# Children's Plasma Cholinesterase Activity and Fatal Methomyl Poisoning

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**Abstract**

There is a case of a couple who intentionally killed their children with methomyl insecticide. This was presented as our initial investigation of plasma cholinesterase (ChE) activity in Thai children. A hundred and five healthy Thai children 5-6 years of age, participated in the project. Their plasma was drawn to measure ChE activity. Mean ± standard deviation of the children ChE was 7,417 ± 1,620 U/L. The enzyme activity of the children was not significantly different between gender and parents' occupations. However, the mean of female ChE activity appeared to be lower than male ChE. Children whose parents were farmers appeared to have lower ChE activity than those whose parents were employees, merchants, government officers, unemployed parents, or private business owners. Two victims of child homicide were presented with ChE activity approximately 6 and 9 per cent of the average, considering healthy children. It was concluded that children's plasma ChE activity lower than 10 per cent of normal, could be a lethal indicator of anti-ChE insecticide poisoning.

**Key word :** Children's Plasma Cholinesterase, Methomyl Poisoning

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Children victimized by their parents, using insecticides, has occurred occasionally over the past 10 years during which there was an HIV epidemic in Thailand. The common insecticide used was methomyl (Lannate®) which is a cholinesterase (ChE) inhibitor[1] and an extremely toxic insecticide[2]. The measurement of serum or plasma ChE activity, is a simple laboratory screening test for monitoring anti-ChE intoxication prior to complicated confirmatory insecticide analysis.

There were several studies of children's ChE activity reported by countries other than Thailand, which indicated children's ChE activity was lower[3] than that of adults. Other studies found children's activity to be higher[4,5] or not different [6]. We presented family cases involving deaths due to methomyl poisoning. This led to our study of ChE activity in normal Thai children and also an investigation of the correlation of those activities to children's age, gender and their parents' occupations. The data is very useful for our laboratory as a reference level of ChE activity in healthy Thai children.

## CASE REPORTS

### Two examples of children who died from methomyl poisoning

A couple with two boys were found dead in their hotel room. Police investigators were notified and came to the scene. A 26 year old man was found dead with a gunshot wound to his forehead. A semiautomatic pistol lay beside his body. Next to the man's body, was a dead 26 year old woman with her two dead children, approximately one, and two years of age. There were two glasses containing a small amount of blue-green liquid found on the floor. No signs of violence were noted.

### Autopsy findings

The bullet which entered the forehead, exited out the back of the head in the occipital region resulting in his death. His blood and gastric contents were collected and sent to the laboratory for toxicological investigation. There were no traumatic wounds noted in the woman and 2 children. Fine froth secretions as well as cyanosis, was present in all of them. Blood and gastric contents were collected from each body.

### Laboratory findings

Choliesterase (ChE) enzyme inhibition and spectrophotometric enzyme assay were used as a screening test for anti-ChE insecticide intoxication. The result was confirmed with thin layer chromatography. Methomyl was detected in those two glasses and in the gastric contents of each body. The serum ChE in the man, woman, younger and older boys, were 360, 1,760, 470 and 680 units/L, respectively. Using indirect ELISA and particle agglutination technique for anti HIV-I, the results showed positive HIV-I in the blood of the man, woman and older boy.

### Cause and manner of death

The cause of death in the man resulted from the gunshot wound, secondary to his successful suicide attempt. The cause of death in the woman and two children was methomyl poisoning. This was thought to be the result of suicide in the case of the woman, but homicide in the case of the children.

In our case conference among staff and medical students, we wanted to know how much ChE activity in healthy Thai children had not been reported. There was an ethical problem and difficulty of obtaining blood from very young children (one and two years of age). A report of Jensen et al[5], found in genotypically normal patients, no age or sex differences were found in plasma ChE activity in children below the age of ten. Therefore, we designed a study to determine plasma ChE activity in children below age six. A hundred and five healthy Thai children between 5 and 6 years of age who came to Maharat-Nakorn Chiang Mai Hospital for their annual vaccinations, were asked to participate in the project. This project was approved by The Research Ethics Committee (Documentary Proof no.10/1999), Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand on April 28th, 1999.

