Acute Health Effects on Planters of Conifer Seedlings Treated with Insecticides

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Objectives: The aim of this study was to assess acute health effects on planters caused by planting conifer seedlings treated with two insecticides, with active ingredients imidacloprid and cypermethrin, in comparison with untreated seedlings.

Methods: The investigation was a double-blind crossover study, which included a follow-up of 19 planters over a 3-week period. During Week 1, the 19 planters handled untreated conifer seedlings while they planted imidacloprid- and cypermethrin-treated seedlings during study Week 2 and 3, respectively. Signs and symptoms of acute health effects were documented by a questionnaire, administered by the field staff, during these 3 weeks. Inflammation markers in the nasal mucous membrane were also measured as an objective test. Exposure to cypermethrin was further assessed by measuring 3-phenoxybenzoic acid (3-PBA) in urine. No validated biomarker was available to assess internal exposure to imidacloprid.

Results: No clear, acute adverse health effects could be found in planters during the week of exposure to conifer seedlings treated with imidacloprid (Merit Forest) or cypermethrin (Forester), as compared to during the week of planting untreated seedlings. During the week of cypermethrin exposure, the individuals had 3-PBA values that were 12–54% higher ($P < 0.05$), depending on the worker, than those observed during the untreated week. There were no statistically significant correlations between the raised levels of 3-PBA and self-reported health problems. These results have been obtained during planting in late summer/early autumn and with good use of protective clothing.

Conclusions: No clear, acute adverse health effects could be found in planters after exposure to conifer seedlings treated with imidacloprid (Merit Forest) or cypermethrin (Forester), as compared with planting untreated seedlings. The metabolite, 3-PBA, was found in low levels in urine and was increased after exposure to cypermethrin. However, no clear relationships could be found between exposure and reported symptoms or between elevated 3-PBA levels and reported symptoms.

Keywords: biological monitoring; health effects; inflammation markers; insecticides; planter

INTRODUCTION

According to the Union of Forest and Wood Workers, there are 2000–3000 individuals involved in planting of forest tree seedlings in Sweden. The total number of individuals who handle insecticide-treated seedlings is estimated to be 1500–2000. During 2006, 40% of all seedlings planted were treated with insecticides.

During 2007, 10 620 tonnes of chemical insecticides were sold in Sweden, of which 54.3 tonnes were insecticides. The usage in forestry alone was 5.5 tonnes, which amounts to 0.1% of the total usage of insecticides (Swedish Chemical Agency, 2007). The sales of imidacloprid (3.3 tonnes) was the same and cypermethrin increased from 0.6 to 5.0 tonnes between 2003 and 2007. These insecticides are used in agriculture, forestry and industry.

The insecticides used in forestry today are Cyper Plus or Forester, with cypermethrin as active substance, and Merit Forest WG, with imidacloprid as active substance. The Swedish Chemicals Agency had approved the usage of these substances with an extension until 31 December 2007 (Swedish Chemical Agency, 2005). This decision covered the usage...
against insect attacks on unbarked timber and conifer seedlings. The Swedish Forest Agency and the Federation of Swedish Farmers are of the opinion that because of the hurricane Gudrun in 2006, there will be an increased damage by pine weevils that will continue at least until 2009. Therefore, there is a need for extending the use of insecticides until new alternative protection methods have been developed.

The two insecticides investigated in this study, cypermethrin and imidacloprid, act by different mechanisms. Cypermethrin is a pyrethroid that targets both the central and peripheral nervous system. Pyrethroids act neurotoxically by extending the opening of sodium channels. A number of transient symptoms have been reported in humans such as abnormal sensations in the face, including burning, itching or prickling sensation, paraesthesia and irritations of the skin, nasal mucous membranes and often with excessive secretion in the nose, eyes and respiratory tract. Non-specific symptoms include headaches, dizziness and nausea (Wieseler et al., 1998). Cypermethrin is rapidly metabolized yielding non-toxic acid metabolites, which are mainly eliminated renally. One of the main metabolites is 3-phenoxbenzoic acid (3-PBA), which is excreted in the urine with a half-life of ~16 h (Woollen et al., 1992). Well-validated analytical methods, for the analysis of 3-PBA in human urine, have been published (Olsson et al., 2004) and have been applied in several studies on occupational exposure (Wieseler et al., 1998; Hardt and Angerer, 2003; Wang et al., 2007).

