special attention to past (long-term) exposure.
The exposed subjects for the health effect study (section 2.3) were selected by means of a mailed questionnaire on working with pesticides. For each member of the exposed group an exposure index (potential exposure over several years) was estimated.

The total investigation included several aspects which are described in the separate parts of the report series:
- an occupational hygiene study (part 2);
- a health effect study to detect early effects on the central nervous system (part 3a) and on the autonomic and peripheral nervous system (part 4);
- health assessment which included a health questionnaire and laboratory assessment of blood and urine constituents, reflecting liver and renal condition (part 5);
- a study of dermal effects (part 6), and neurophysiological assessment of the central nervous system (part 3b) in a part of the population;
- biological monitoring studies for the pesticides dichloropropene (part 7), zineb, maneb, and captan (part 8), and pirimicarb (part 9).

Figure 2 presents the general design.

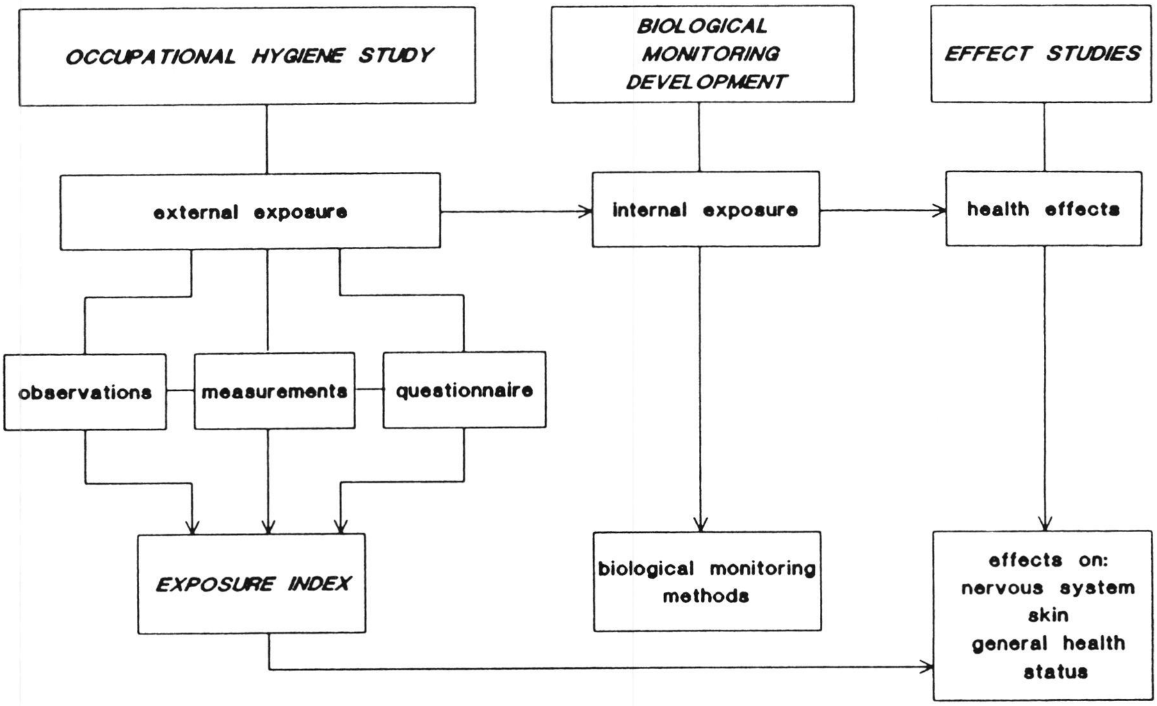

Figure 2 General study design

2.1 Occupational hygiene study

The description of the design of the occupational hygiene study is here confined to the farmers and employees who participated in the health effect study. The investigation of the contracters exposed to 1,3-dichloropropene is described in 2.2.1.

2.1.1 Questionnaires and observations

To select farms for an in-depth survey of the different applications of pesticides around the year, 15 farms were visited. Eight farms were selected representing the different application techniques used in this region. Based on this and on information from the literature (Arbeidsinspectie, 1984; Liem and de Groot, 1984) and on consultations of local experts, a postal questionnaire was developed on working with pesticides in the flower bulb culture. The questionnaire was tested for completeness and accuracy. After correction, it was submitted to 382 farmers. The first selection of the exposed group for the health effect study was based on the results of this questionnaire.

A second postal questionnaire was submitted to a subset of 187 responders to obtain more detailed information on the techniques for bulb disinfection, in order to select farms for a field study.

