

# Inter and intraindividual variations in plasma cholinesterase activity and substance concentration in employees of an organophosphorus insecticide factory

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## Abstract

**During a period of 10 months, inter and intraindividual variations in plasma cholinesterase (ChE) activity were studied in 331 employees of an organophosphorus insecticide factory, and in 193 healthy volunteers without occupational exposure to known ChE inhibitors. Repeated (n = 6) measurements of ChE activity and ChE substance concentration were performed in 410 subjects. The study showed substantial intraindividual variations of ChE activity and ChE substance concentration (up to 40%) in the employees and in the reference group. When effects due to sex, ChE-1 phenotype, body weight, and height were considered, one subgroup of employees of the organophosphorus insecticide factory showed a significantly lower average ChE activity than other subgroups; as ChE substance concentrations were found to be proportionally decreased, it was concluded that the low ChE activity was unrelated to occupational exposure. A combined determination of ChE activity and ChE substance concentration is recommended as a rational diagnostic tool when an unexpected decrease of plasma ChE activity is registered in people joining organophosphorus insecticide health surveillance programmes.**

Organophosphorus insecticides are highly toxic substances to man as well as to pests. Persons with risk for substantial occupational exposure to such substances are employees of organophosphorus insecticide factories and pesticide operators within gar-

dening and agriculture. Because even a few mg may possess appreciable toxic effects in man, and as absorbed organophosphorus insecticides are excreted as non-specific metabolites (for example, p-nitrophenol from parathion and methyl-parathion), most programmes for health surveillance are based on measurements of plasma cholinesterase activity (ChE, pseudocholinesterase, butyrylcholinesterase, acylcholine acylhydrolase, EC 3.1.1.8), which is strongly inhibited by all organophosphorus insecticides.<sup>1</sup> In such health surveillance programmes (as in other areas of clinical medicine) decisions should be made from rational interpretations of observed variations based on knowledge of spontaneous inter and intraindividual variations in the population.<sup>2</sup>

Biosynthesis of the 574 amino acid ChE subunit<sup>3</sup> is controlled by the ChE-1 locus on chromosome No 3, where a number of alleles (ChE<sup>u</sup>, ChE<sup>a</sup>, ChE<sup>f</sup>, ChE<sup>s</sup>, ChE<sup>i</sup>, ChE<sup>b</sup>) define the actual genotype<sup>3</sup>; the genotype ChE<sup>u</sup>ChE<sup>u</sup> (u = usual) is found in about 95% of Caucasian populations. About 4% should be expected to be ChE<sup>u</sup>ChE<sup>a</sup> (a = atypical); the atypical ChE has recently been shown to be an Asp-70 → Gly mutation owing to a change of the actual ChE<sup>a</sup> codon from GAT to GGT.<sup>5</sup> Within a Caucasian population one person in 100 000 will be expected to be of ChE-1 genotype ChE<sup>s</sup>ChE<sup>s</sup> (s = silent) without detectable ChE catalytic activity in plasma. Such people, who are found much more frequently among the Inuits of the north-western parts of Canada than among a general population (Dr Alison Dinwoodie, University of Alberta, Canada, personal communication), are healthy and without any increased risk of death unless exposed to suxamethonium to which they are extremely sensitive.<sup>4</sup>

Previous studies<sup>6-10</sup> have analysed the well known, wide interindividual variation in plasma ChE activity in healthy subjects. Such variations, which are independent of electrophoretic heterogeneity,<sup>11</sup> are statistically related to physiological factors such as body weight, height, and sex.<sup>7,10</sup> Four of these studies<sup>6,8-10</sup> also describe substantial intraindividual variations that are independent of age, body weight,

height, sex, and ChE-1 phenotype.<sup>10</sup> The main problem in the interpretation of significant variations of plasma ChE activity (which is further influenced by a variety of other physiological and pathological conditions<sup>4</sup>) is to decide whether a decrease in catalytic activity is caused by a reduced ChE substance concentration (for example, due to pre-analytical errors, reduced ChE synthesis, or increased ChE degradation) or by a partial inhibition of the catalytic activity (for instance, due to absorbed organophosphorus insecticides).

