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Study of acute effects on tree-planters planting out conifer saplings treated with insecticides

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Summary

This study is a questionnaire study combined with biological monitoring of tree-planters and acute health effects of planting insecticide treated conifer saplings. The investigation is a double-blind crossover study over tree weeks.

This study involved the evaluation of health effects based on questionnaires covering problems with the respiratory tract, and also objective tests on nasal mucous membranes. No clear, acute negative health effects could be found in planters after exposure to coniferous plants treated with imidacloprid (Merit Forest) or cypermethrin (Forester), as compared with exposure to untreated plants. The breakdown product, 3-PBA, from cypermethrin was, however, increased and can therefore be used as an indicator of exposure to this insecticide. We could not, however, find any statistically-significant correlation between the acute symptoms and health problems in planters, and the questionnaire responses or 3-PBA levels. These results have been obtained during planting in late summer/early autumn and with good use of protective clothing.

Preface

The following people from the Department of Occupational and Environmental Medicine in Uppsala participated in this study: Dr Lena Elfman was project leader and Carl Hogstedt was research assistant. Together they performed all field work. Dr Karin Engvall (behaviourist) has assisted in the preparation and evaluation of the questionnaire and was also present when the questionnaire was tested in the field. Statistical calculations were performed by Erik Lampa M.Sc. Dr Robert Wålinder has been the medical doctor in the project and has helped in discussions on the evaluation of results. Jolanta Jamrozy has translated the questionnaire and letter to Polish.

Martin Lindell from Svenska Skogsplantor AB (SSP)**,** was responsible for coordination of the forestry part of the project. Andreas Hugosson (SSP Lugnet Nursery) was responsible for treatment, packing, marking and distribution of the plants together with Jörgen Jonsson (SSP). Ulf Allvin (Sveaskog) and Elin Larsson (SSP/Sveaskog) have planned the planting work. Four entrepreneurs and a foreman have worked as contact people and nineteen tree-planters have participated in the study.

Analyses of inflammation markers in nasal lavages (a test panel for mucous membrane reactions) were performed by Kerstin Lindblad and Eva Heldesjö Blom, of the department of Clinical Chemistry and Pharmacology, Akademiska Sjukhuset, Uppsala. Assistant Professor Christian Lindh, at the Department of Occupational and Environmental Medicine Clinic, University Hospital in Lund was responsible for the analysis of breakdown product of cypermethrin-containing Forester, 3-PBA (3-phenoxybenzoic acid) in urine. Dr Margareta Littorin was the principle medical doctor at the Department of Occupational and Environmental Medicine Clinic, University Hospital in Lund

Finally, we would like to thank all who helped in the performance of this study.

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Introduction

During 2006 more than 9 685 tonnes of chemical insecticides were sold in Sweden, which is an increase of nearly 395 tonnes compared with the year before. The usage of imidacloprid and cypermethrin has increased with 2.4 tonnes (96%) and 1.4 tonnes (156%), respectively, between 2002 and 2006. These insecticides were used in agriculture, forestry and industry. The usage in forestry alone amounts to 0.05% of the total usage of insecticides [1].

Since 1992 Skogsbrukets Plantskyddskommitté has worked on reducing the amount of chemical insecticides used in the forestry. Research has focused on creating a barrier protection against insects consisting of wax, glue and sand. These barriers are expected to have a protective effect for approximately 2 years and have a lower environmental impact than today's insecticides. Presently, there is one alternative barrier-protection (Bugstop) used at Bergvik Skog AB. About 5 million saplings have been treated per year, which should be compared with the annual total need for treating approximately 100 million saplings. Alternative barriers are under development, but the technology for mass application has not yet been developed [Plantskyddskommittén, internal report, 2007].

The insecticides used in forestry today are Cyper Plus, with cypermethrin as active substance, and Merit Forest WG, with imidacloprid as active substance. The Swedish Chemicals Agency approved the usage of these substances until 2005-12-31 with an extension until 2007-12-31 [2]. This decision covered the usage against insect attacks on un-barked timber and conifer saplings. The Swedish Forest Agency and Federation of Swedish Farmers, Forestry division, are of the opinion that, because of the hurricane Gudrun, there will be an increased damage by pine weevils until 2008/2009. In order to secure an effective barrier protection against pine weevils they suggest prolonging the usage of these insecticides even after 2007-12-31.

Aim

Occupational and Environmental Medicine at Uppsala University Hospital in Uppsala, has at the request of Plantskyddskommittén, Skogforsk, Uppsala, performed a study among tree-planters in Sweden in relation to the usage of insecticides in forestry. The aim of the investigation was to determine if exposure of humans to the two insecticides cypermethrin (Forester) and imidacloprid (Merit Forest WG) resulted in acute health effects.

Material and Methods

Study group

According to the union of forest and wood workers there are 2-3000 individuals involved in planting in Sweden. During last year 40% of all saplings planted were treated with insecticides. Since some of the planting personnel plant out both untreated and treated saplings, the total number of individuals who handle insecticide-treated saplings is estimated at 1500-2000.

This investigation was a double-blind cross-over study, in which each planter was his own control. The planting personnel planted out untreated saplings and Forester- and Merit Forest-treated conifer saplings for 5 days/week for in total 3 weeks. The weekend was a wash-out period between each treatment. The saplings were treated at Lugnet Nursery, Bålsta. The

saplings were distributed to the various planting sites with a code (A,B,C), which was not known by the planters and the personnel performing the field-studies.

There were 19 planters in the study group, representing four teams with five persons in each team, except one team with 4 individuals. The working teams were spread over the country with one team in the neighbourhood of Skinnskatteberg in the county of Västmanland, one in Hallsberg in Närke, one in Hok/Vrigstad, and one in Växjö/Nybro in Småland. According to the original research plan the study should have contained a larger number of individuals. The number was, however, limited due to the relatively short planting season (August to September) at our disposal, and the large number of conifer saplings that had to be treated (over half a million).

This project has been granted ethical approval by the regional ethical committee of Uppsala University (Dnr 2007/144).