2.1.2 Field study and exposure index

Field studies were conducted to obtain data on potential dermal and respiratory exposure to pesticides during mixing and loading of pesticides for spraying, and during bulb disinfection. Zineb and maneb, active ingredients of formulations which are extensively used for both crop protection and bulb disinfection were chosen as indicator compounds for other pesticides similarly formulated. For exposure measurements during mixing and loading, 58 farmers were selected from the responders on the first questionnaire. Selection criteria were application of zineb/maneb (wettable powder) formulations, tractor-mounted spraying, and frequency of spraying. By telephone, information was obtained on their mixing and loading methods. Twenty-four farmers using one of three selected methods of mixing and loading (direct tank filling, premix-pouring and premix-scooping) were selected for this part of the field study.

Thirty-four farmers were selected for measurement of dermal exposure during bulb disinfection from the responders on a second postal questionnaire. Selection criteria were use of zineb/maneb formulations and bulb disinfection with a drive-in vessel or a dipping bath with baskets. Five farmers used other bulb disinfection methods than indicated in the questionnaire. The exposure measurements could be completed for only 14 of the 34 farmers, since a recent study had shown better phyto-pathological results for another pesticide.

A measure for total exposure over the years is necessary to relate health effects with long-term exposure. It was not feasible to estimate the exposure for each applied pesticide separately, because of the large variety of pesticides used, the lack of knowledge among the farmers on the type and extent of pesticides used in the past, and the difficulty of measuring such variation in exposure. Selection of pesticides with neurotoxic properties is also quite difficult because data on neurotoxicity of pesticides other than organophosphorous compounds are very sparse.

Several activities in the bulb growing process have a concomitant exposure to various pesticides. These activities were extensively surveyed and an attempt has been made to emphasize the degree of potential dermal and respiratory exposure for these activities. The major exposure to pesticides of the workers participating in the health effect study occurs during field spraying and bulb disinfection. Minor exposure is due to weed control, whereas disinfection of barns and soil are often performed by contractors. The dermal exposure route is considered to be the major source of exposure to pesticides, as was actually confirmed in the field study. Therefore, it was decided to develop an index for (total) potential exposure. Potential exposure means, in contrast to actual exposure, the exposure of the worker independent of the use of protection (*e.g.* protective gloves). Data for calculating method specific levels of exposure index were obtained from log books, own field studies and a questionnaire on application of pesticides in the past. Since the levels of exposure depend to a large degree on the actual work methods, they were determined for various methods that could be distinguished. From the observed degree of exposure, method-specific (average) levels of exposure were obtained.

Data on potential dermal exposure to pesticides during crop protection using tractor-mounted spraying and knapsack spraying were collected from the literature on field studies performed in the U.K. (Ministry of Agriculture, Fishery and Food, 1986). These data were adjusted with data from log books on application rate, dose and speed, which were completed by 16 farmers (for details, *see* part 2). The calculations were based on the most frequently used pesticide mixture zineb/maneb. The exposure index is considered an estimate for the 'historical exposure' of all participating workers.

It is indicated here that the exposure index is only a rough estimate of the actual exposure which, although calculated from data of questionnaires related to individual workers, is based on averages of exposure per method in use. Further, the extrapolation from potential to actual exposure, *i.e.* exposure on the skin, is not straightforward, due to the unknown use of protective clothing in the past and even in the present, in the sense that the degree of protection depends on many factors, which are not known in detail.

The moderate correlation between lifetime exposure and years of exposure ($r = 0.45$; $n = 127$) is considered to be an indication that the use of data on application techniques, frequency of application and area treated are important for long-term exposure assessment. It is concluded that the use of such exposure indices is very helpful, especially for the formulation of hypotheses for further studies.

The observed levels of potential dermal and respiratory exposure indicate the need for several hygienic measures to be taken by the workers. Further, several techniques and work methods lead to unnecessary high levels of exposure, which should therefore be discouraged.

2.2 Biological monitoring

The aim of the biological monitoring studies was to develop methods to measure internal exposure to pesticides for different exposure situations in the flower bulb culture. For dichloropropene-, zineb/maneb-, captan-, and pirimicarb-containing pesticides analytical methods were developed to measure metabolites excreted in the urine. For dichloropropene these data on internal exposure were correlated with data on external exposure obtained through environmental monitoring.

2.2.1 1,3-Dichloropropene

Twelve contractors applying undiluted 1,3-dichloropropene (DCP) by injection into the soil for disinfection were studied (for details, *see* part 7). For two workers incomplete data were available. As DCP is a volatile substance, respiratory exposure was considered to be the predominant route of exposure.

