Cholinesterase Activity in Female Greenhouse Workers—Influence of Work Practices and Use of Oral Contraceptives

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Abstract: Cholinesterase Activity in Female Greenhouse Workers—Influence of Work Practices and Use of Oral Contraceptives: Jesper B Nielsen, et al. Environmental Medicine, University of Southern Denmark—Objectives—Associations between serum butyryl cholinesterase activity in female greenhouse workers and information from a questionnaire on work, use of personal protective equipment, and lifestyle were evaluated. Methods—Data were gathered for 571 female greenhouse workers by means of a questionnaire and serum cholinesterase analyses (butyrylthiocholin-assay). Results—The mean concentration of butyryl cholinesterase in serum was 6.50 kU/l. Serum butyryl cholinesterase activity was significantly decreased by the use of oral contraceptives (p<0.001). Among women with re-entry intervals less than 24 h, serum butyryl cholinesterase activities were significantly reduced. Within this group of women, only those not using gloves during the manual handling of plant cultures had significantly reduced serum butyryl cholinesterase activities (p=0.028). Conclusions—Use of oral contraceptives is an important confounder in studies on serum butyryl cholinesterase activity. If re-entry intervals were below 24 h, serum butyryl cholinesterase activity was significantly inhibited, but use of gloves protected the women from such exposures. (J Occup Health 2002; 44: 234–239)

Key words: Pesticides, Cholinesterase activity, Occupational exposure, Re-entry intervals, Confounding, Oral contraceptives

Biomarkers of exposure are normally developed with the purpose of specificity with respect to the chemical exposure. But if several chemicals act on the same receptor and ultimately cause identical effects, a biomarker of exposure with a wider specificity with respect to chemicals but a high specificity with respect to the mechanism or metabolism may be preferred. This is especially useful for screening purposes. Cholinesterase inhibition is often used to screen for exposure to the organophosphates and carbamates, the advantage being that the biomarker (e.g., butyryl cholinesterase in serum) reflects the integrated exposure to a mixture of chemicals. A biomarker will, however, reflect the total exposure irrespectively of occupational or non-occupational origin. The use of a biomarker in occupational settings therefore depends on information not only on potential confounders, but also on non-occupational exposures potentially affecting the biomarker.

In acute poisoning with organophosphates or carbamates, the cholinesterase activities are 30 to 50 percent of normal by the time symptoms appear1), but the reference interval for cholinesterase activity is wide. After cessation of organophosphate exposure, the plasma cholinesterase will normally return to baseline activity within 4–6 wk2), the return to baseline being considerably faster after cessation of carbamate exposure. A number of factors are known to affect cholinesterase activity in humans, and are normally taken into consideration when evaluating and comparing cholinesterase activity in different groups of individuals: body weight, height, sex, genetic polymorphism in metabolising enzymes, and age2, 3). The influence of age has been most extensively discussed in relation to cholinesterase activity in women. In postmenopausal women, the cholinesterase activity is significantly higher than in premenopausal women of the same age4), whereas the variation in cholinesterase activity with age in adult premenopausal women is insignificant as long as the women do not use oestrogen-containing contraceptives or are pregnant5, 6).

Cholinesterase activity is also known to vary over time and with the season2, and the problem with intra-individual variation has often been dealt with by trying to get baseline values before or after a certain exposure
situation. Studies on larger cohorts, however, demonstrate that the influence of variance components due to intra-individual variations is marginal compared to that of the inter-individual variance\(^2\). These authors therefore conclude, that repeated measurements of plasma or serum cholinesterase activity within the same individual will only reduce the total variation insignificantly, and that the inter-individual variation should be reduced by using matched groups of individuals\(^2\)–\(^3\).

Women working in greenhouses are exposed occupationally to a wide selection of chemicals, but may also have relevant non-occupational exposures. To reduce the risk of handling hazardous chemicals or plants recently treated with pesticides (re-entry exposure), the advice is often to use personal protective equipment (PPE). As the main route of occupational exposure to chemicals (i.e. pesticides) among these women is dermal, the women are often advised to use gloves. Whether gloves actually protect the women from unwanted exposure is seldom evaluated.

