[Environmental Health and Preventive Medicine 1, 119-127, October, 1996]

Original Article

Effects on Acute Organophosphorus Poisoning in Rats in Aging and Solubility of Organophosphates

Sumie YAMANAKA*[1], Kaoru OHTA*[1], Yumiko TOMITA*[2], Atsushi TAKAYANAGI*[1], Toshio NOMURA*[1] and Yoshinori TAKAESU*[1]

*[1] *Department of Hygiene and* *[2] *Department of Biochemistry, Tokyo Dental College, Chiba*

Abstract

Various organophosphorus compounds with low acute toxicity are predominantly used as insecticides worldwide. Human acute organophosphorous poisoning often occurs as a result of accidental, criminal or suicidal ingestion. We determined the effect of rat age and lipid solubility of organophosphates on acute organophosphorus poisoning.

After trichlorfon with high water solubility was administered to rats, it and its metabolite, dichlorvos, rapidly disappeared from blood, liver, kidneys and fat-tissues, and the ChE activity in the serum, erythrocytes and brain was rapidly normalized. Dichlofenthion disappeared very slowly from poisoned rats due to its fat-solubility. ChE activity was inhibited for a long time by dichlofenthion released from adipose reservoirs in the whole body, especially in 40-week-old rats, and normal and obese rats at 80 weeks of age. Three-week-old rats, which were at a sexually immature developmental stage, showed mild symptoms of dichlofenthion poisoning. By contrast, 7-week-old rats were poisoned most severely with dichlofenthion and their ChE activity was the most severely inhibited among 3-, 7-, 40-and 80-week-old rats. The recovery of ChE activity in rats poisoned with fenitrothion was the most protracted because of the rapid aging of ChE phosphorylated by fenitrothion, although fenitrothion disappeared more rapidly from rat tissues than dichlofenthion.

These findings in rats demonstrated that the pattern of recovery and the degree of symptoms of acute organophosphorus poisoning differed with age and the organophosphate.

Key words: Acute organophosphorus poisoning, Lipid-solubility, Rats in aging, Cholinesterase (ChE) activity.

Introduction

Organophosphorus compounds are the most commonly used agricultural chemicals in the world. The mechanism of the development of their toxicity is relatively well understood [1-4]. In Japan, since various organophosphorus compounds are widely used as anti-epidemic pesticides not only for agricultural but also for domestic use, cases of poisoning have been frequently reported [4].

At present, organophosphorus compounds with low acute toxicity such as fenitrothion (trade name: Sumithion), malathion (Malathon), and trichlorfon (Dipterex) are primarily used instead of those with high acute toxicity such as parathion and tetraethyl pyrophosphate (TEPP). However, much attention to organophosphorus compounds has been paid owing to recent cases of poisoning by nerve gas such as sarin (GA) and a V-agent (VX), which are highly toxic organophosphorus compounds. In the research field, there are such problems to solve the mechanism of the development of organophosphorus ester-induced delayed neuropathy, so that further advances have been made in studies using cultured nerve cells [5, 6].

All organophosphorus compounds have a common action, i. e., inhibition of cholinesterase (ChE) activity, but differ in the rate of *in vivo* metabolism and the effectiveness of 2-pyridine aldoxime methiodide (PAM) as an antidote [7]. Compared with organochlorine pesticides, organophosphorus compounds are rapidly metabolized in general, only slight amounts remaining in the body. However, some organophosphorus compounds are

Reprint requests to:
Sumie Yamanaka,
Department of Hygiene, Tokyo Dental College,
1-2-2 Masago, Mihama-ku, Chiba 261, Japan
Tel: 043-270-3766
Fax: 043-270-3769

highly fat-soluble and remain for a long period in the body, causing persistent toxic symptoms[8-10]. The solubility of organophosphorus compounds markedly differs from relatively water-soulble trichlorfon to negligibly water-soulble dichlofenthion (VC-13). The rates of spontaneous recovery and aging of ChE bound with organophosphorus compounds have also been reported to differ[7]. In cases of poisoning, the course differs considerably among compounds. We previously reported marked differences in the course according to sex and age even in human poisoning by the same compound[4,8].

To clarify the effects of the solubility of organophosphorus compounds and the rat age in acute poisoning, we orally administered 3 organophosphorus compounds that differ in solubility (trichlorfon, fenitrothion and dichlofenthion) gradually from immature rats to aged ones and evaluated each compound in the body and the course of recovery of ChE activity.

Materials and Methods

1. organophosphorus compounds

The three organophosphates used in this experiment are shown in Table 1 with the trade names, chemical names, LD_{50} values per oral in rats and reported water solubilities[11,12].