Treatment of conifer saplings

Saplings used in this study were treated and distributed by Lugnet Nursery. The study started during week 37/2007. The sapling was the conifer Starpot 75, i.e. a form of plant with covered roots. The saplings were treated in three ways:1) Merit Forest (Bayer, approved by Swedish Chemicals Agency for treatment against pine weevils, registration no. 4605, class 2L; 1% solution); 2) Forester (cypermethrin cis/trans 40/60, producer and licence holder Agropfar, Belgium; 2% solution); 3) no treatment. Interagro Skog AB is the distributor of Forester in Sweden, which is not yet approved or registered in Sweden. Interagro was granted an exemption from Swedish Chemicals Agency (Diary number 730-773-07) to be included in this investigation. Earlier treatment involved Cyper Plus (cypermethrin, cis/trans 80/20, approved by the Swedish Chemicals Agency, registration no. 4580, class 2L).

The three treatments were performed and each box was marked (A,B,C) according to a system only known to two people at Lugnet Nursery. Each individual included in the study had to report which letter was on the box they planted out each week. They planted saplings with the same treatment for one week i.e. for 5 days followed by 2 work-free days.

All boxes with saplings were provided with a warning label for both Merit Forest and Cyper plus, irrespective of which treatment they had. In this study Forester was used instead of Cyper plus, but since there is as yet no warning label for this preparation the label for Cyper plus was used. Both insecticides contain cypermethrin as active substance. Lugnet Nursery packed and distributed the following for each treatment: A) 173 000 saplings, B) 173 000 saplings, C) 170 000 saplings. This made a total of 516 000 saplings for this study. All saplings were delivered punctually to the teams in Götaland and Svealand. Andreas Hugoson, production manager at Lugnet Nursery, was responsible for treatment and logistics.

Questionnaire

The basis of our questionnaire, *Insecticidal treatment of conifer saplings effect on planting personnel,* was an earlier questionnaire developed by Kolmodin Hedman and co-workers in a similar study with the preparation permethrin. A first draft of the questionnaire was discussed with representatives from Plantskyddskommittén. All questions were discussed with respect to aim, relevance and personal integrity. The research group visited a planting team in the forest to gain a better knowledge of the planters working situation.

Later the questionnaire was tested by mailing it to eight planters, who were asked to answer the questions and give their point of view regarding both content and design. The questionnaire was updated with these comments in mind. The questionnaire was also translated into Polish, since seven of the planters included in the study were Polish citizens.

The questionnaire consisted of background questions about sex, age, and nationality, if they have or have had symptoms of asthma, hay fever, eczema or allergy, and for how long time they had been planting saplings during 2007. Exposure to other insecticides was checked with a question on use of insecticides outside work.

The most important part of the questionnaire concerned the presence of various health symptoms or pains after each exposure week. The various symptoms asked about in the questionnaire were; 'irritation of eyes', 'hazy-sight', 'irritation of airways', 'irritating, blocked or runny nose', 'itching', 'reduced perception or shooting pain, or burning sensation in face, arms or legs', and 'feeling sick' or 'trouble with dry skin'.

Another important question regarding exposure and effect on humans concerns the nature of the occupational environment, and whether the planters wore the protective clothes while planting out the saplings. The first question was if the employer had provided the workmen with protective clothes, followed by how often he/she used them, and how in general the individual was dressed while performing the task.

There is also a potential risk that insecticides can come in contact with hands and may be ingested. We were therefore interested in the hygienic situation during working hours and asked questions about 'how often the individual washed his/her hands during the day', in conjunction with 'eating or drinking', and 'smoking or using snuff'. Irritations on the skin of the body, as an effect of exposure to insecticides, can be reduced by ready access to shower/bath and washing machines. The participants were asked if they had access to these.

Besides questions related to exposure and health effects we also asked the planters if they noticed any annoying smell from the treated saplings when they put them into their planting-bags. Finally, each person could give his or her own viewpoint in relation to occupational environment and health. All planters were asked to answer the questionnaire after each week of exposure, i.e. in total three times. All 19 planters participated at all three occasions.

Biological monitoring

Biological monitoring of inflammation markers in upper airways.

Different effects on upper airways after exposure to the two insecticides used in this study were followed with several tests which have been widely used for environmental studies at department of Occupational and Environmental Medicine in Uppsala[4]. Signs of inflammation of the nasal mucosa can be studied by performing nasal lavage (NAL) [5]. Physiological saline solution (5ml) was injected into each nostril with the aid of a syringe and an olive (used to get a tight seal in the nostril) after which the solution was rinsed 5 times up and down the nostril. The solution from the two rinses of the nose were combined in a sample tube and centrifuged to remove cells. NAL solution was kept frozen until analysis of inflammation markers: lysozyme as a marker for parasympathetic stimulation of secretory

cells and albumin, present in the plasma, as a marker of vascular leakage from capillary vessels in the nasal mucosa [6].

Biological monitoring of metabolites in urine.

Cypermethrin is metabolised in humans in the liver to a number of products such as 3-phenoxybenzoic acid (3-PBA), which is excreted in the urine. There is no such marker for the substance imidacloprid, and this part of the study is therefore only valid for exposure to the insecticide Forester.

The planters handed over a urine sample after 4-5 working days of exposure and the following morning. This procedure was repeated during the three study weeks, that is with the three different treatments. Urine samples collected during the week with untreated saplings were used as controls for each individual, since these were collected after 6-7 days of non-exposure to insecticides [7]. Samples from the week when individuals were exposed to imidacloprid were not analysed.

The Department of Occupational and Environmental Medicine in Lund developed the method for analysis of a general urine metabolite, 3-PBA, from pyrethroids [8]. The method has previously been used in a study on the public in a project supported by the Swedish Environmental Protection Agency.

The biomarker 3-PBA in urine samples was measured using liquid chromatography coupled to a tandem mass-spectrometer (LC/MS/MS). Urine samples were hydrolysed to break any conjugates. The products were purified by solid-phase extraction, and analysed with 'selected reaction monitoring' (SRM) in negative ion mode. 3-PBA labelled with [$^{13}C_6$] was used as internal standard. Mass numbers $[213.1]^- \rightarrow [93.0]^-$ and $[219.1]^- \rightarrow [99.1]^-$ were used as internal standards. The detection limit was set to 0.4 ng/ml urine.