Environmental monitoring was performed by personal air sampling in the breathing zone on 1-3 separate days during the whole working period. Biological monitoring was carried out by measurement of thioethers and the mercapturic acid metabolites of both the E- and the Z-isomer in urine portions. Parameters of liver and renal condition in blood and urine were measured, both before and at the end of the application season. In addition, possible effects on glutathione conjugation were studied.

There was a linear relationship between the concentration of DCP in inhaled air and the total amount of mercapturic acid metabolites and thioethers excreted in urine. Elimination half-lives of both mercapturic acids were between five and six hours.

The respiratory exposure was measured and related to specific application methods and techniques. On several days the average inhaled air concentration over 8 hrs exceeded the TLV of 5 mg/m^3 (Brouwer *et al.*, 1991a).

It is concluded that measurement of mercapturic acid conjugates of DCP is useful for the purpose of biological monitoring (Welie *et al.*, 1991). Regarding liver and kidney parameters, a decrease in total serum bilirubin in combination with increased gamma-glutamyltranspeptidase during the application season indicated liver moderate enzyme induction. The glomerular function parameters albumin in urine and creatinine in serum, as well as the tubular function parameter retinol binding protein changed significantly during the season. Therefore, a (subclinical) effect on renal function of subchronic exposure to DCP is likely to exist. A decrease in erythrocyte glutathione-S-transferase activity and blood glutathione concentration indicated an effect of DCP on glutathione conjugation capacity (Brouwer *et al.*, 1991b).

A control group was not used in this part of the study; the results are considered not to be alarming. However, the exposure levels and the observed effects indicate that a reduction of the exposure levels to DCP need to be achieved.

2.2.2 Pirimicarb

Pirimicarb is a cholinesterase inhibitor which is frequently used both in the flower bulb culture and in other branches such as flower culture. Exposure may occur dermally and through inhalation. After absorption it is extensively metabolized, the most important products being 2,2-dimethylamino-4-hydroxy-5,6-dimethyl pirimidine and 2-methylamino-4-hydroxy-5,6-dimethyl pirimidine. These substances were synthesized for the development of an assay method in urine.

Ten farmers collected urine before, during, and after pirimicarb application. The samples of three of them have been analyzed as yet. Both metabolites could be detected in the urine after exposure, the concentration of the latter metabolite being the highest. Both substances were shown to be subject to rapid elimination. For details, *see* part 9.

2.2.3 Zineb, maneb and captan

Captan, a bulb disinfectant, is absorbed after exposure and metabolized to 2-thiothiazolidine-4-carboxylic acid (TTCA) and tetrahydrophthalimide (THPI). Analytical methods were developed for these metabolites. Six workers collected urine before and after bulb disinfection. In all samples both TTCA and THPI could be measured; THPI was consistently and significantly increased after occupational exposure. Although more data have to be collected to substantiate a proper relationship between captan exposure and THPI excretion, the latter metabolite may be used for measuring internal exposure to captan-containing pesticides. For details, *see* part 8.

Zineb and maneb are frequently used fungicides applied in both crop protection and bulb disinfection. Amongst others, they are metabolized to TTCA and ethylene thiourea (ETU), both of which metabolites are excreted into the urine. The same analytical method for TTCA as used to measure captan exposure was applied to zineb and maneb exposure. In addition, a HPLC-UV method for ETU was developed. The detection limit of this method applied to human urine was sufficient to measure exposure in seven farmers. Further improvement of the method is necessary to allow its use for quantification of the internal exposure to zineb and maneb.

2.3 Health effect study

Health effects of long-term exposure to pesticides in the flower bulb culture were studied in a group of 137 workers who had been intensively involved in the application of pesticides. The results were compared with a control group of 73 workers, matched for sex, age, physical activity, education and alcohol consumption.

2.3.1 Study groups

The selection of the study group is extensively described in this paragraph. A summary of the data is presented in the appendix.

The exposed group: farmers

In the the Bulb Region 827 flower bulb farms were identified in 1987. More then 90% of the farms in this region are concentrated in the five municipalities Noordwijk, Noordwijkerhout, Voorhout, Hillegom and Lisse. For practical reasons the study area was restricted to these five municipalities.

The branch organisation for farmers in agriculture and horticulture was contacted in order to obtain addresses of the farmers. A letter was sent by the organisation to a random sample of 615 farmers, to ask their permission for giving their address to the investigators. Three hundred and eighty two farmers responded positively (62%), and a questionnaire on exposure to pesticides was mailed to them. The farmers were asked to complete an enclosed form if they were *not willing* to participate in the study. Table I presents the reasons for not participating. The total response was 321 (84%; 295 questionnaires). Among the 295 farmers who completed the questionnaire, 23 were not willing to participate in the study.