This paper describes the inter and intraindividual variations in catalytic and immunoreactive<sup>12</sup> plasma ChE activity in a group of employees of an organophosphorus factory. It also evaluates the variations by a multiple regression model that is appropriate for a healthy population group without occupational exposure to known ChE inhibitors.<sup>10,13</sup>

## Materials and methods

### SAMPLE

During a period of 10 months (November 1987–September 1988) heparin stabilised venous blood samples were taken, at intervals of four to six weeks, from 331 employees of an organophosphorus insecticide factory (319 men, 12 women) and 193 healthy volunteers (122 men, 71 women) not exposed to known ChE inhibitors. Plasma was separated from the blood cells within 24–30 hours after withdrawal and stored at  $-20^{\circ}\text{C}$ . Detailed data concerning the 193 volunteers have already been published.<sup>10,13</sup>

All employees of the organophosphorus insecticide factory (Cheminova, Lemvig, Denmark) were subdivided by the Health Service and the local safety committee into four groups according to their actual risk for exposure to toxic organophosphorus compounds as follows: group 1 (high risk for exposure); persons occupied with production of diethyl-phosphorochloridothionate; persons handling repairs and maintenance all over the factory; group 2 (medium risk for exposure); persons occupied with production

of dimethyl-phosphorochloridothionate, parathion, methyl-parathion, malathion, pirimiphos, dimethoate, and p-nitrophenol; persons occupied with unspecified assistance, drawing, and handling of waste water; persons employed in quality control and development; persons occupied with laboratory routines: group 3 (low risk for exposure); persons occupied with production of dimethyl- and diethyl-dithiophosphoric acid; persons taking care of lubrication, fire control, etc.; engineers, supervisors, etc.: group 4 (negligible risk for exposure to toxic organophosphorus compounds); persons occupied with production of phosphoropentasulfide; persons within planning and management.

Age, body weight, height, previous liver disease, use of oral contraceptives, and any regular intake of drugs were recorded from information given by the Health Service. Of the 331 employees, 318 completed the full programme (six samples from each subject) and so did 131 of the 193 volunteers (table 1).

### ANALYTICAL METHODS

Determination of plasma ChE activity was performed on a Cobas FARA centrifugal analyser by a continuous assay using 6 mmol/l butyrylthiocholine iodide (Merckotest 3337) as substrate.<sup>10,11</sup> Day to day reproducibility was assessed from parallel determinations of lyophilised quality control material (M + D Monitrol I-E and Monitrol II-E); coefficient of variation (CV) = 1.34% (mean = 4.38 kU/l, SD = 0.059 kU/l, n = 125) for lot No PTD 108; CV = 1.92% (mean = 6.38 kU/l, SD = 0.123 kU/l, n = 44) for lot No LTD 209; and CV = 1.36% (mean = 6.51 kU/l, SD = 0.089 kU/l, n = 81) for lot No LTD 210.

Phenotypes of ChE-1 (ChE types) were classified from inhibition of the catalytic activity by dibucaine and fluoride under reaction conditions as recommended by the Association of Clinical Pathologists.<sup>14,15</sup> Of the 316 employees of the organophosphorus insecticide factory, 18 men were classified as ChE<sup>a</sup>ChE<sup>a</sup>. The remaining 298 were classified as ChE<sup>a</sup>ChE<sup>b</sup>. Of

Table 1 Population groups from which six blood samples were obtained. Risk group 1, high risk for exposure to organophosphorus insecticides; 2, medium risk; 3, low risk; 4, negligible risk; Reference group, subjects without occupational exposure to known ChE inhibitors

Population group	No of subjects	Mean body weight (kg (SD))	Mean height (cm (SD))	Mean age (y (SD))
Employees	318	79.6 (10.8)	176.5 (6.5)	40.2 (9.7)
Risk group 1	75	81.3 (9.7)	176.0 (5.8)	40.8 (7.5)
Risk group 2	200	78.4 (11.0)	176.2 (6.6)	39.3 (10.2)
Men	188	79.2 (10.2)	176.8 (6.0)	39.9 (10.0)
Women	12	66.2 (15.4)	166.8 (9.0)	30.1 (8.9)
Risk group 3	29	81.8 (12.3)	179.2 (7.9)	41.9 (9.4)
Risk group 4	14	82.5 (9.9)	178.6 (4.8)	46.1 (10.5)
Reference group	131	71.8 (13.0)	173.0 (8.7)	39.0 (9.7)
Men	75	79.5 (9.8)	178.3 (6.1)	38.9 (9.6)
Women	56	61.5 (9.0)	165.8 (6.0)	39.2 (9.8)