## MATERIAL AND METHOD

### Instruments and chemicals

Spectrophotometer model Milton Roy Spectronic 401 (BecThai Company), cuvette 10x90 mm and autometric pipette (Socorex ISBA S.A., Switzerland) 10-100 μL were used. Butyrylthiocholine iodide (Sigma, St.Louis, MO), 5,5-dithiobis-2-nitro-

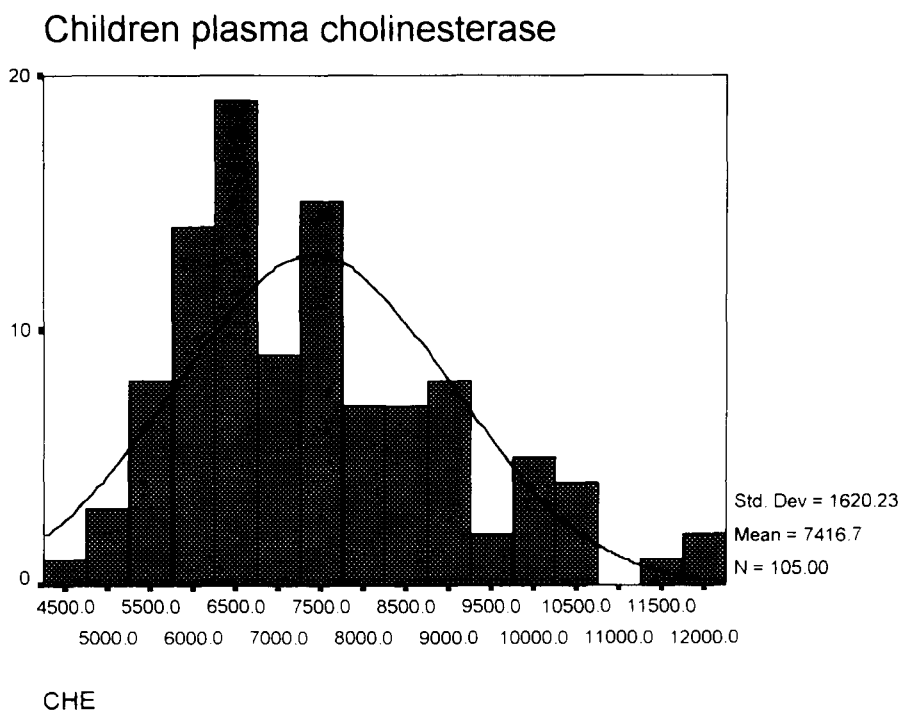


**Fig. 1. Distribution of Thai children's plasma cholinesterase (n=105, mean = 7,417 U/L, median = 7,130 U/L).**

benzoic acid (Fluka A.G., Switzerland), dipotassium hydrogen phosphate and disodium hydrogen phosphate were purchased from E. Merch, F.R. Germany *via* a local agency.

### Obtaining plasma samples

About 100 μL of blood was drawn from the children's finger tip filling two heparinized hematocrit capillary tubes after the consent form was signed by the parents with the children's willingness. Age, gender and parents' occupations were recorded *via* interview prior to blood drawing.

### Plasma ChE determination

Plasma ChE was determined by the method of Pongraveevongsa and Ruangyuttikarn[7]. Twenty μL of plasma was pipetted from the heparinized hematocrit capillary tube after centrifugation at 12,000 rpm for 5 minutes. 5 per cent w/v of butyrylthiocholine was used as a substrate in phosphate buffer pH 7.7. The substrate was hydrolyzed to be thiocholine which reacted with 0.25 mM 5, 5-dithiobisnitrobenzoate. A yellow compound of 2-nitro-5-mercaptobenzoate was formed and measured at wavelength 405 nm at room temperature for 30 seconds interval, over a period of 2 minutes. The concentration of ChE was calculated according to Widdop's method[8].

### Data analysis

Data was analyzed by a statistical SPSS software program.