Neurophysiological measurements

The nervous system is the target organ for pyrethroids, which affect both CNS and PNS. Pyrethroids act neurotoxically by extending the opening of sodium-channels. A number of transient symptoms have been reported in humans: abnormal sensations in the face, including burning, itching or prickling sensation; paresthesia; and irritations of the skin, nasal mucus membranes (often with excessive nasal secretion), eyes and respiratory tract. Non-specific symptoms include headaches, dizziness, and nausea [9]. Neurophysiological measurements were planned for those individuals who had answered yes to the most common symptoms in the questionnaire, such as numbness and prickling sensations in arms and face.

Imidacloprid is a nicotine-like insecticide that exerts its neurotoxicity by partially binding to the nicotine-like acetylcholine receptor and thus disturbing nerve-muscle or nerve-nerve contacts. Imidacloprid is a so-called agonist i.e. it imitates nicotine's effect on the nervous system and gives rise to an unregulated increase in nerve impulses which can lead to blockage of the nervous system. Imidacloprid analogues or metabolites that bind with high affinity to insects' acetylcholine receptors bind with low affinity to human receptors.

During the course of the project it was decided not to conduct this investigation within the framework of the current study for technical reasons. This decision had no negative effects on

the study since only one individual reported numbness in arms and legs in connection with work involving plants treated with imidacloprid and cypermethrin.

Statistical methods

A logistic regression model was used to analyse data from the questionnaire. The model accounted for the serial correlation present among the repeated measurements on the same subject. The outcome variables were the presence of different symptoms and the different treatments were used as independent variables. The weeks with imidaclorprid and cypermethrin were compared independently with the untreated week.

Data on levels of lyzozyme and albumin in nasal lavages, and 3-PBA in urine, were analysed using a so-called linear mixed effects regression model. The mixed model accounts for the correlation between repeated measurements on the same individual as mentioned above [10]. When analysing data for 3-PBA, adjustments were also made for the correlation between the two test weeks 1 (untreated) and 3 (cypermethrin).

Many statistical analyses require that the residuals (differences between the measured values and the model values) should be normally distributed and independent. This may sometimes demand transformation of data, and in the current case the values for 3-PBA levels were log-transformed. Interpretation of regression coefficients therefore involved changes in percent of the geometrical mean.

Results

This study is a questionnaire combined with biological follow-up to determine health effects on tree-planters. All individuals completed the questionnaire and provided nasal-lavage and urine samples. The study was of the double-blind crossover type, which means that the research leader, the research personnel and the participants did not know which preparation was used for each week. The code was broken on completion of all fieldwork and the treatment was as follows:

week 1: Preparation A = untreated
week 2: Preparation B = Merit Forest WG (imidacloprid)
week 3: Preparation C = Forester (cypermethrin).

Results from the questionnaire

The tree-planters answered the questionnaire at the end of every working week. The questionnaire consisted of three parts: background, health problems and symptoms, and working environment and safety. The results were divided up into background questions such as sex, age, working environment, and housing conditions, and also questions on various symptoms and health problems. The number of responders is given in the tables unless otherwise stated. The answers are shown in table form in Appendix 1. Cross-over results for health problems and symptoms are presented as two figures in Appendix 2. The results are summarised below.

Tree-planters

Seven of the nineteen tree-planters were Polish men. The remainders were Swedish, including two women. The age distribution was relatively even with eight being 30 years old or younger, eight between 31 and 49 years old, and 3 older than 50. The majority had worked with tree-planting for at least two months during 2007, before the study began. The majority (16 of 19) lived in their normal home, and three lived in a temporary home. All had access to showers as well as washing and cooking facilities in their homes.

Protective clothes and hygiene

All tree-planters were provided with protective clothing by their employers and gloves and long trousers were used during all planting work. Nearly all tree-planters also wore long-sleeved shirts and a hat. The majority usually ate food or a snack twice during the working day, and washed their hands as often. Just under half of the tree-planters (9 of 19) smoked or used snuff, and many stated that they *often* washed their hands before smoking or using snuff.

Self-reported health issues

The questionnaire included two questions concerning health: "Have you or have you had asthma, hay fever, eczema or allergy?" and "Have you experienced any of the following symptoms or problems during work this week?" in which case they had to note the presence of nine symptoms. Of the nineteen tree-planters one had asthma, one had hay fever, and three had allergy. The most common symptoms for all weeks were "itching, smarting pain, irritation in the eyes" and "blocked or runny nose".

A study of the occurrence of health problems after the exposure weeks indicates that the greatest number of planters complained of problems with eyes, irritated throat or nose and also dry skin after the week handling untreated plants. The reported frequency of eye irritation was still high after the week of exposure to imidacloprid and an additional person suffered nasal irritation. There was also an increase in the number of tree-planters who reported itching on the face and dry skin. Eye irritation, reported by 6 of the 19 subjects, was still dominant after the week of exposure to cypermethrin. The frequency of facial itching was unchanged, whilst problems with dry skin had reduced. There were no significant differences in the occurrence of the symptoms between weeks, but it would be difficult to detect statistically significant changes with such a small data set.

Crossover analysis enabled the study of the reported symptoms for each individual after each week of exposure in relation to the week when tree-planters were not exposed to treated plants (Figures 1 and 2 in Appendix 2). Figure 1 shows that there were symptoms that were not reported after the week with untreated plants but were reported by some of the tree-planters following the week of exposure to imidacloprid. Two people stated that they had suffered itching in the face, two people had problems with dry skin, one person had "reduced sense of touch in the hands and arms", and one person had "prickly, burning sensation in the hands and arms". A number of tree-planters who had reported problems after the first week (with untreated plants) reported no such problems after subsequent weeks when plants had been treated. This suggests that the evaluation procedure itself affected the result during the first week. The results indicate the type of health problems that might occur in connection with treatments with the insecticides even if they cannot be shown to be statistically significant due to the limited data set.

The tree-planters were also asked if they experienced any annoying smells when placing the plants in the bags. Four of nineteen reported this after the week with untreated plants. None reported annoying smells after handling the imidacloprid-treated plants and seven of the nineteen reported annoying smells after handling the cypermethrin-treated plants.

The numbers of participants who answered each question in the questionnaire are presented as frequency tables in Appendix 1. Figures illustrating the results of the crossover analysis are shown in Appendix 2.