Table I Reasons of exposed farmers for not participating in the study (n=26)

Reason	n
not or almost not applying pesticides	7
no time	6
no longer working on a flower bulb farm	4
retired or near retirement	3
other reasons	6

The exposed group: employees

Addresses of employees were provided by the farmers, the Food and Allied Workers Union (FNV) and the Agricultural Social Insurance Fund (ASF). A questionnaire was submitted to a total of 307 employees to obtain information about their handling of pesticides. The total response was 229 (75%) (92%, 37%, and 74% respectively for the different sources of addresses). All employees who completed the questionnaire were willing to participate in the consecutive part of the study.

To confine the study to the workers (farmers and employees) with the potentially highest degree of exposure, those workers were selected who did apply pesticides in both crop protection and bulb disinfection for at least 10 years. Farmers with flower culturing as the main activity were excluded from the study in order to make the exposure characteristics more homogeneous. Moreover, the study group was restricted to workers aged 30 to 55 years, because age might affect the effect parameters differently in relatively young and old workers. After this first selection a second postal questionnaire was submitted to 123 farmers and 45 employees concerning life style, and presence of the following exclusion criteria in order to prevent interference with the neurotoxic effect indicators:

- brain haemorrhage in the past
- meningitis in the past
- ever been unconscious for more than one hour because of a concussion
- epilepsy and use of anticonvulsants
- diabetes mellitus
- alcohol consumption of more than 40 glasses a week
- use of drugs possibly interfering with the neurotoxic effect indicators

Finally, 137 workers (102 farmers and 35 employees) took part in the health effect study.

The control group

Almost all workers of flower bulb farms are directly or indirectly exposed to pesticides. Therefore, it was decided not to select a control group of workers working on flower bulb farms. Since in all other branches of agriculture or horticulture in The Netherlands pesticides are applied, these sources for composing a control group could not be used. In a pilot study, exposed workers were asked to find a relative or acquaintance not exposed to pesticides or neurotoxic agents and with no more than five years of difference in age. This turned out not to be successful: only eight out of 14 farmers were able to find a person who met the inclusion criteria. Therefore, it was decided to find a control group in another way. A random sample of 1150 men, aged 30 to 55 years was drawn from the register of population in the five selected municipalities. A letter was sent to them explaining the study design and objectives, emphasizing the relevance of a control group. All participants were offered Hfl 75.- for their participation. A short questionnaire was enclosed with questions concerning the matching and exclusion criteria.

In addition to the exclusion criteria for the exposed group, the following exclusion criteria for the controls were added:

- working in agriculture or horticulture
- working with pesticides
- occupational exposure to lead or lead compounds and mercury or mercury compounds for more than once a month
- working in a job with regular exposure to glue, solvents, paint or paint solvents
- unemployed for more than one year

They were asked to return an enclosed form if they were not willing to participate. The reasons for not participating in the study are given in table II. The total response was 647 (56%; 556 questionnaires and 91 forms).

Table II Reasons of potential control subjects for not participating in the study (n=91)

Reason	Controls n (%)
no time	18 (20)
working on a flower bulb farm	17 (19)
no interest	10 (11)
working with chemicals/pesticides	7 (8)
under medical treatment	6 (7)
moving shortly	6 (7)
older than 55 years	4 (4)
incapacitated	3 (3)
mentally deficient	2 (2)
other reasons	13 (14)
no reason	5 (6)

Sixty-six subjects were selected for the control group, mainly persons with physically active jobs. Together with the eight workers asked by the farmers themselves a group of 74 workers with the same distribution for age, education, and alcohol consumption as the exposed group was composed. One person withdrew from the study for unknown reasons, so 73 control persons were examined in the health effect study.
In a sample of both the exposed group and the control group, information was obtained about the reasons to participate. Tabel III presents the reasons of 96 exposed and 56 control persons. Several persons mentioned more than one reason. There are no major differences between the two groups in their reasons for participation.

Table III Reasons of exposed and controls for participation

Reason	Exposed (n=96) % (n)	Controls (n=56) % (n)
knowledge about own health status	25 (24)	43 (24)
cooperation in a useful study	13 (12)	20 (11)
knowledge about effects of pesticides on own health	16 (15)	
it matters to all	9 (9)	16 (9)
asked by an acquintance		14 (8)
working with pesticides	9 (9)	
knowledge about effects of pesticides	6 (6)	9 (5)
interesting	6 (6)	7 (4)
important for own health	6 (6)	
health complaints	2 (2)	4 (2)
anxious about own health	2 (2)	
other reasons	9 (9)	11 (6)
unknown	14 (14)	13 (7)

2.3.2 Nervous system

The study on neurotoxic effects was performed between November 1988 and March 1989. In the winter hardly any pesticides are applied in the flower bulb culture.