Imidacloprid is a neonicotinoid with chloropyridylmethyl substituents that exerts its neurotoxicity by partially binding to the nicotine-like acetylcholine receptor and thus disturbing nerve–muscle or nerve–nerve contacts. However, imidacloprid analogues or metabolites that bind with high affinity to insects’ acetylcholine receptors bind to human receptors with low affinity. Imidacloprid seems to give only a few clinical signs after skin or inhalation exposure. However, some formulations have been reported to be slight or mild skin and eye irritants. Inhalation studies at high concentrations in rats showed some transient clinical signs of difficulty in breathing, reduced mobility and slight tremor. Studies with mammals suggest that imidacloprid is rapidly and completely eliminated in the urine and faeces (Anatra-Cordone and Durkin, 2005). It has been suggested that one of the metabolites, 6-chloronicotinic acid could be used for biological monitoring. Validated analytical methods have been presented, but 6-chloronicotinic acid has not been found in human urine after exposure to imidacloprid (Uroz et al., 2001; Proenca et al., 2005). A recent study in mice has shown that 6-chloronicotinic acid is a minor metabolite of imidacloprid (Ford and Casida, 2006). Thus, there are no well-validated biomarkers in urine for assessment of exposure to imidacloprid.

This study was performed on planters of forest tree seedlings in Sweden. The aim of the investigation was to determine if exposure to the two insecticides cypermethrin (Forester) and imidacloprid (Merit Forest WG), through working with insecticide-treated conifer seedlings, resulted in any acute health effects such as symptoms of the nasal mucosa, skin irritations or the nervous system.

MATERIALS AND METHODS

Study group

Nineteen planters were included in the study group: 17 men and two women. These formed four teams with five persons in three teams each and one team with four persons. The teams were selected mainly from the middle and south counties of Sweden, where the usage of insecticides is most frequent. The number of planters was limited due to the relatively small study group available and the short planting season (August to September). Another limiting factor was the large number of conifer seedlings that had to be treated and distributed, which means that the logistics of this study were substantial. Half a million seedlings were used during the 3 weeks of planting, and they had to be delivered at the right place and at the right time.

This investigation was a double-blind crossover study in which each planter served as their own control. The main advantage with this study design is that within-planter confounding factors, such as age, smoking status etc., are controlled for by the design. The planting personnel planted untreated seedlings, Forester- or Merit Forest-treated conifer seedlings for 5 days per week for a total of 3 weeks. The weekend was a washout period between each treatment, which is sufficient since pyrethroids are rapidly metabolized in humans (Woollen et al., 1992). The seedlings were treated at a nursery (Svenska Skogsplanter AB, Bälsta, Sweden). The seedlings were distributed to the various planting sites with a code (A, B and C), which was not known by the planters or the personnel performing the field studies.

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

Treatment of conifer seedlings

Conifer seedlings were treated and distributed by the nursery. The seedling used was the conifer Starpot 75, i.e. a form of plant with soil-covered roots. The seedlings were treated in three ways:

- Merit Forest WG (imidacloprid, Bayer, approved by Swedish Chemicals Agency until 31 December 2008 for treatment against pine weevils, registration no. 4605, class 2L; 1% solution);


- Forester (cypermethrin cis\textit{trans} 40/60, producer and licence holder Agrophar, Belgium, approved by the Swedish Chemicals Agency until 31 December 2011; 2% solution);
- no treatment.

Interagro Skog AB is the distributor of Forester in Sweden, which was not yet approved or registered in Sweden at the time of the field study. Interagro Skog AB was granted an exemption from the Swedish Chemicals Agency (Diary number 730-773-07) to be included in this investigation. Earlier treatment used Cyper Plus (cypermethrin, cis\textit{trans} 80/20, approved by the Swedish Chemicals Agency, registration no. 4580, class 2L). Since then, Forester has from 5 December 2007 been approved by the Swedish Chemicals Agency until 31 December 2011.