Biological monitoring

The analysis of inflammation markers in nasal lavage was used as an objective measurement of exposure to the insecticides Merit Forest and Forester, and their effect on the nasal mucous membranes. The markers were lysozyme and albumin and both were analysed in the same sample. The results of the analyses are presented in figures and tables below, and were also compared with results from earlier studies performed on people with other occupations.

Lysozyme

The measurement of lysozyme levels was performed to determine if exposure to insecticides had lead to increased secretion in nasal mucous membranes. Figure 1 shows the individual data points for each week. There was no apparent difference between the groups for untreated and treated weeks. The median value for lysozyme was, however, higher than in earlier studies [11][4].

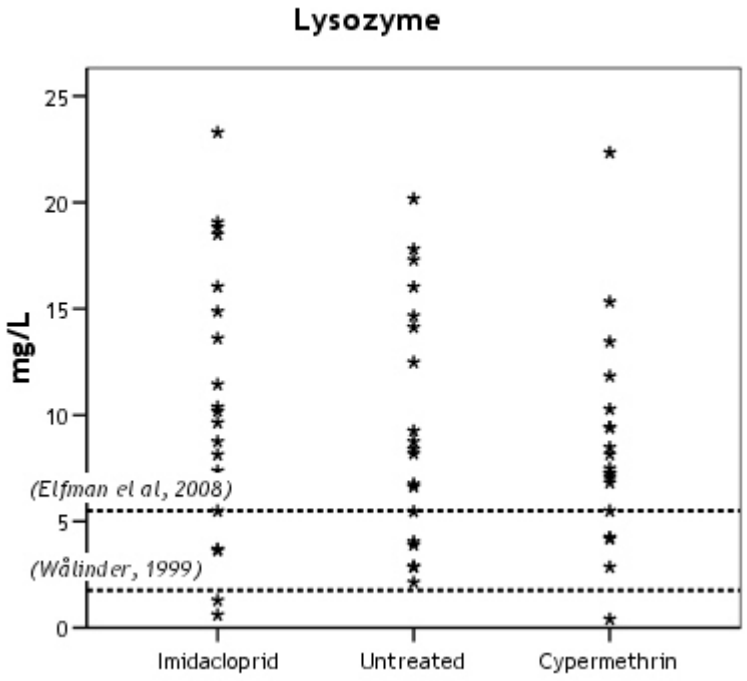

Figure 1: Lysozyme values in NAL from the planters for respective week.

Table 1: Levels of lysozyme, with the number of tree-planters in each category where the untreated week is compared with weeks with treatments.

	N	Untreated <cypermethrin <imidacloprid	Untreated >cypermethrin <imidacloprid	Untreated <cypermethrin >imidacloprid	Untreated >cypermethrin >imidacloprid
Number	19	8	4	2	5

Table 1 shows that 8 people had lower levels of lysozyme during the week of untreated plants in comparison with the weeks with treated plants, whilst five people had higher levels.

Table 2: Lysozyme values in planters during the three weeks

	N	Percentiles		
		P_{25}	Median	P_{75}
Imidacloprid	19	5,5	10,1	16,0
Untreated	19	4,0	8,4	14,7
Cypermethrin	19	5,5	7,5	10,2

Lysozyme values (mg/L) for 25th, 50th and 75th percentiles.

There was no significant difference in levels of lysozyme between weeks planting untreated plants and those weeks when the planters were exposed to Merit Forest (imidacloprid) and Forester (cypermetrin), *table 2*.

Albumin

The level of albumin was used as a measure of vascular leakage in the nasal mucous membranes. During the three weeks only 26 % of the planters had levels over the lower detection limit of 5 mg/L albumin. *Figure 2* shows the detectable values for all three weeks.

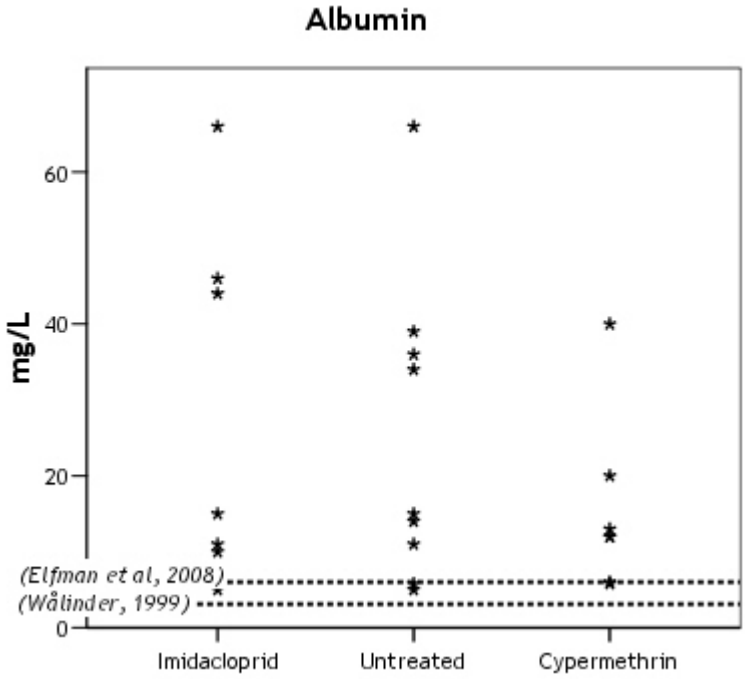

Figure 1: Albumin levels in NAL from the planters for respective weeks
The dotted lines indicate the median values for earlier studies [11,12].

Table 3: Categories based on the level of albumin after exposure to untreated plants and compared to those treated with Merit Forest or Forester.

	N	N <d.l.	Untreated <cypermethrin <imidacloprid	Untreated >cypermethrin <imidacloprid	Untreated <cypermethrin >imidacloprid	Untreated >cypermethrin >imidacloprid
Number	19	14	3	0	2	0

d.l. = detection limit 5mg/L

The data in *Table 3* shows that 14 planters had albumin levels that were below the detection limit. Of the five people who showed detectable albumin levels, three had lower levels during the week with untreated plants compared with the weeks with treated plants, whilst two had higher levels.

Table 4: Albumin levels in planters during the three weeks

	N	Percentiles		
Week		P_{25}	Median	P_{75}
Imidacloprid	19	10,5	15,0	45,0
Untreated	19	11,0	15,0	36,0
Cypermethrin	19	9,0	12,0	16,5

Albumin values (mg/L) for 25th, 50th and 75th percentiles.