The examinations were done in a sporting centre in the flower bulb region. This took four hours per person. Exposed and control subjects were seen, as far as possible, at the same time of the day to minimize possible circadian effects on the indicators to be measured.

An evaluation of effects on the nervous system was divided in two parts. Each subject first performed the central nervous system tests. Subsequently the neurophysiological measurements were carried out.

The investigators were not informed of the exposure status of the subjects.

The central nervous system

Neuropsychological functioning was evaluated using the Neurobehavioral Evaluation System (NES) (Baker and Letz, 1986), which is an automated test battery designed to assess several basic dimensions of neuropsychological functioning (Hooisma *et al*, 1990). Furthermore, three questionnaires, the SCL-90 Symptom Checklist, the Mood States questionnaire, and the Neurotoxic Anxiety Scale were administered to all participants to collect data about affective symptomatology or subjective complaints (for details, *see* part 3a).

A group of 129 exposed workers and 64 controls was studied. Statistical analysis of combined test scores showed that performance speed in the domains of *attention* and *perceptual coding* was significantly slower (5-8%) in pesticide-exposed workers than in controls. Further, significant relationships were found between the degree of exposure and test scores related to the functional domain of perceptual coding. Statistical analyses showed no differences in the prevalence of psychiatric symptoms or subjective symptoms between the two groups.

In an additional study (for details, *see* part 3b), a clinical neurological examination was performed, electroencephalographic monitoring (EEG) was conducted and Visual Evoked Potentials were measured in a subset of 62 exposed and 28 controls. This study was performed in the Westeinde Hospital in The Hague several weeks after the first test described above. The electro-encephalographic study demonstrated an increase in beta-activity in the central and parieto-occipetal areas after computerized analysis of the EEGs. Such an increase is also observed in patients taking hypnotic and sedative drugs. Since subjects taking these medicines were excluded from the study, it is not unlikely that this observation is related to pesticide exposure. Evoked potentials did not differ substantially between the two groups.

The peripheral nervous system

In 131 exposed and 67 controls, the effects of long-term exposure to pesticides on the peripheral nervous system was investigated with electrodiagnostic tests which consisted of

nerve conduction velocity measurements, including distal latency and amplitude on the median, ulnar, peroneal, and sural nerves. Only surface electrodes were used. In addition the distributions of conductivity velocities and of refractory periods were measured. These parameters may be valuable as early indicators of toxic neuropathy (Ruijten *et al.*, 1990;1991).
To investigate *autonomic nerve function* the variability of the heart rate during rest (Resting Arrhythmia), and the response to deep breathing (Forced Respiratory Sinus Arrhythmia) and to isometric muscle contraction (Muscle-Heart Reflex) were determined (for details, *see* part 4).
In the median nerve a decrease of 2-4% in conduction velocity was observed, which correlated with the individual exposure index (*see* 2.1.2). In the two nerves of the legs, conduction velocity was approx. 2-3% lower than in controls. The conduction velocity in the slow fibres of the peroneal nerve was also shown to be decreased. The refractory period in the sural nerve appeared to be prolonged by 5-10%. For some parameters there appeared to be a relation with the exposure index, indicating a causal relation between exposure to pesticides and decrease in peripheral nerve function.

Because of the good comparability of the control group in this study, the observed effects on the central and peripheral nervous system are probably due to pesticides. If a 'healthy worker effect' would yet exist, the measured effects would even be larger. No specific substances can be labelled as playing a causative role: a contribution of mercury compounds -used by approx. 90% of the exposed subjects in the past- cannot be excluded.
Although the *average* negative effects are (relatively) small when considered on a group basis, the impact on the *individual* pesticide worker is difficult to envisage. The *average* effects are so small that one would be unable to demonstrate such effect in an individual. Nevertheless, the occurrence of signs and symptoms of nervous system dysfunction could be accelerated in addition to the normal process of ageing, especially in bulb growers with relatively high exposure.
Longitudinal studies are necessary to fully evaluate the long-term consequences of these effects for worker safety and health.

2.3.3 General health assessment

The health assessment study included a postal health questionnaire and laboratory assessment of blood and urine constituents reflecting liver and renal condition.
A standard questionnaire, the 95% VOEG (Questionnaire to Study the Experienced Health Status) was used to obtain data on the general health status (Jansen and Sikkel, 1981).