The three treatments were performed and each box was marked (A, B and C) according to a system only known to two people at the nursery. Each individual included in the study had to report which letter was on the box with seedlings they planted out each week. They planted seedlings with the same treatment for 1 week i.e. for 5 days followed by two work-free days.

All boxes with seedlings 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 was no warning label for this preparation at the time of the study, the label for Cyper Plus was used. Both these insecticides contain cypermethrin as active substance. The nursery packed and distributed the following number for each treatment: A, 173 000 seedlings; B, 173 000 seedlings and C, 170 000 seedlings. This made a total of 516 000 seedlings used for this study.

\textit{Questionnaire study}

The questionnaire, ‘Insecticidal treatment of conifer seedlings effect on planting personnel’, was based on an earlier questionnaire developed by Kolmodin-Hedman \textit{et al.} (1995) in a similar study using the preparation permethrin. A first draft of the present questionnaire was discussed with representatives from the Swedish Committee for Seeding Protection. 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 their comments in mind. The final 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 they had been planting seedlings 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’.

Other questions concerned whether the planters wore protective clothes while planting out the seedlings. 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 work.

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 facilities.

Besides questions related to exposure and health effects, we also asked the planters if they noticed any annoying smell from the treated seedlings 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. Each week, after having filled in the questionnaire, the fieldworkers also asked each person if they knew which treatment the seedlings had that week. This was done in order to check the effectiveness of the blinding procedure. All 19 planters participated on all three occasions.

\textit{Biological monitoring}

\textit{Biological monitoring of inflammation-markers in upper airways}. Different health 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 the Department of Occupational and Environmental Medicine in Uppsala (Wålinder, 1999). Signs of inflammation of the nasal mucosa were
studied by performing nasal lavage (NAL) (Wålinder, 1999; Wålinder et al., 2000). Physiological saline solution (5 ml) 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 flushed five 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 at −20°C until analysis of the following inflammation markers: lysozyme as a marker for parasympathetic stimulation of secretory cells and albumin, present in the plasma, as a marker for vascular leakage from capillary vessels in the nasal mucosa (Raphael et al., 1989).

Biological monitoring of metabolites in urine. Urine samples were collected from the planters after four to five working days of exposure (first sample) and the following morning (second sample). The urine samples were collected in polypropylene tubes and immediately frozen and kept at −20°C until analysis. This procedure was repeated during the three study weeks, that is, with the three different treatments. Urine samples collected during the week with untreated seedlings were used as controls for each individual since these were collected after 6–7 days of non-exposure to insecticides. Samples from the week when individuals were exposed to imidacloprid were not analysed due to the lack of a good biological marker.

The biomarker 3-PBA was measured in urine using liquid chromatography coupled to a tandem mass spectrometer, in principle according to a method described earlier (Olsson et al., 2004; Lindh et al., 2008). 3-PBA labelled with [213.1] was used as an internal standard. Urine samples were hydrolysed to break any conjugates. The products were purified by solid-phase extraction and analysed using selected reaction monitoring in the negative ion mode using the transitions [213.1]− → [93.0]− for 3-PBA and [219.1]− → [99.1]− for the internal standard. The detection limit was 0.4 ng ml−1 urine. The analyses of 3-PBA were part of the Round Robin interlaboratory comparison programme (Prof. Hans Drexler, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany). The analytical results were within the tolerance limits set by the interlaboratory comparison programme. Density and creatinine levels were also determined in all urine samples to be able to correct for dilution. A hand refractometer was used to determine the urine density. The creatinine levels were analyzed using an enzymatic method described by Mazzaachi et al. (2000). The urine concentrations, adjusted for density (d.a.), were calculated as $C_{\text{d.a.}} = C_{\text{obs}} \times \frac{1.016 - 1}{(\rho - 1)}$. The $C_{\text{d.a.}}$ is the corrected concentration, $C_{\text{obs}}$ is the observed concentration, $\rho$ is the specific density and 1.016 is the assumed average density as described by Boeniger et al. (1993). The ratio between $C_{\text{obs}}$ and creatinine level was used for the creatinine correction.