There was no significant difference, *table 4*, in levels of albumin in planters between weeks planting untreated plants and weeks when they were exposed to Merit Forest (imidacloprid) and Forester (cypermethrin).

3-PBA

Figure 3 shows a plot of the values for 3-PBA for the week with untreated plants (sample 1 and 2) and the week with cypermethrin-treated plants (sample 1 and 2). The values are adjusted for the density of urine (d.j.) This adjustment is done since the concentration of urine depends on liquid intake. Another way of adjusting for this involved measuring the level of creatinine in the urine and then adjusting in a similar way (creatinine-adjusted c.j.). 3-PBA is not present in the urine as a breakdown product after exposure to imidacloprid (Merit Forest).

The analysis indicates an increase of 30% in 3-PBA after adjusting by density ($p<0.05$ CI: 12% - 52%, d.l. <0.4mg/ml), whilst the increase was 33% after adjusting with creatinine (c.j.; $p<0.05$, CI: 11% - 58%, d.l.<0.1). The level of 3-PBA in samples from the week with cypermethrin-treated plants increased from sample 1 to sample 2, taken a day later. This difference was, however, not significant. The individual levels of 3-PBA increased by 12 – 52% during the week of planting cypermethrin-treated plants. The levels were perhaps highest the morning after planting. These increases could not be explained by smoking habits or the use of snuff.

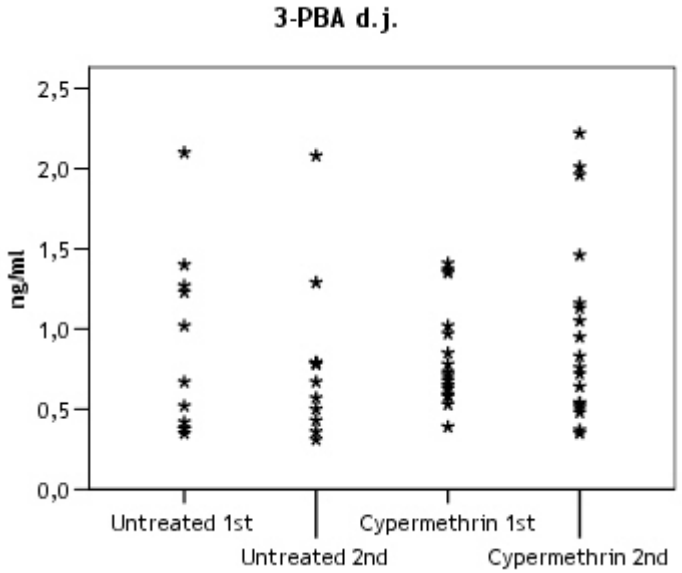

Figure 3: Individual 3-PBA values (adjusted by density) for respective week

Table 5: 3-PBA levels in urine – comparison between untreated and week with cypermethrin-treated plants

Type of correction	week	Sample	N	Proportion (%)> d.l	GM	GSD	P_{25}	Median	P_{75}
3-PBA d.j.									
ng/ml	Untreated	1:a	19	58 %	0.22	1.36	<d.l.	<d.l	0.84
d.l.<0.4		2:a	19	58 %	0.40	1.36	<d.l	<d.l	0.72
	Cypermetrin	1:a	19	84 %	0.63	1.26	0.56	0.68	0.91
		2:a	19	95 %	0.78	1.23	0.52	0.76	1.15
3-PBA k.j.									
nmol/mmol	Untreated	1:a	19	58 %	0.22	1.56	<d.l.	0.19	0.42
d.l.<0.10		2:a	19	58 %	0.21	1.56	<d.l.	0.20	0.44
	Cypermetrin	1:a	19	84 %	0.36	1.28	0.28	0.42	0.59
		2:a	19	95 %	0.40	1.41	0.25	0.34	0.72

Density-adjusted (d.j) and creatinine-adjusted (c.j); d.l. detection level; N, number of planters; GM= geometric mean ; GS,= geometric standard deviation; P_{25}, 25th percentile; $P_{75,}$ 75th percentile.

Table 6: 3-PBA levels - comparison between week with untreated and week with cypermethrin-treated plants

	N	Untreated <cypermethrin	Untreated >cypermethrin
Density-adjusted	19	15	4
Creatinine-adjusted	19	15	4

Table 6 shows the number of planters in each category where average 3-PBA levels after handling the untreated- and cypermethrin-treated plants are compared. Seventy-nine percent of planters had levels of 3-PBA that were higher after handling cypermethrin-treated plants than after handling untreated plants.

Discussion

It is generally difficult to make correlations between pesticide-exposure and symptoms with the help of a questionnaire [9]. One important reason is that health problems are affected by many factors including sex, age, employment time and oversensitivity. It is therefore important that the effect of these factors is considered in correlation analysis. The effectiveness of this study was increased by doing it as a double-blind crossover study with a modified questionnaire used earlier by Kolmodin-Hedman and co-workers [3]. In addition, biological monitoring was included to evaluate objectively any health effects that depended on exposure to the insecticides Merit Forest and Forester.

During the spring of 1991, Birgitta Kolmodin Hedman, Department of Occupational Medicine, Karolinska Institute, lead an investigation into the health problems experienced by planters who worked with conifer plants with soil-covered roots and treated with permethrin. The investigation, which was also a double-blind study and included 18 people, showed that the planters did not have more health problems after planting permethrin-treated plants than after planting untreated plants [3]. This agrees with the results obtained in the current study.

The results from determination of the levels of inflammation markers in nasal lavage do not indicate any clear effects of imidacloprid and cypermethrin on human airways. The albumin levels were not increased in comparison with the plasma-leakage as can be observed in, for example, rhinovirus infections. The median level of lysozyme was increased during all three weeks, which can indicate an increase in parasympathetic secretion in connection with outdoor work. The levels were higher than those obtained for both indoor workers such as teaching and office-workers [4, 5] and stable personnel working both indoors and outdoors [11].