This was supplemented with a questionnaire on health effects reported in the literature as related to exposure to pesticides, and questions about reproduction and past diseases. The questionnaire was submitted and completed in advance and finalized when the participants were examined.

Subjective symptoms

Symptoms regarding experienced ill-health were not reported more often by the exposed subjects than by the controls. Similar results were obtained with a questionnaire directed towards symptoms potentially being caused by pesticides. The only single symptom occurring more frequently in the exposed group was a burning sensation of the eyes: 24% *vs* 9%.

Clinical biochemistry

Blood and urine samples were taken to determine liver or renal impairment. The samples were collected in the study centre and transported to the laboratory. The parameters were measured with standard clinical chemical methods (for details, *see* part 5).

Among the parameters measured in serum of the exposed and control subjects were alkaline phosphatase, aspartate aminotranferase, alanine aminotransferase, gamma-glutamyltranspeptidase, lactate dehydrogenase, urea, uric acid, and creatinine. The percentage of subjects with one or more test results outside the normal range did not differ between both groups. The results do not indicate that liver and kidney condition in the members of the exposed and control groups do differ.

2.3.4 Skin

In a part of the exposed and control group a dermatological study was performed to investigate the prevalence of skin disorders. All participants to the study on neurotoxic effects were asked to take part in the dermatological study. Both in the exposed and the control group three subjects refused. A dermatological screening was performed in 75% (n=103) of the exposed group and 67% (n=49) of the control group. Subsequently, 22 subjects (19 exposed and three controls) with past or present skin disorders were tested epicutaneously for allergic reactions (for details, *see* part 6).

The exposed and unexposed subjects visited a dermatologist, who inspected the skin of at least hands and forearms. Signs of damage or intensive irritation of the skin were observed in 30% of the exposed group and in 8% of the control group; eczema in 11% and 10%, respectively. Exposed subjects often reported irritation due to contact with plants: *e.g.,* 19% had severe and 26% had moderate complaints of lily rash; 14% complained of tulip fingers; hyacinths often caused severe itching. Thirteen exposed subjects reported symptoms ascribed to one or two specific pesticides and had stopped using these. They, as well as those subjects with eczema, were invited for patch-tests for allergy: ten out of 19 exposed subjects showed a positive reaction to a pesticide (captafol, 5 times) and four out of 19 to bulb extracts (tulip, daffodil). In the controls one out of three showed allergic reactions to bulb extracts (tulip), another to chemicals related to his work.

3 GENERAL REMARKS

In an attempt to relate the exposure to pestides during various activities in the bulb growing culture to the possible occurrence of health effects, the investigations were directed towards the development of biological monitoring for a number of important pesticides, the estimation of exposure during various activities and the measurements of various health parameters, mainly for the nervous system.

Analytical methods have been developed for metabolites of several pesticides in urine. This was succesful for dichloropropene. In this case it was possible to use the method in field experiments where contractors used this compound for fumigation of soil. The relatively high exposure during these activities could be related to the use of specific techniques. Furthermore, it could be shown that the respiratory exposure to dichloropropene was highly correlated to the amount of metabolites in urine, indicating that this method for biological monitoring can be applied in practice to estimate exposure and possibly potential health risk. For the workers using dichloropropene, moderate enzyme induction and subclinical nephrotoxicity was observed. On the basis of the relatively high exposure and these effects the occurrence of long-term health effects due to dichloropropene cannot be excluded.

The analytical methods for metabolites of maneb/zineb, captan and pirimicarb have been used in some preliminary experiments, which indicate that internal exposure to these compounds does occur in practice. Further studies are needed, however, to be able to interpret such data in detail.

So far, biological monitoring could not be used to relate the uptake of pesticides to neurotoxic effects.

For the major activities in the bulb growing process which may lead to exposure to pesticides method-specific exposure data were established for zineb/maneb. This compound was chosen, since it is the major pesticide in use for crop protection and amongst the major pesticides for bulb disinfection. This choice was further considered acceptable since the use of pesticides throughout the bulb growing in the Bulb Region is largely homogeneous. On the basis of data which are important for the total potential dermal exposure over large periods of time, an exposure index for past exposure was estimated for each worker that entered the health effect study. Although the precision of such an exposure index is limited for individuals, due to individual differences in hygienic practice, etc., it roughly indicates differences in exposure between workers, and may therefore be used as an indicator for total exposure in the health effect study.