Statistical methods

A series of logistic regression models, one model per symptom, were used to analyse the questionnaire. The outcome variable in the models was the presence of the symptom (yes/no) and the different treatments were used as independent variables with the untreated week as the reference. The regression models included a random intercept for each planter to accommodate for the serial correlation arising from repeated measurements on the same planter (Brown and Prescott, 2001).

The relationship between pesticide exposure and levels of lysozyme and albumin in NAL and 3-PBA in urine was analysed using linear mixed-effect models. The models included a random intercept for each planter and the model for 3-PBA in urine also included a random intercept for the measurement occasion within each exposure, thus accounting for serial correlation within each planter as well as within each exposure (Brown and Prescott, 2001). Due to a highly right-skewed distribution of 3-PBA, the values were log-transformed to normalize their distribution and the regression coefficients were therefore interpreted as percentage change in the geometric mean value. Values below the limit of detection (d.l.) were assigned the value d.l./2. All analyses were made using STATA v9.2 (StataCorp LP, 2005).

RESULTS

Questionnaire

Planters. Seven of the nineteen planters were Polish men. The remaining planters 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 three older than 50 years. The majority had worked with planting for at least 2 months before the beginning of the study. 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 planters were provided with protective clothing by their employers and gloves and long trousers were used during all planting work. Nearly, all 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 just as often. Just under half of the 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 symptoms.** Of the 19 planters, one had asthma, one had hay fever and three had allergy to tree or grass pollen and mites. The most common symptoms for all weeks were ‘blocked or runny nose’, ‘irritation in the eyes’, itching and ‘shooting pain’.

A study of the occurrence of health problems after the exposure weeks indicated that the planters complained mostly of problems with irritated throat or nose, irritation of eyes and also dry skin irrespective of treatment week. After the week of exposure to imidacloprid, 6 of 19 planters reported irritated and blocked nose; eye irritation was reported by 4 of 19 planters and one person suffered from numbness. There were also a number of planters who reported itching on the face and dry skin. After the week of exposure to cypermethrin, 7 of 19 planters reported irritated and blocked nose and 4 of 19 facial itching. Problems with dry skin were reported by 3 of 19 individuals. Problems with blocked and irritated nose was also high (7 of 19) during the untreated week, followed by irritation of the airways (six persons) and itching and irritation of the eyes (five persons). There were no significant differences in the occurrence of the symptoms between weeks.

Crossover analysis enabled the study of the reported symptoms for each individual after each week of exposure in relation to the week when planters were exposed to untreated seedlings. The study shows that there were symptoms that were reported by some of the planters following the week of exposure to imidacloprid but were not reported after the week with untreated seedlings. A number of planters who had reported problems after the first week (with untreated seedlings) reported no such problems after subsequent weeks when seedlings had been treated with insecticides (Fig. 1).

The planters were also asked if they experienced any annoying smells when placing the plants in the bags. None reported annoying smells after handling the imidacloprid-treated seedlings but 7 of the 19 reported annoying smells after handling the cypermethrin-treated seedlings. Four of 19 reported annoying smells after the week with untreated seedlings. None of the planters could tell which insecticide was being used on the seedlings during each week. This was used as evidence of the effectiveness of the blinding procedure.

**Biological monitoring**

The analysis of inflammation markers in NAL 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 Fig. 2 and Table 1 below and were also compared with results from earlier studies performed on people with other occupations.

**Lysozyme.** The measurement of lysozyme levels in NAL was performed to determine if exposure to insecticides had lead to increased secretion in nasal mucous membranes. Figure 2a 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 (Wållinder, 1999; Elfman et al., 2009). There was no significant difference in levels of lysozyme between weeks planting untreated seedlings and those weeks when the planters were exposed to Merit Forest (imidacloprid) and Forester (cypermethrin), Table 1.