## RESULTS

Fig. 1 shows the distribution of plasma cholinesterase activity in 105 healthy Thai children with a mean ± S.D. at the concentration of 7,417 ± 1,620 U/L. The median (7,130 U/L) was slightly lower than the mean value. The ChE activity of male and female children was not significantly different (data shown in Table 1), even though the mean of female ChE activity appeared to be lower

**Table 1. Plasma cholinesterase activity in healthy Thai children characterized by gender.**

| Children | n | ChE (U/L) Mean ± SD | Maximum value | Minimum value | %CV |
|---|---|---|---|---|---|
| Male | 48 | 7,512 ± 1,643 | 11,690 | 4,850 | 21.87 |
| Female | 57 | 7,335 ± 1,611 | 11,800 | 4,680 | 21.96 |
| Total | 105 | 7,417 ± 1,620 | 11,800 | 4,680 | 21.84 |

ChE = Cholinesterase

**Table 2. Plasma cholinesterase activity in healthy Thai children characterized by parents' occupations.**

| The occupation of the children's parents | n | ChE (U/L) Mean ± SD | Maximum value | Minimum value | %CV |
|---|---|---|---|---|---|
| Employees | 43 | 7,156 ± 1,544 | 11,690 | 4,680 | 21.58 |
| Merchants | 14 | 7,937 ± 1,703 | 11,800 | 4,850 | 21.46 |
| Government officers | 9 | 7,059 ± 1,443 | 9,870 | 5,320 | 20.44 |
| Unemployed parents | 24 | 7,508 ± 1,596 | 10,700 | 5,180 | 21.26 |
| Farmers | 6 | 6,920 ± 1,272 | 8,660 | 5,370 | 18.38 |
| Private business owners | 5 | 7,744 ± 2,031 | 10,620 | 5,720 | 26.23 |
| Unknown | 4 | 8,995 ± 2,254 | 11,750 | 6,790 | 25.06 |
| Total | 105 | 7,417 ± 1,620 | 11,800 | 4,680 | 21.84 |

ChE = Cholinesterase

than that of the male ChE. The ChE activity in children aged 5 was not significantly different from children age 6 (data not shown). The occupations of the children's parents were separated into 7 groups shown in Table 2. There was also no significant difference of ChE activity among the children whose parents had different occupations. However, children whose parents were farmers appeared to have lower ChE activity than the others.

## DISCUSSION

It has been reported that there was no significant difference between plasma and serum ChE activities[9]. Both can be used as an effective indicator of anti-ChE insecticide poisoning. Our results show that average plasma ChE activity in children is higher than adult ChE activity. This supports findings of Genc et al[4] and Jensen et al [5] but not Chan's report[3]. Strauss and Modanlou [6] reported that there were no statistically significant differences in abnormally low enzyme levels, in preterm or term infants, and adults. Table 3 shows a comparison of plasma ChE activity in both children and adults, found by several laboratories.

ChE activities in male and female children are not significantly different but there is a tendency showing that the level of ChE in females is lower than that of males. In addition, we found that the enzyme had a high inter-individual variability in both genders of adults and children.

Accommodating the environment is another factor that influences the enzyme level. Children who live with parents who are farmers appear to have lower ChE activity than children who live with parents with other occupations (result in Table 2). The result corresponds to adult ChE activity[7], in which farmers appear to have lower serum ChE level than monks, traders, soldiers, policemen, the unemployed, civil servants, and students. Richter et al[15] also reported that small and presumably inconsequential in-season reductions in plasma and whole blood ChE activities were seen in field workers and residents exposed to spray drift, in contrast to non-exposed residents.