Eczema was observed as frequent in farmers (bulb growers) as in controls, whereas skin irritation was more frequent amongst the farmers. This may be important since the penetration of pesticides through the skin is most probably enhanced by damages in the skin.
In the study that was aimed at the elucidation of possible differences in neurotoxic parameters significant differences were detected between exposed workers and controls, nearly all indicated that the exposed workers were negatively affected. For some parameters there was a significant relationship between the magnitude of the exposure index and the magnitude of the (negative) effect. It is, however, very difficult to interpret these data for specific compounds, since for the various pesticides that are used in the bulb growing process it is not known which compounds may be considered responsible for the neurotoxic effects. Neither is it known whether the observed effects are reversible and if so, to what extent. Nevertheless, the mere observation of the significant differences for almost all parameters indicates an unwanted situation. In view of the observed relatively high exposure with several application techniques, a decrease in exposure is desirable and feasible; this will probably also prevent a further increase of the unwanted effects.

4 CONCLUSIONS

- The level of exposure to dichloropropene in soil fumigation is higher than is considered acceptable.

- Several analytical methods for the determination of metabolites from dichloropropene, zineb/maneb, captan and pirimicarb have been developed. The method for dichloropropene has been proven to be very useful for biological monitoring.

- Large differences in dermal exposure are observed between the various techniques that are used for the application of pesticides in the bulb growing culture.

- Clear indications have been obtained for negative effects on several peripheral and central nervous system parameters due to exposure to pesticides in the flower bulb culture.

5 RECOMMENDATIONS

- The application methods with the lowest intrinsic exposure to the workers should be used. For most situations these are tractor-mounted techniques for spraying and drive-in vessels for disinfection, using liquid formulations.

- Further research is required into the effects of hygienic measures on exposure and health effects during soil fumigation with dichloropropene.

- Further development of methods for biological monitoring is required to study the effect of various conditions in the field (hygiene, application methods, etc.) on the uptake in the body of pesticides.

- In the present study the exposure during re-entry (*e.g.* contact with treated bulbs) has not been considered. Such studies are required to obtain a valid picture of the exposure in bulb growing cultures.

- Effective education on health risks of pesticides and training of workers is required. This may be done through the development of occupational health and hygiene services in agriculture.

- A longitudinal study in the present groups will show the course of the observed effects and its relation with pesticide exposure. For this, further knowledge on neurotoxic properties of the pesticides is required.

FURTHER READING

The following detailed reports, pertaining to this study, will appear in this Scientific Series.

Brouwer D.H., Brouwer E.J., Hemmen J.J. van, Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 2. Estimation of long-term exposure to pesticides.

Emmen, H.H., Hooisma, J., Kulig, B.M. and Brouwer, E.J., Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 3a. Neurobehavioral assessment of workers occupational exposed to pesticides in the bulb growing industry.

Weerd A.W. de, Jonkman E.J., Poortvliet D.C.J., Veldhuizen R.J., Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 3b. Effects on the central nervous system: quantitative EEG and evoked potential.

Ruijten M.W.M.M., Sallé H.J.A., Smink M., Verberk M.M., Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 4. Effects on the peripheral and autonomic nervous system.

Brouwer E.J., Wolff F.A. de, Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 5. Health evaluation study.

Bruynzeel D.P., Haan, P. de, Brouwer, E.J., Boer E.M. de, Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 6. Dermal study.

Welie R.T.H. van, Duyn P van, D.H.Brouwer, J.J. van Hemmen, Vermeulen N.P.E., Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 7. Monitoring of exposure to dichloropropene.

Welie R.T.H. van, Duyn P van, Vermeulen N.P.E., Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 8*. Biological monitoring of zineb, maneb and captan.

Sjardin W., Kom J.F.M. de, Wolff F.A. de, Health in relation to occupational exposure to pesticides in the Dutch flower bulb culture. Part 9*. Biological monitoring of pirimicarb.

* Parts 8 and 9 will not appear in the Scientific Series but are available at the pertaining research institute.

REFERENCES

Arbeidsinspectie, Rapport betreffende een beeldvormend vooronderzoek naar de arbeidsomstandigheden bij het werken met bestrijdingsmiddelen in de bloembollen/knollenteelt. Zoetermeer, 1984.

Baker E.L., Letz R., Neurobehavioral testing in monitoring hazardous workplace exposures. J. Occup. Med. 1986, 28, 987-990.

Baron S., Mazliah J., Gordon M., Field screening for neurotoxic effects in agricultural workers exposed to pesticides: lessons from a preliminary study. In: Neurobehavioural methods in occupational and environmental health. WHO, Copenhagen, 1985.

Brouwer D.H., Brouwer E.J., Vreede J.A.F. de, Welie R.T.H. van, Vermeulen N.P.E., Hemmen J.J. van, Inhalation exposure to 1,3-dichloropropene in the Dutch flower bulb culture. Part 1. Environmental monitoring. Arch. Environ. Contam. Toxicol., 1991a, 20, 1-5.