We have collected blood samples from 571 female greenhouse workers and analysed the blood samples for butyryl cholinesterase (BuChE) activity. These data have been related to information from an extensive questionnaire on the traditional confounding variables, non-occupational exposures and very specific information on work practices and use of protective equipment. Therefore, the purpose of the present study was to evaluate associations between occupational exposure including handling practices and serum BuChE in female greenhouse workers.

**Materials and Methods**

**Cohort establishment**

Employers: A letter was sent to all greenhouse owners on the island of Funen, Denmark asking whether they would accept participation in a study, which would initially include allowing us to visit their greenhouses and invite their female employees to participate in the study. If the female employees agreed to participate, the employer would give information on use of insecticides, fungicides, and growth retardants (hereinafter grouped together as pesticides) during the last 3 months. Among the 220 greenhouse gardens on Funen, 100 (45%) of the owners agreed to participate after telephone contact, whereas 35% did not meet the inclusion criteria (had no female employees) and 20% did not want to participate, so that the participation rate among eligible employers was 69%.

Employees: All female employees at the 100 greenhouse gardens were informed orally and in a letter of invitation about the project. Among the women working in the greenhouses 602 (appr. 75%) agreed to participate and blood samples and questionnaires were successfully collected from 571 women (95%). The overall participation rate was 72% among employees. Questionnaires and blood samples were collected at the workplace in order to minimize time consumption for the participating women and their employer.

**Questionnaire**

All participants received a questionnaire with detailed questions regarding job description, user-frequency and names of products used in gardening, personal handling of pesticides, re-entry intervals before entering greenhouses where pesticides had been applied, handling plant cultures recently treated with pesticides, and the use of PPE. The women were also asked about age, educational background, smoking habits, non-occupational exposure to pesticides (used at home for pets or during gardening) and use of oral contraceptives. All information on exposure reflected the last three months before blood sampling.

**Use of pesticides**

A total of 172 pesticides with 128 different active ingredients were used in the greenhouses. The five most frequently used insecticides included three carbamates (methomyl, pirimicarb, and methiocarb), one organophosphate (dichlorvos), and one pyrethroid (deltamethrin). Two growth retardants (chlormequat-chloride and daminozid) were used at frequencies comparable to the carbamates.

**Blood samples**

Venous blood samples were collected in Venoject tubes between 9.00 and 16.00. All samples were taken by the same phlebotomist, and blood samples were centrifuged to separate the serum within 1 h after sampling and frozen in 1 ml aliquots within 4 h at –80°C for later analysis.

**Analytical methods**

Serum cholinesterase (EC 3.1.1.8) was quantitatively measured with Vitros CHE slides (Vitros 126 2096, Johnson & Johnson Clinical Diagnostics, Rochester NY, US) with butyrylthiocholine as substrate. Cholinesterase hydrolyses butyrylthiocholine to thiocochline, which reduces potassium hexacyanoferrate III to potassium hexacyanoferrate II. The rate of colour loss is monitored spectrophotometrically at 400 nm. Assay was performed at 37°C. Reproducibility was assessed from repeated parallel determinations of two standard samples. Within-run imprecision was 1.3–3.2% (CV-values).

**Statistics**

Analyses were performed with the SPSS software (SPSS, Chicago IL) on log-normalized cholinesterase data. The independent t-test was used for bivariate comparisons between means. Correlations between continuous variables with near-normal distribution were
studied by using the Pearson correlation coefficient. Backward stepwise regression was used for multivariate analyses. Two-tailed p-values are given throughout.

**Ethics**

All participants gave informed consent after receiving oral as well as written information on the project. The study followed the guidelines of the second Helsinki declaration and was approved by the regional ethical review committee.

**Results**

**Serum butyryl cholinesterase level**

The mean concentration of BuChE in serum from the 571 women of an average age of 34.8 yr was 6.50 kU/l (geometric mean) with a 95% confidence interval of 6.38–6.62 kU/l. As analysis of the distribution revealed that a better fit to a Gaussian distribution was obtained if the BuChE variable was log-normalized, all further analyses are performed on log-normalized data.