**Albumin.** The level of albumin in NAL was used as a measure of vascular leakage in the nasal mucous membranes. During the 3 weeks, only 26% of the planters had levels over the lower detection limit of 5 mg l⁻¹ albumin. Figure 2b shows the detectable values for all 3 weeks.

Fourteen 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 seedlings compared with the weeks with treated seedlings, while two had higher levels. There was no significant difference (Table 1) in levels of albumin in planters between weeks planting untreated seedlings 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 seedlings (first and second sample) and the week with cypermethrin-treated seedlings. The
values are adjusted for the density of urine (d.a.) or creatinine (c.a.) (Table 2). This adjustment was done since the concentration of urine depends on liquid intake. 3-PBA is not present in the urine as a metabolite after exposure to imidacloprid (Merit Forest). Seventy-nine percent of planters had levels of 3-PBA that were higher after handling cypermethrin-treated seedlings than after handling untreated seedlings. The analysis indicates an average increase of 30% in 3-PBA after adjusting with density ($P < 0.05$, confidence interval (CI): 12–52%, d.l. 0.4 ng ml$^{-1}$, confidence interval (CI): 12–52%, d.l. 0.4 ng ml$^{-1}$), while the increase was 33% after adjusting with creatinine ($P < 0.05$, CI: 11–58%, d.l. 0.1 nmol mmol$^{-1}$ creatinine) during the week of planting cypermethrin-treated seedlings. The level of 3-PBA in samples from the week with cypermethrin-treated seedlings increased from Sample 1 to Sample 2, taken a day later. This difference was, however, not significant. These increases could not be explained by smoking habits or the use of snuff. However, it may indicate a delayed dermal uptake.

**DISCUSSION**

Exposure to insecticides by planters is likely to be by inhalation, skin absorption and ingestion, of which skin absorption is probably the most important route in this study. However, earlier studies indicate that pyrethroids are absorbed less effectively through the skin (Woollen et al., 1992) and together with good use of protective clothing we can conclude that the exposure levels were probably low among these planters.

It is generally difficult to make correlations between pesticide exposure and symptoms with the

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**Table 1.** Lysozyme and albumin levels in planters during the 3 weeks

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<th>Lysozyme</th>
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<td></td>
<td>Percentiles</td>
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<tr>
<td></td>
<td>P25, Median</td>
<td>P50, Median</td>
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<tr>
<td>Imidacloprid</td>
<td>19 5.5, 10.1</td>
<td>16.0, 10.5</td>
</tr>
<tr>
<td>Untreated</td>
<td>19 4.0, 8.4</td>
<td>14.7, 11.0</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>19 5.5, 7.5</td>
<td>10.2, 9.0</td>
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</table>

Lysozyme and albumin values (mg l$^{-1}$) for 25th, 50th and 75th percentiles.
help of a questionnaire (Wieseler et al., 1998). One important reason is that health problems are affected by many factors including sex, age, employment time and hypersensitivity. It is therefore important that the effect of these factors is considered in correlation analysis. The effectiveness of this study was increased by performing it as a double-blind cross-over study with a modified questionnaire used earlier by Kolmodin-Hedman et al. (1995). The present study suggests that the evaluation procedure itself affected the result during the first week, probably because of increased awareness by the planters, which then reduced during the following two study weeks. The results of the questionnaire study indicated the type of health problems that might occur in planters when working with insecticide-treated seedlings. However, it could not be shown to be statistically significant, due to the limited data set or simply because there were no significant health effects of the insecticides used.

During the spring of 1991, Kolmodin-Hedman et al. (1995) performed an investigation into the health problems experienced by planters who worked with conifer seedlings with soil-covered roots that were treated with permethrin. The investigation, which was also a double-blind study and included 18 individuals, showed that the planters did not have more health problems after planting permethrin-treated seedlings than after planting untreated seedlings (Kolmodin-Hedman et al., 1995). This agrees well with the results obtained in the current study.