Brouwer E.J., Evelo C.T.A., Verplanke A.J.W., Welie R.T.H. van, Wolff F.A. de, Biological effect monitoring of occupational exposure to 1,3-dichloropropene; effects on liver function and on glutathion conjugation. Br. J. Ind. Med., 1991b, 48, 169-172.

Emmen H.H., Hoogendijk E.M.G., Hooisma J, Orlebeke J.F., Uijtdehage S.H.J., Adaptation of two standardized international test batteries for use in the Netherlands for detection of exposure to neurotoxic compounds, MBL-report 1988-18, 1988.

Hooisma J, Emmen H.H., Kulig B.M., Muijser H., Poortvliet D., Factor analysis of test from the Neurobehavioral Evaluation System and the World Health Organization Neurobehavioral Core Test battery. In: Johnson B.L., Xintaras Ch., Proceedings of the Third International Symposium on Neurobehavioral Methods in Occupational and Environmental Health, 1990.

Jansen M.E. and Sikkel D., Verkorte versies van de VOEG schaal. G & S, 1981, 2, 78-82.

Jusic A., Juvenic D. and Milic S., Electromyographical neuromuscular synapse testing and neurological findings in workers exposed to organophosphorus pesticides. Arch. Environ. Health 1980, 35 168-175.

Kerklaan P.R.M., Vermeulen N.P.E. and Wolff F.A. de, Gezondheidseffecten van werken met bestrijdingsmiddelen in de bloembollenteelt. Onderzoeksvoorstel, Leiden, 1985.

Koemeester A., Gezondheidseffecten van bestrijdingsmiddelen bij mensen werkzaam in de bollenteelt. Onderzoeksverslag en onderzoeksvoorstel. Leiden, 1984.

Liem K.O. and Groot W.T. de, Bestrijdingsmiddelen in de bloembollenteelt: een verkenning van risico's van bestrijdingsmiddelen voor bewoners van de Bloembollenstreek. Deel 1. Theoretische emissies van bestrijdingsmiddelen bij de bloembollenteelt in de Bloembollenstreek. Leiden, 1984.

Ministry of Agriculture, Fishery and Food, Joint Medical Panel of the Scientific Subcommittee on Pesticides and the British Agrochemical Association. Estimation of exposure and absorption of pesticides by spray operators. Internal Report. United Kingdom, 1986.

Ruijten M.W.M.M., Sallé H.J.A., Verberk M.M. and Muijser H., Special nerve functions and colour discrimination in workers with long-term low-level exposure to carbon disulphide (CS_2). Brit. J. Ind. Med., 1990, 47, 589-595.

Ruijten M.W.M.M., Sallé H.J.A. and Verberk M.M., Nerve functions in workers with long-term exposure to trichloroethene. Brit. J. Ind. Med., 1991, 48, 87-92.

Welie R.T.H., Duyn P. van, Brouwer D.H., Hemmen J.J. van, Brouwer E.J., Vermeulen N.P.E., Inhalation exposure to 1,3-dichloropropene in the Dutch flower bulb culture. Part II. Biological monitoring by measurement of urinary excretion of two mercapturic acid metabolites. Arch. Environ. Contam. Toxicol., 1991, 20, 6-12.

Welie R.T.H. van, Duyn P van, Vermeulen N.P.E., Determination of two mercapturid acid metabolites of 1,3-dichloropropene inhuman urine with gas chromatography and sulfur-selective detection. J. Chromatogr. Biomed. Appl. 1989, 496, 463-471.

APPENDIX Selection of the study groups

	Farmers	Employees	Controls
Total	827	ca. 1100	
Letter sent for permission	615		
Questionnaire sent	**382**	**307**	**1150***
Response	321	229	647
- forms	26		91
- questionnaires	295		556
Willing to participate	**272**	**229**	**556**
Exclusion criteria I#:			
- age (<30 and > 55 yr)	- 67	- 42	
- applying pesticides (<10 yr)	- 22	- 40	
(no more)	- 35	- 81	
- only crop protection or bulb disinfection	- 55	- 73	
Second questionnaire sent to	**123**	**45**	
Response	115	42	
Exclusion criteria II:			
- no bulb disinfection or crop protection	- 3	- 3	
- illness	- 4	- 2	
- other reason	- 6	- 2	
Included in study group	**102**	**35**	**73°**

more than one reason for exclusion is possible
* selected for sex and age from the general population in the five communities
° matched with the exposed group