Table 2 Mean (SD) ChE activity in each of six plasma samples (ChE-1–ChE-6) from 318 employees of an organophosphorus insecticide factory and in 131 healthy subjects without occupational exposure to known ChE inhibitors (reference group)

Population group	No of subjects	ChE-1 (kU/l (SD))	ChE-2 (kU/l (SD))	ChE-3 (kU/l (SD))	ChE-4 (kU/l (SD))	ChE-5 (kU/l (SD))	ChE-6 (kU/l (SD))	Standardised ChE <sub>mean</sub> (kU/l (SD))
Employees	318	8.78 (1.79)	8.83 (1.81)	8.78 (1.85)	8.78 (1.81)	8.76 (1.89)	8.57 (1.85)	8.21 (1.64)
Risk group 1	75	8.56 (1.56)	8.65 (1.65)	8.63 (1.69)	8.64 (1.75)	8.65 (1.76)	8.48 (1.80)	7.92 (1.58)
Risk group 2	200	8.94 (1.88)	8.96 (1.88)	8.92 (1.93)	8.89 (1.84)	8.88 (1.95)	8.66 (1.91)	8.39 (1.66)
Men	188	8.99 (1.84)	9.04 (1.84)	9.00 (1.88)	8.95 (1.81)	8.95 (1.91)	8.73 (1.76)	8.39 (1.65)
Women	12	8.08 (2.43)	7.86 (2.21)	7.57 (2.29)	7.99 (2.13)	7.75 (2.29)	7.67 (2.47)	8.31 (1.88)
Risk group 3	29	8.21 (1.58)	8.24 (1.64)	8.25 (1.65)	8.16 (1.65)	8.09 (1.62)	8.03 (1.52)	7.60 (1.36)
Risk group 4	14	8.93 (1.71)	9.09 (1.86)	8.81 (1.79)	9.18 (1.94)	8.97 (1.99)	8.89 (1.83)	8.40 (1.78)
Reference group	131	8.26 (1.92)	8.15 (1.85)	7.92 (1.79)	8.03 (1.85)	8.00 (1.83)	8.17 (1.93)	8.25 (1.36)
Men	75	8.81 (1.91)	8.75 (1.84)	8.46 (1.84)	8.58 (1.82)	8.49 (1.83)	8.65 (2.02)	8.26 (1.49)
Women	56	7.53 (1.68)	7.34 (1.54)	7.20 (1.46)	7.29 (1.62)	7.33 (1.63)	7.53 (1.61)	8.25 (1.18)

the 131 volunteers of the reference group, seven were classified as ChE<sup>u</sup>ChE<sup>s</sup>, and the remaining 124 were classified as ChE<sup>u</sup>ChE<sup>u</sup>.

Determination of immunoreactive ChE substance concentration was performed by an enzyme linked immunosorbent assay (ELISA) using polyclonal (rabbit) antihuman ChE antibodies combined with a highly specific monoclonal (mouse) antihuman ChE antibody and a peroxidase conjugated (rabbit) antibody against mouse immunoglobulins as signal carrier.<sup>12</sup> Day to day reproducibility was assessed from parallel determinations of lyophilised quality control material (M + D Monitrol I–E and Monitrol II–E); CV = 4.97% (mean = 4.62 mg/l, SD = 0.23 mg/l, n = 73) for lot No LTD 210; and CV = 5.56% (mean = 3.42 mg/l, SD = 0.19 mg/l, n = 83) for lot No PTD 109.

#### STATISTICS

Results were evaluated from a multiple regression analysis, an analysis of variance covariance, and a repeated measure analysis of variance.<sup>10,13</sup> All calculations were performed on an IBM PC/AT using the SPSS/PC+ software package (SPSS Inc, Chicago, IL).