Even though there is an intra- and inter-individual variability in plasma ChE activity, the ChE in plasma is still very useful for monitoring field workers[14-16], environmental insecticide con-

**Table 3. Comparison of plasma cholinesterase activity in children and adults from previous studies to the present study.**

| References | n | Plasma samples from | ChE (U/L) |
|---|---|---|---|
| The present study | 105 | Children | 7,416 ± 1,620 |
| Pattarawadee & Ruangyuttikarn 1999[7] | 503 | Adult : male | 6,123 ± 1,243 |
| | 65 | : female | 5,421 ± 1,103 |
| Suppaiboonkit & Ruangyuttikarn 1994[10] | 323 | Adult | > 4,900 |
| McConnell et al 1999[11] | 43 | Children | 5,800 |
| Genc et al 1997[4] | 1,967 | Adult : male | 9,180 ± 2,760 |
| | | : female | 8,340 ± 2,390 |
| | | Children : male | 10,750 ± 3,000 |
| | | : female | 10,280 ± 2,960 |
| Chan 1995[3] | 50 | Adult | 5,420 ± 1,500 |
| | | Elderly | 4,480 ± 1,160 |
| | | Infants | 4,460 ± 1,540 |
| Chan 1994[12] | 359 | Adult : male | 5,520 ± 1,500 |
| | 48 | : female | 4,740 ± 1,400 |
| Crook et al 1994[13] | 22 | Adult : male | 6.2 ± 1.8 UI/L |
| | 24 | : female | 6.4 ± 1.5 UI/L |
| Huizenga et al 1985[14] | | Adult : male | 3,110 ± 570 |
| | | : female | 2,500 ± 430 |

ChE = Cholinesterase

tamination[10,17], primary affective disorders[18], attempted suicide, and accidental poisoning[19-22].

In our two cases of victimized children, their ChE activities were 470 and 680 U/L or approximately 6 and 9 per cent of the average value of the normal ChE activity in healthy Thai children. It could be concluded that if the plasma ChE level in children decreases below 10 per cent of the normal level, it would be a lethal indication of anti-ChE insecticide poisoning.

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# พลาสม่าโคลีนเอสเทอเรสในเด็ก และกรณีศึกษาเด็กที่เสียชีวิตเนื่องจากพิษเมทโธมิล

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ได้นำเสนอกรณีศึกษาพ่อแม่ฆาตกรรมลูกของตัวเองด้วยการให้กินสารกำจัดแมลงเมทโธมิล ซึ่งเป็นเหตุเริ่มของการศึกษาระดับเอนไซม์โคลีนเอสเทอเรสในเด็กไทยในงานวิจัยครั้งนี้ที่ยังไม่เคยมีรายงานที่ไหนมาก่อน โดยได้ศึกษากับเด็กไทยสุขภาพดีอายุ 5–6 ขวบ จำนวน 105 ราย พบว่าค่าเฉลี่ย ± ค่าเบี่ยงเบนมาตรฐานของระดับเอนไซม์โคลีนเอสเทอเรสในเด็กไทยคือ 7,417 ± 1,620 U/L และไม่มีความแตกต่างกันระหว่างเพศ หรือระหว่างกลุ่มเด็กที่พ่อแม่มีอาชีพต่างกันอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามพบว่าเด็กผู้หญิงมีแนวโน้มของระดับเอนไซม์โคลีนเอสเทอเรสต่ำกว่าของเด็กผู้ชาย และเด็กที่มีพ่อแม่มีอาชีพเป็นชาวนามีระดับเอนไซม์โคลีนเอสเทอเรสโดยเฉลี่ยต่ำกว่าเด็กที่พ่อแม่มีอาชีพเป็นลูกจ้าง พ่อค้า ข้าราชการ แม่บ้าน หรือนักธุรกิจ เด็กที่ถูกพ่อแม่ฆาตกรรม 2 รายที่นำเสนอในครั้งนี้ ตรวจพบว่ามีระดับเอนไซม์โคลีนเอสเทอเรสประมาณร้อยละ 6 และ 9 ของระดับเอนไซม์เฉลี่ยในเด็กปกติ ดังนั้นสรุปได้ว่าเด็กที่ได้รับสารกำจัดแมลงจนระดับเอนไซม์โคลีนเอสเทอเรสเหลือต่ำกว่าร้อยละ 10 ของระดับปกติ สามารถใช้เป็นค่าบ่งชี้ที่ทำให้เด็กเสียชีวิตได้

**คำสำคัญ** : พลาสม่าโคลีนเอสเทอเรสในเด็ก, พิษเมทโธมิล

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