Pyrethroids are rapidly metabolised in the liver, resulting in an effective detoxification [12]. The literature indicates that the level of breakdown products in people working with insecticides in agriculture are under the detection limit whereas levels are detectable in those working in greenhouses. The highest levels have been measured in people working with pest-control indoors [9,13,14]. Littorin and co-workers have used a newly developed method that is one hundred times more sensitive than the ones used in earlier studies. Preliminary data showed the presence of the metabolite 3-PBA in 20% of urine samples from the public, which is presumably a result of consuming food products that have been treated with insecticides, or the result of contact with insecticides in the home or garden. None of those studied had used any insecticides against hair lice, which also contain pyrethroids. The planters in this study had levels that were increased by 12-52 %, which is clearly higher than the level in the general population. These increased levels represent a measure of exposure to insecticides containing cypermethrin. It is, however, unclear if these levels have any connection with acute health effects.

From a study in Germany it was concluded that one could not expect negative health effects from pyrethroids in personnel, providing the pyrethroids were applied in a careful way and with good working routines, protective clothes, and regular changes of protective gloves. Exposure can be reduced through good hygiene such as washing hands and the face before meal times and breaks, as well as at the end of the day [14], which was also the case in this study.

Conclusion

This study involved the evaluation of health effects based on questionnaires covering problems with the respiratory tract, and also objective tests on nasal mucous membranes. No clear, acute negative health effects could be found in planters after exposure to coniferous plants treated with imidacloprid (Merit Forest) or cypermethrin (Forester), as compared with exposure to untreated plants. The breakdown product, 3-PBA, from cypermethrin was, however, increased and can therefore be used as an indicator of exposure to this insecticide. We could not, however, find any statistically-significant correlation between the acute symptoms and health problems in planters, and the questionnaire responses or 3-PBA levels. These results have been obtained during planting in late summer/early autumn and with good use of protective clothing.

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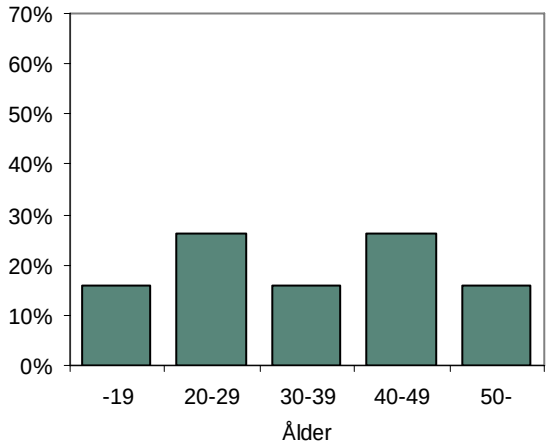
Results from the questionnaire study

Listed below are questions from the questionnaire followed by answers to the respective question presented as frequency. In some of the tables there is one line "yes, no time given". This means that the planter has answered yes to the question, but he/she has not chosen any of the time alternatives.

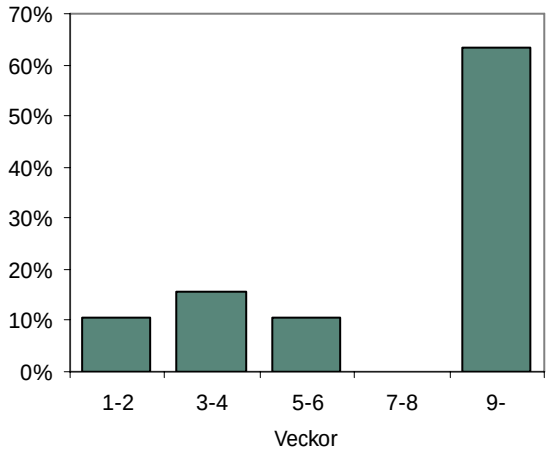
Background

3. Age:



Age	*%*	*n*
-19	16%	3
20-29	26%	5
30-39	16%	3
40-49	26%	5
50-	16%	3
no answer	0 %	0
Sum	100	19

4. In total, how many weeks have you been working with tree-planting during 2007?



No. of weeks	*%*	*n*
1-2	11%	2
3-4	16%	3
5-6	11%	2
7-8	0%	0
9-	63%	12
no answer	0%	0
Sum	100	19

5. The saplings you are working with have been treated with insecticides. Have you during the last week been in contact with insecticides outside work for example: while gardening, when doing pest control of ants, or ticks on pets, or by treating head-lice?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes	1	1	0
No	18	18	19
no answer	0	0	0
Sum	19	19	19

6. Have you or have you had asthma symptoms, hay fever, eczema or allergy?

	Asthma	*Hay fever*	*Eczema*	*Allergy*
Yes	1	1	0	3*
No	18	18	19	16
No answer	0	0	0	0
Sum	19	19	19	19

Those who answered yes to allergy (3), said they were allergic to tree and grass pollen, and mites.

Health problems and symptoms

7. Have you during work this week experienced any of the following health problems or symptoms?

a) Itching, smarting pain or irritation in the eyes?

	Week1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	4	4	3
Yes, to the following day	1	0	0
Yes, the whole week	0	0	0
Yes, no time given	0	0	0
No	14	15	16
No answer	0	0	0
Sum	19	19	19

b) Hazy-sight

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	2	0	0
Yes, to the following day	1	0	0
Yes, the whole week	0	2	1
Yes, no time given	2	0	2
No	14	17	16
No answer	0	0	0
Sum	19	19	19

c) Hoarseness, dry throat, irritation of the airways?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	2	0	2
Yes, to the following day	1	1	0
Yes, the whole week	1	0	0
Yes, no time given	2	1	1
No	13	17	16
No answer	0	0	0
Sum	19	19	19

d) Irritated, blocked or runny nose?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	1	2	1
Yes, to the following day	0	0	3
Yes, the whole week	4	3	2
Yes, no time given	2	1	1
No	12	13	12
No answer	0	0	0
Sum	19	19	19

e) Itching in face, on hands/arms or legs?

	face			*Hands/arms*			*legs*		
Week	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*
Yes, 1-2 hours	0	2	0	0	1	0	0	0	0
Yes, to the following day	0	0	0	1	0	0	0	0	0
Yes, the whole week	0	0	2	0	0	0	0	0	0
Yes, no time given	0	0	1	1	0	1	0	0	0
No	19	17	16	17	17	17	19	18	17
No answer	0	0	0	0	1	1	0	1	2
Sum	19	19	19	19	19	19	19	19	19

f) Numbness, reduced sense of feeling in face, hands/arms or legs?