## Results

### CHOLINESTERASE ACTIVITY

Table 2 shows the total ChE activity in six plasma samples (ChE-1–ChE-6) from the 449 subjects (employees 318; reference group 131) who completed the full programme. It shows the well known wide interindividual variation that may conceal a treatment effect when comparing unmatched groups and subgroups. As described earlier,<sup>10</sup> this problem is partly overcome by comparing “standardised” ChE activities (from which the effect of varying body weight, height, sex, and ChE-1 phenotype is eliminated) instead of “raw” ChE activities. The right column of the table shows such standardised mean ChE activities (model: ChE<sub>stand</sub> = ChE<sub>mean</sub> –

(weight–75) × 0.085 + (ChE<sub>type</sub>–1) × 2.77 + (height–180) × 0.102 + (sex–1) × 1.09; ChE<sub>type</sub>: ChE<sup>u</sup>ChE<sup>u</sup> = 1, ChE<sup>u</sup>ChE<sup>s</sup> = 2; sex: men = 1, women = 2; weight in kg and height in cm). Standardised mean ChE activities of employees in risk group 3 were significantly lower than those of men in the reference group (t = 2.15, 0.01 < p < 0.05), whereas standardised mean ChE activities of male employees in other risk groups did not differ from each other or from the men in the reference group. Female employees (risk group 2) showed the same standardised ChE activity as women in the reference group.

Table 3 shows maximum intraindividual variations (differences between a person's highest and lowest ChE as a percentage of the person's mean activity) and intraindividual variations expressed as individual SDs (n = 6). Like intraindividual variations in the reference group,<sup>10</sup> intraindividual variations of the employees of the organophosphorus insecticide factory showed a skew to the right; average maximum variation was 12.6% (range: 3.4–38.9%) independently of the actual risk group and equal to that of the reference group (mean: 14.0%, range: 3.0–41.8%). In 26 of the 318 employees, and in 23 of 131 referents the difference between highest and lowest ChE activity exceeded 20% of the mean

Table 3 Intraindividual variation of ChE activity (n = 6) in employees of an organophosphorus insecticide factory and in healthy subjects without occupational exposure to known ChE inhibitors (reference group)

Population group	No of subjects	ChE activity	
		Maximum variation (mean and range)	Individual SD range (kU/l)
Employees	318	12.6 (3.4–38.9)	0.11–1.02
Risk group 1	75	12.7 (4.3–32.0)	0.12–0.90
Risk group 2	200	12.9 (3.4–38.9)	0.11–1.02
Risk group 3	29	10.9 (3.4–21.5)	0.15–0.63
Risk group 4	14	11.3 (3.7–18.6)	0.18–0.62
Reference group	131	14.0 (3.0–41.8)	0.10–1.12

Table 4 Immunoreactive ChE substance concentration (mean of six samples) in 316 employees of an organophosphorus insecticide factory and in 94 healthy subjects without occupational exposure to known ChE inhibitors (reference group)

Population group	No of subjects	ChE substance concentration (mean mg/l (SD))	Standardised ChE substance conc (mean mg/l (SD))
Employees	316	5.47 (1.14)	5.13 (1.09)
Risk group 1	74	5.42 (1.06)	4.96 (1.06)
Risk group 2	199	5.53 (1.19)	5.23 (1.11)
Men	187	5.58 (1.16)	5.23 (1.11)
Women	12	4.77 (1.36)	5.20 (1.16)
Risk group 3	29	5.12 (0.99)	4.78 (0.91)
Risk group 4	14	5.62 (1.09)	5.26 (1.13)
Reference group	94	5.01 (1.11)	5.28 (0.91)
Men	43	5.42 (1.23)	5.28 (1.09)
Women	51	4.66 (0.88)	5.28 (0.74)

activity. In four of the employees and in three of the referents the maximum intraindividual variation exceeded 30%. The maximum intraindividual variation (expressed relative to the mean activity) was independent of ChE activity.<sup>10</sup>

A quantitative estimate of the intraindividual variance of the 318 employees was obtained from a repeated measures analysis of variance, which also gives a quantitative estimate of the influence by risk group on the intraindividual variation of ChE activity from one sample to another (second sample *v* first sample, third sample *v* first and second, fourth sample *v* first, second, and third, etc). The repeated measures analysis of variance showed an average  $SD_{intra}$  of 0.43 kU/l without influence of actual risk group ( $F = 1.05$ ,  $p > 0.10$ ). In the same way, the average  $SD_{intra}$  of the reference group was 0.44 kU/l.