In addition, biological monitoring was included to evaluate objectively any health effects that depended on exposure to the insecticides Merit Forest and For ester. The results from determination of the levels of inflammation markers in NAL do not indicate any clear effects of imidaclorpid 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 3 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 (Wålinder, 1999; Wålinder et al., 2000) and stable personnel working both indoors and outdoors (Elfman et al., 2009).

The planters in this study had median 3-PBA levels <0.4 ng ml\(^{-1}\) (d.a.) during the week working with untreated seedlings and 0.7 ng ml\(^{-1}\) during the week with cypermethrin-treated seedlings, which is clearly higher than the levels obtained in the general population (Fortin et al., 2008) but much less than in people working with pest control (Llewellyn et al., 1996; Wieseler et al., 1998). These increased (12–52%) levels represent a measure of exposure to insecticides containing cypermethrin. It is, however, not clear if these levels have any connection with acute health effects.

Pyrethroids are rapidly metabolized in the liver, resulting in an effective detoxification (Woollen et al., 1992). The literature indicates that the level of metabolites in people working with insecticides in agriculture under good hygienic measures using protective clothing 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 (Llewellyn et al., 1996; Wieseler et al., 1998; Heudorf and Angerer, 2001; Hardt and Angerer, 2003). Furthermore, workers spraying pyrethroids had higher values (geometric means) in summer (12.2 μg g\(^{-1}\) creatinine) than in winter (3.9 μg g\(^{-1}\) creatinine) (Wang et al., 2007). In a study performed among professional users spraying permethrins, the 3-PBA levels ranged between non-detectable and 46.2 nmol mmol\(^{-1}\) creatinine, with >90% of the values being <10 nmol mmol\(^{-1}\) creatinine (Llewellyn et al., 1996). The operatives with the highest contamination levels were working in confined areas.

In a recent study performed among the general population of Quebec (120 adults), the median daily 3-PBA level was 0.17 μg l\(^{-1}\) urine (range 0.01–15.5 μg l\(^{-1}\)) (Fortin et al., 2008). Preliminary data from a general population in Sweden (227 adults) showed the presence of the metabolite 3-PBA in 14% of urine

<table>
<thead>
<tr>
<th>Type of correction</th>
<th>Treatment</th>
<th>Sample</th>
<th>n</th>
<th>Proportion (%) &gt;d.l.</th>
<th>GM</th>
<th>GSD</th>
<th>P25</th>
<th>Median</th>
<th>P75</th>
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<tr>
<td>3-PBA d.a., ng ml(^{-1})</td>
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<td>First</td>
<td>19</td>
<td>58</td>
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<td>1.36</td>
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<td>84</td>
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<td>3-PBA c.a., nmol mmol(^{-1})</td>
<td>Untreated</td>
<td>First</td>
<td>19</td>
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n = number of planters; GM = geometric mean; GSD = geometric standard deviation; P25, 25th percentile; P75, 75th percentile.
samples (>0.4 µg l⁻¹ and range 0.4–3 µg l⁻¹, Associate Prof. CH Lindh, University of Lund).

From two studies in Germany (Wieseler et al., 1998; Hardt and Angerer, 2003), it was concluded that one could not expect adverse health effects from pyrethroids in workers, providing the pyrethroids were applied in a careful way and with good working routines, protective clothes and regular changes of protective gloves. The study in Japan (Wang et al., 2007) showed no significant difference in any exposure-related subjective symptoms or laboratory test results and physical examination revealed no signs of suggestive neurotoxicity in any subject, which is in accordance with the results obtained in this study. 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 (Hardt and Angerer, 2003) 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, skin and nervous system and also objective tests of biomarkers on nasal mucous membranes and urine. No clear, acute adverse health effects could be found in planters after exposure to conifer seedlings treated with imidacloprid (Merit Forest) or cypermethrin (Forester), as compared with exposure to untreated seedlings. The metabolite, 3-PBA, was found in low levels in urine and was increased after exposure to cypermethrin. However, we could not find any statistically significant correlations 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 use of protective clothing, which probably resulted in relatively low exposure levels.

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REFERENCES


StatCorp. (2005) STATA, statistical software: release 9. College Station, TX. StataCorp LP.