	face			*hands/arms*			*legs*		
Week	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*
Yes, 1-2 hours	0	0	0	0	1	1	0	0	0
Yes, to the following day	0	0	0	0	0	0	0	0	0
Yes, the whole week	0	0	0	0	0	0	0	0	0
Yes, no time given	0	0	0	0	0	0	0	0	0
No	19	19	19	19	18	18	19	19	19
No answer	0	0	0	0	0	0	0	0	0
Sum	19	19	19	19	19	19	19	19	19

g) Shooting pain or burning sensation in face, hands/arms or legs?

	face			*hands/arms*			*legs*		
Week	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*	*1 Untr*	*2 Imid*	*3 Cyp*
Yes, 1-2 hours	2	0	1	0	0	0	1	0	0
Yes, to the following day	0	0	0	0	0	0	0	0	0
Yes, the whole week	0	0	1	0	0	0	0	0	0
Yes, no time given	0	1	0	0	1	0	0	0	0
No	17	18	17	19	18	19	18	19	19
No answer	0	0	0	0	0	0	0	0	0
Sum-	19	19	19	19	19	19	19	19	19

h) Nausea?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	0	0	0
Yes, to the following day	1	1	0
Yes, the whole week	0	0	0
Yes, no time given	0	0	0
No	0	0	1
No answer	18	18	18
Sum	19	19	19

i) Dry skin?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, 1-2 hours	1	2	1
Yes, to the following day	0	1	0
Yes, the whole week	2	2	1
Yes, no time given	0	0	1
No	16	14	16
No answer	0	0	0
Sum	19	19	19

Occupational environment and safety

8. Has the employer provided you with any type of protective clothes such as gloves or overalls?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes	19	19	19
No	0	0	0
No answer	0	0	0
Sum	19	19	19

9. When you are planting how often have you used during this week: gloves, long-sleeved shirt/coat, long trousers, cap/hat?

	Gloves			*Long-sleeved shirt/coat*			*Trousers*			*Cap/hat*		
week	*1 unt*	*2 Imi*	*3 Cyp*	*1 unt*	*2 Imi*	*3 Cyp*	*1 unt*	*2 Imi*	*3 Cyp*	*1 unt*	*2 Imi*	*3 Cyp*
Always	19	19	19	16	16	17	19	19	19	14	15	15
Often	0	0	0	2	2	2	0	0	0	4	1	2
Sometimes	0	0	0	0	1	0	0	0	0	1	3	2
Rarely	0	0	0	1	0	0	0	0	0	0	0	0
Never	0	0	0	0	0	0	0	0	0	0	0	0
No answer	0	0	0	0	0	0	0	0	0	0	0	0
Sum	19	19	19	19	19	19	19	19	19	19	19	19

10. During this week have you noticed any annoying smell while you were putting the plants in the planting bag?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes	4	0	7
No	15	19	12
No answer	0	0	0
Sum	19	19	19

11. How often do you wash your hands during a working day?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Once	4	2	5
2-3 times	13	13	10
4- times	2	4	4
After work	0	0	0
Never	0	0	0
No answer	0	0	0
Sum	19	19	19

12. How often do you eat or drink during a working day?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Once	5	5	5
Twice	12	13	13
3- times or more	2	1	1
Never	0	0	0
No answer	0	0	0
Sum	19	19	19

13. Have you smoked or used snuff during working hours this week?
If yes – do you generally wash your hands before you smoke or use snuff?

	Week 1 Untreated	*Week 2 Imidacloprid*	*Week 3 Cypermethrin*
Yes, always	1	2	2
Yes, often	3	2	2
Yes, sometimes	1	2	3
Yes, rarely	3	3	1
yes, never	1	0	1
No	10	10	8
No answer	0	0	2
Sum	19	19	19

14. Where do you live while you are working as a tree-planter?

	n
Permanent home	16
Temporary home	3
Hotel/hostel	0
Camping van/caravan	0
Tent	0
Other	0
No answer	0
Sum	19

15. Does your home/lodging provide possibilities to the following:

	Shower/bath	*Cooking*	*Washing clothes*
Yes	19	19	19
No	0	0	0
No answer	0	0	0
Sum	19	19	19

16. If you have any further comments regarding your working environment and health that you want to present, please write below. You may also use the next page if there is not enough space.

- ”During planting I generally get dry crusts in my nose, which last during the whole tree-planting season. I never get that while doing other forest work”.

- ”Back burden/strain from planting bags, stress and fear of failure”

- ”When planting in spring and summer in strong sunlight and heat: dry throat, smell from plants, itching and rash on lower arms. (due to less clothing, only longsleeved shirt)”.

- ”I have had back problems during the whole week. I think that the planting bags that we use distribute the weight onto the back in the wrong way, causing back pain”.

- ”Having dry hands can just as well depend on the use of rubber gloves all day”

- ”I have problems in the spring with dryness in the mouth and throat, a runny nose, and even itching in the face”

- ”Stress ryggbelastning”

- ”Stress due to straining the back”

- ”I have the same view this week regarding my back”

- ”It hasn’t rained this week and therefore no dry hands or runny nose (perhaps)”

- ”Strained back, stress, shoulders”

Results from cross-over study

These two figures show if the planters changed their answers to the questions from week 1 with untreated tree-plants to weeks with treated plants, e.g. week 2 with imidacloprid and week 3 with cypermethrin