**Non-occupational exposures, oral contraceptives, smoking and age**

The questionnaire included information on non-occupational exposure to potential cholinesterase inhibitors such as oral contraceptives, anti-flea insecticides used for pets, and use of all pesticides during hobby gardening. Use of different pesticides at home was reported by 16% of the participants but did not affect the serum BuChE activity (Table 1). Serum BuChE activity was, however, significantly affected by the use of oral contraceptives (p<0.001). Thus, the serum BuChE activity in those 30% of the women using oral contraceptives was reduced by 12.5% as compared to non-users (Table 1). Serum BuChE activity was positively and significantly correlated with age, but the significance of this correlation disappeared after adjustment for the use of oral contraceptives. Serum BuChE was not affected by smoking.

**Use of pesticides**

Close to 88% (505/571) of all women confirmed working at gardens where pesticides were used (Table 1). The mean BuChE level in serum was marginally higher in this group than in women with no potential occupational pesticide exposure, but the difference was not significant (Table 1). Twenty percent of the women had personally mixed or distributed pesticides within the last three months before blood sampling, but their serum BuChE activities were not significantly different from those of the majority of women not involved in these work practices (Table 2). Furthermore, analysis of serum BuChE activities in groups of women dipping cuttings did not reveal significant differences in activity of BuChE in serum as compared with women not performing this function (Table 2).

**Handling and dermal contact with flowers or vegetables**

All women employed at a place where pesticides were used, were asked whether they within the last three months had handled flowers or vegetables that had been treated with pesticides less than a week or less than 24 h before sampling. Ninety percent (N=400) of the women had handled flowers or vegetables that had been treated less than a week before handling, but their serum BuChE activities were not significantly different from those of the remaining ten percent (N=43; Table 3). But among the 276 women (65%) who reported handling plant cultures which had been treated less than 24 h before handling (re-entry interval less than 24 h), serum BuChE

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**Table 1.** Mean serum butyryl cholinesterase (BuChE) activity in selected groups of the female greenhouse workers (N=571) with occupational or non-occupational exposure to pesticides or use of oral contraceptives. Data on BuChE are given as geometric means with 95% CI

<table>
<thead>
<tr>
<th></th>
<th>N*</th>
<th>BuChE activity (kU/l)</th>
<th>different from previous group (p)</th>
<th>mean ratio with 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-occupational exposure to insecticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>91</td>
<td>6.580 (6.281–6.894)</td>
<td></td>
<td>1.015 (0.965–1.069)</td>
</tr>
<tr>
<td>no</td>
<td>472</td>
<td>6.481 (6.347–6.618)</td>
<td>0.561</td>
<td></td>
</tr>
<tr>
<td>Use of oral contraceptives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>173</td>
<td>5.936 (5.744–6.134)</td>
<td>&lt;0.001</td>
<td>0.876 (0.842–0.912)</td>
</tr>
<tr>
<td>no</td>
<td>393</td>
<td>6.773 (6.627–6.923)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesticides used at the work place</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>505</td>
<td>6.516 (6.383–6.639)</td>
<td>0.118</td>
<td>1.109 (0.974–1.263)</td>
</tr>
<tr>
<td>no</td>
<td>12</td>
<td>5.876 (5.048–6.839)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Deviation from N=571 is due to missing answer to the specific question. *As BuChE is log-transformed, back transformation of mean differences becomes ratios, i.e. the H_0 hypothesis is that the ratio is 1.
activities were reduced and the difference was significant \((p=0.029; \text{Table 3})\). Regression analysis, including the use of oral contraceptives as a predictor for BuChE, demonstrated that adjustment for this confounder did not weaken the observed association between BuChE and handling plant cultures within 24 h after pesticide treatment (Table 3). Further analysis demonstrated that within the group of women handling plant cultures within 24 h of pesticide treatment, only those not using gloves during the manual handling of plant cultures had significantly reduced serum BuChE activities \((p=0.028; \text{Table 3})\). Actually, those using gloves had almost the same BuChE activity as those who did not handle flowers or vegetables treated within the last 24 h (Table 3). Regression analysis confirmed that within this group of women the use of gloves was a significant predictor for BuChE (Table 3).