#### CHOLINESTERASE SUBSTANCE CONCENTRATION

Of the 449 subjects who completed the full programme, immunoreactive ChE substance concentrations were measured in 316 of the 318 employees, and in 94 of the 131 subjects of the reference group. Table 4 shows the immunoreactive ChE substance concentrations of all 410 subjects studied. Like ChE activities, ChE substance concentrations are influenced by body weight, height, sex, and ChE-1 phenotype;<sup>13</sup> therefore, "standardised" mean ChE substance concentrations (model:  $ChE_{stand} = ChE_{mean} - (weight-75) \times 0.048 + (ChE_{type} - 1) \times 1.10 + (height-180) \times 0.064 + (sex-1) \times 0.85$ ) are preferable when comparing groups and subgroups. Like standardised ChE activity, standardised ChE substance concentration of employees in risk group 3 differed significantly ( $t = 2.12$ ,  $0.01 < p < 0.05$ ) from that of men in the reference group; no other male groups differed from each other. Female employees showed the same standardised ChE substance concentration as women in the reference group.

Table 5 shows the maximum intraindividual variations and intraindividual variations expressed as individual SD ( $n = 6$ ). As in the reference group,<sup>13</sup> the distribution of intraindividual variations of the employees of the organophosphorus insecticide factory was skewed to the right. Average maximum variation was 18.7% (range: 3–43%), independent of actual risk group, and similar to that of the reference group (mean: 20%, range: 6–43%). Within the 316 employees, a repeated measures analysis of variance showed an average  $SD_{intra}$  of 0.41 mg/l (reference group:  $SD_{intra} = 0.44$  mg/l).

#### Discussion

The use of plasma ChE activity measurements for biological monitoring should not be confused with relating the enzymatic activity to clinical or toxicological effects. Earlier systematic studies of ChE after exposure to the toxic organophosphorus compound DFP (diisopropylfluorophosphate) showed up to 95% reduction of plasma ChE activity after parenteral administration of 0.5–3.0 mg.<sup>16–18</sup> No close relation between toxic symptoms and changes in plasma ChE activities were seen. Although distinct organophosphorus insecticides inhibit plasma ChE to a varying extent,<sup>1</sup> and although plasma ChE differs in amino acid sequence from the target enzyme for organophosphorus insecticides (acetylcholinesterase EC 3.1.1.7), the use of plasma ChE activity for monitoring exposure to organophosphorus insecticides offers great advantages compared with erythrocyte acetylcholinesterase, which (contrary to plasma ChE) appears quite unstable, even when stored at  $-20^{\circ}C$  (A Brock, unpublished observations).

When comparing plasma ChE activity in unmatched groups or subgroups, influence by factors other than organophosphorus inhibitors has to be considered. The effects by ChE-1 phenotype and sex are well known,<sup>4,7,14</sup> but also (as in other liver synthesised plasma enzymes<sup>19</sup>) varying body weight and height has appeared to affect the interindividual variation of plasma ChE activity<sup>10</sup> as well as plasma ChE substance concentration.<sup>13</sup> Even when considering all

Table 5 Intraindividual variation of ChE substance concentration ( $n = 6$ ) in employees of an organophosphorus insecticide factory, and in healthy subjects without occupational exposure to known ChE inhibitors (reference group)

Population group	No of subjects	Maximum variation (mean and range)	Individual SD range (mg/l)
Employees	316	18.7 (3.2–43.0)	0.06–1.20
Risk group 1	74	17.1 (5.4–38.0)	0.11–0.89
Risk group 2	199	19.4 (3.2–43.0)	0.06–1.20
Risk group 3	29	18.2 (10.4–29.7)	0.18–0.69
Risk group 4	14	18.2 (7.8–28.8)	0.18–0.66
Reference group	94	20.1 (6.4–43.2)	0.11–1.35

Table 6 ChE specific catalytic activity (mean of six samples) in 316 employees of an organophosphorus insecticide factory and in healthy subjects without occupational exposure to known ChE inhibitors (reference group)