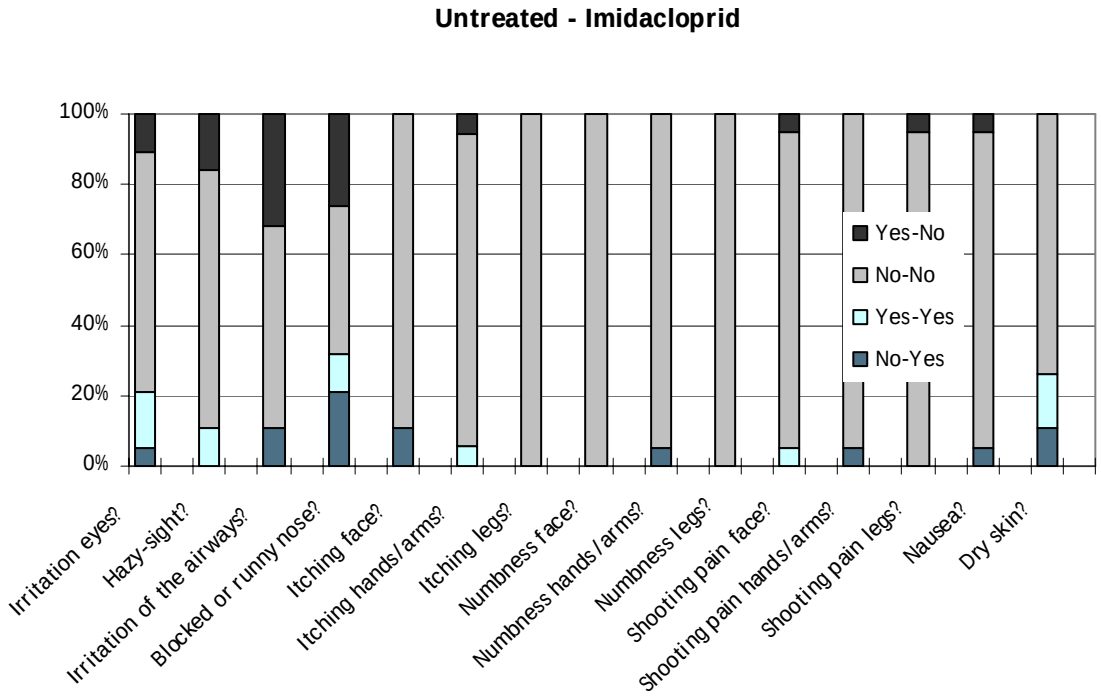


Figure 1: Percentage who answered 'yes' or 'no' during week 1/untreated and then answered 'yes' and 'no', respectively, when the plants had been treated with imidacloprid/week 2.

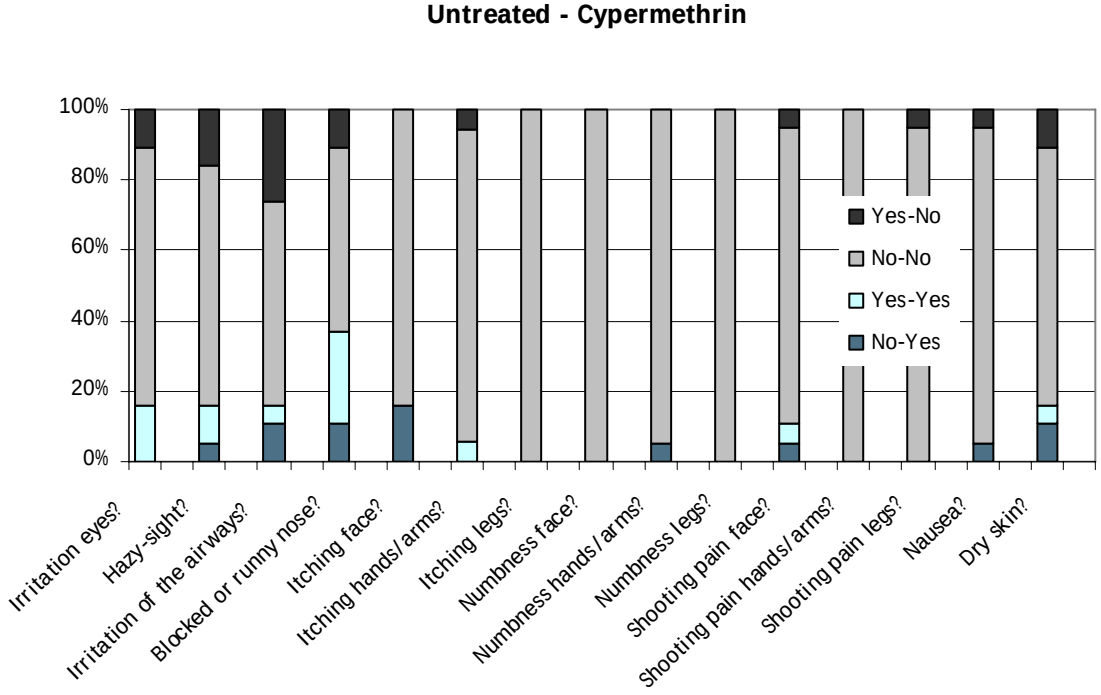


Figure 2: Percentage who answered 'yes' or 'no' during week 1/untreated and then answered 'yes' and 'no', respectively, when the plants had been treated with cypermethrin/week 3.

Levels of Lysozyme and Albumin in earlier studies

The results in *table 1* show that the group of tree-planters generally had a high median level of lysozyme. This was presumably because they worked outdoors, in contrast with stable personnel, who work only partly outdoors, and the lowest levels were obtained for school-, hospital- and office personnel who work indoors.

Table 1: Lysozyme values from earlier studies on various working environments

Environment	N	P_{25}	Median	P_{75}
Stable[1]	13	3,1	5,5	8,6
School[2]	234	1,8	3,1	4,8
Hospital[2]	88	0,57	1,0	1,6
Office[2]	89	0,32	0,73	1,6
Total[2]	411	0,70	1,76	3,64

Lysozyme values (mg/L) for 25th, 50th and 75th percentiles from two earlier studies;
[1] Influence of stable environment on human airways [11]
[2] Nasal reactions and the school environment [4]

The results in *table 2* show that the group of tree-planters generally had a high median level of albumin. This was presumably because they worked outdoors, in contrast with stable personnel, who work only partly outdoors, and the lowest levels were obtained for school-, hospital- and office personnel who all work indoors.

Tabell 2: Albumin levels from earlier studies on various working environments

Environment	N	P_{25}	Median	P_{75}
Stable[1]	13	3,3	6	27,3
School[2]	234	3	5,6	10,9
Hospital[2]	88	<3	<3	<3
Office[2]	89	<3	<3	<3
Total[2]	411	1,5	3,1	7,4

Albumin values for 25th, 50th and 75th percentiles for two earlier studies
[1] Influence of stable environment on human airways [11]
[2] Nasal reactions and the school environment [4]

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Study of acute effects on tree-planters planting out conifer saplings treated with insecticides

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