**Discussion**

Serum BuChE inhibition has been used as a biomarker for exposure to organophosphates and carbamates in epidemiological studies and screening programmes. The
median degrees of inhibition in the study groups are most often within the range where no clinical signs of toxicity would be expected. Nevertheless, as the inter-individual variation is large, even a minor shift of the distribution curve to the left may bring susceptible individuals in the population below their individual threshold concentrations for sub-clinical effects. In a cohort of younger females, this is especially important since recent laboratory animal studies indicate that even minor inhibition of cholinesterase activity may cause developmental toxicity in the offspring\(^5,9\).

The mean concentration of BuChE in the whole cohort (n=571) was 6.5 kU/l with significantly higher concentrations in women without occupational exposure and no use of oral contraceptives. This mean concentration is within the expected range observed in previous population-based studies using the same assay method (7.51 ± 1.53 kU/l for women)\(^3,4\). Our finding stresses the importance of considering the use of oral contraceptives as an important and influential confounder in epidemiological and clinical studies with BuChE as a biomarker. We report 12.5% reduced serum BuChE in women using oral contraceptives, which is in accordance with a previous study demonstrating an approximately 12% lower serum BuChE activity in women using oral contraceptives\(^9\). The regression analyses, however, demonstrate that adjustment for this confounder is possible.

The suggested mechanism for this confounder, which was already described in 1967\(^10\), should be through changes in the oestrogen concentration in the blood causing either a depression of the hepatic synthesis or release of the enzyme\(^5,6\). In occupational settings an inhibited cholinesterase is normally used as a biomarker for exposure to organophosphates or carbamates. But, as other chemicals, including other pesticides, have demonstrated some estrogenic potency in cell systems\(^11\) and as different detergents have inhibited the cholinesterase activity in mussels\(^12\), the specificity of serum BuChE as a marker for exposure to organophosphates and carbamates could be questioned. These observations might of course limit the use of the marker in some instances, but in other situations it might even be an advantage with a wider specificity with respect to chemical exposures affecting the biomarker.

Almost all persons in our cohort of female greenhouse workers were employed at work places where pesticides were used regularly, but the serum BuChE activity was not statistically lower than in the few persons with no occupational exposure to pesticides. In the questionnaires we had a strong focus on work practices and the use of personal protective equipment. Work involving mixing and distributing pesticides was not associated with changed concentrations of BuChE activity. These tasks are also characterized by strict regulations concerning the training and education of workers and use of protective equipment to avoid inhalation and skin contact with chemicals. As information from the questionnaire also revealed that the women were generally using the prescribed protective equipment, it is reassuring that the biological exposure marker demonstrates an equivalent result.

Due to the regulations and the general compliance with the rules during mixing and distribution, exposure of greenhouse workers is predominantly dermal and occurs through handling of flowers or vegetables previously treated with pesticides. The two most important determinants for this dermal exposure are the time between when the chemicals are distributed on the flowers and the handling (re-entry interval), and the use of gloves. In the present study, we were not able to detect any significant effect on the biomarker if the re-entry interval was more than a week, but if re-entry intervals were reduced to 24 h, the serum BuChE activity was significantly inhibited in the women with the shorter re-entry interval, thus indicating exposure and absorption of cholinesterase inhibitors. Further analysis of this group of women with re-entry intervals below 24 h demonstrated that women not using gloves were the driving force in this shift in distribution. Thus, women with re-entry intervals less than 24 h using gloves during handling of plant cultures had serum BuChE concentrations at the same level as those who did not have a short re-entry interval.

Based on the findings of the present study, we must conclude that 1) use of oral contraceptives is an important confounder in studies on serum BuChE activity; 2) mixers and distributors of pesticides did not, if prescribed protective equipment were used, absorb sufficient chemicals to affect the serum BuChE activity; 3) No effect on serum BuChE activity was seen if re-entry intervals were more than one week; 4) If the re-entry interval was less than 24 h, serum BuChE activity was significantly inhibited, but the use of gloves protected the women from exposures associated with reduced serum BuChE activity.

References


4) Lepage L, Schiele F, Gueguen R, Siest JG. Total cholinesterase in plasma: biological variations and