Population group	No of subjects	ChE specific activity (mean kU/mg (SD))
Phenotype ChE <sup>a</sup> ChE <sup>a</sup>	387	1.61 (0.05)
Employees:		
Risk group 1	67	1.62 (0.05)
Risk group 2	190	1.62 (0.05)
Risk group 3	28	1.61 (0.05)
Risk group 4	13	1.62 (0.04)
Reference group	89	1.59 (0.05)
Phenotype ChE <sup>b</sup> ChE <sup>b</sup>	23	1.30 (0.07)
Employees:		
Risk group 1	7	1.33 (0.03)
Risk group 2	9	1.32 (0.04)
Risk group 3	1	1.38 (—)
Risk group 4	1	1.33 (—)
Reference group	5	1.22 (0.09)

these factors, however, unjustified conclusions may be drawn. In the present paper standardised ChE activities of employees in one risk group (group 3, low risk for exposure to organophosphorus insecticides) were significantly lower than in other groups and subgroups. To examine the question "Were workers in risk group 3 subjected to exposure to ChE inhibitors?" ChE specific catalytic activities (ChE activities relative to ChE substance concentrations) of all 410 subjects were compared. Table 6 shows ChE specific catalytic activities by ChE-1 phenotype, which is the only physiological factor known to influence this quantity.<sup>13</sup> The specific catalytic activity of subjects in risk group 3 equals those of the other groups and subgroups. As inhibition of ChE activity by organophosphorus insecticides (phosphorylation of the catalytic active site<sup>1</sup>) does not influence the plasma turnover rate,<sup>4</sup> it was concluded that risk group 3 was not subjected to disproportionate exposure.

In health surveillance programmes, changes in plasma ChE activity are used to indicate occupational exposure of the person to organophosphorus insecticides. In accordance with earlier studies<sup>6,9</sup> (each based on 22–24 subjects) the present study, including

Table 7 Intraindividual variation of ChE specific activity (n = 6) in employees of an organophosphorus insecticide factory and in healthy subjects without occupational exposure to known ChE inhibitors (referenced group)

Population group	No of subjects	Maximum variation (mean and range)	Individual SD range (kU/mg)
Employees	316	15.8 (3.2–30.8)	0.02–0.18
Risk group 1	74	14.3 (4.0–30.8)	0.02–0.18
Risk group 2	199	16.0 (3.6–29.7)	0.02–0.17
Risk group 3	29	16.3 (7.8–27.2)	0.05–0.16
Risk group 4	14	19.1 (5.8–28.4)	0.03–0.16
Reference group	94	17.5 (5.7–37.1)	0.03–0.21

a more detailed analysis of the reference group,<sup>10</sup> shows substantial intraindividual variations of plasma ChE activity even in healthy unexposed persons. The magnitude of such normal variations are crucial in diagnostic decision making<sup>20</sup> and for defining appropriate analytical goals.<sup>21,22</sup> Analytical imprecisions of the actual ChE activity assay are immaterial compared with the biological intraindividual variations.<sup>10</sup> Hence, analytical improvements will not significantly reduce the observed intraindividual variations, and strategies of health surveillance programmes are forced to accept such large variations in plasma ChE activity. According to the British Health and Safety Executive<sup>23</sup> persons should be medically examined "if, during routine monitoring, plasma ChE activity has been shown to have fallen by more than 30% of pre-exposure levels." This strategy, which corresponds with the intraindividual variation of ChE activity found in the present study (only four of the 318 employees showed intraindividual variations higher than 30%), might be replaced by a strategy based on variations of ChE specific activity that within healthy persons exhibit insignificant biological intraindividual variations.<sup>13</sup> Unfortunately, the actual analytical imprecision of the ELISA assay for ChE substance concentrations (CV = 5–5.5%) so far eliminates most of the theoretical advantages of a strategy based on this quantity (table 7).

Until a real improvement in analytical precision of the ChE substance assay is achieved, ChE specific catalytic activity will be of limited value when dealing with minor intraindividual changes of ChE activity. When comparing groups and subgroups within a health surveillance programme and when unexpected large intraindividual variations in plasma ChE activity are to be interpreted, measurement of plasma ChE specific activity must be considered as a rational diagnostic tool.

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