2. Animals and experimental treatment

Male Wistar rats from Sankyo Labo Service Co. (Tokyo, Japan) were fed solid food (type F-2) purchased from Funabashi-Farms. They were housed in stainless steel cages in a well-ventilated room maintained at 23 ± 2 ℃ on a 12 hour light/12 hour dark cycle, until they reached 3, 7, 40 or 80 weeks of age. Obese aged rats 80-week-old were obtained through abnormally enhanceing eating activity by injuring of the satiety center of the VMH (ventromedial hypothalamus) at 7 weeks of age[13]. Rats in this study were immature rats at 3 weeeks, young rats at 7 weeks, adult rats at 40 weeks, and normal aged and obese aged rats at 80 weeks of age. Their mean weights of body and epididymal fat pads are shown in Table 2. After fasting for 16 hours, they were orally administered half of the LD_{50} value of each organophosphate by stomach syringe i. e., trichlorfon, 310mg/kg; fenitrothion, 170mg/kg; dichlofenthion, 120mg/kg. The animals were killed by ether anesthesia at 90 minutes, 3, 6, 24, and 48 hours, and 4 and 7 days after administration of organophosphates. Their blood, kidney, liver, brain and fat tissues in the perirenal, periepididymal, femoral, scapular and costal areas were obtained at once for analysis.

Table 1 Trade names, chemical names, LD_{50} values and water solubilities of organophosphorus compounds used in this experiment

Organohposphorus compounds	Trade names	Chemical names	LD_{50} value p.o. in rats	Water solubility (ppm)*
Trichlorfon	Dipterex	dimethyl-2, 2, 2-trichloro-1-hydroxyethyl-phosphonate	620mg/kg	154000
Fenitrothion	Sumithion	O, O'-dimethyl O- (3-methyl-4-nitro-phenyl) phosphoro-thioate	340 (1100**)	30.0
Dichlofenthion	VC-13	O, O'-diethyl-O-2, 4-dichloro phenyl-phosphorothioate	240	0.245

*: Water solubility reported at 20-25°C[11,12]
**: LD_{50} value at p.o. in mice

Table 2 Weight of body and epididymal fat pad in rats at various ages and in 80-week-old obese rats

Age	Body weight (g) : A	Epididymal fat pad weight (g) : B	% of B to A*
3-week-old	68 ± 5.6	-------	-------
7-week-old	195 ± 14	1.2 ± 0.1	0.6
40-week-old	530 ± 48	7.9 ± 2.1	1.5
80-week-old	650 ± 68	15.9 ± 4.6	2.4
Obese 80-week-old	710 ± 98	21.5 ± 7.6	3.0

(Mean ± SD of 5 rats)
* : % of epididymal fat pad weight to body weight

Table 3 Gas chromatography conditions for analysis of organophosphorus compounds

Detector	: FTD-NPD
Column	: DB-1, DB-17, 15m × 0.25mm I. D., 0.25 μm film
Carrier gas	: He, 12ml/min
Hydrogen	: 3.5ml/min
Air	: 120ml/min
Injection temp.	: 230°C
Column temp.	: 60-230°C (Program: 20°C/min)
Detector temp.	: 240°C
Purge time	: 0.5min

3. Determination of organophosphorus levels in tissues of rats

Blood was added to 4 volumes of 0.1N HCl solution, and brain, liver, kidney and fat tissues were homogenized with 0.1N HCl solution. Organophosphate in rat tissues was extracted in the ethyl acetic acid phase, and one μl of ethyl acetic acid was prepared for analysis by gas-liquid chromatography (GLC)[14]. The gas-liquid chromatographer used was a Hewlett-Packard Model 5890 equipped with a simplified splitter model for the capillary column. The conditions for GLC analysis of organophosphates are shown inTable 3.

4. Assay of ChE activity in tissues of rats

ChE activity in plasma and erythrocytes of rats was determined by the DTNB colour method of Voss et al[15]. Brain and liver were homogenized with 10 and 5 volumes of saline solution, respectively; then the ChE activity in these tissues was measured by the DTNB method. Iodide butyryl thiocholine and iodide acetyl thiocholine purchased from Sigma Co. were used as the substrates for analysis of ChE activity in plasma and liver, and in erythrocytes and brain, respectively.

5. Statistical analysis

Statistical significance between mean values was assessed by Student's t-test or the Scheffe method.

Results

1. Differences in the disappearance patterns and ChE activities of organophosphorus compounds

Figure 1 shows the disappearance patterns of trichlorfon, fenitrothion, and dichlofenthion in the rat blood, kidneys, and fat tissue. In the trichlorfon-administration group, trichlorfon and its metabolite (dichlorvos; DDVP) were detected, but their disappearance from the body was rapid. In the dichlofenthion-administration group, disappearance from the body, especially

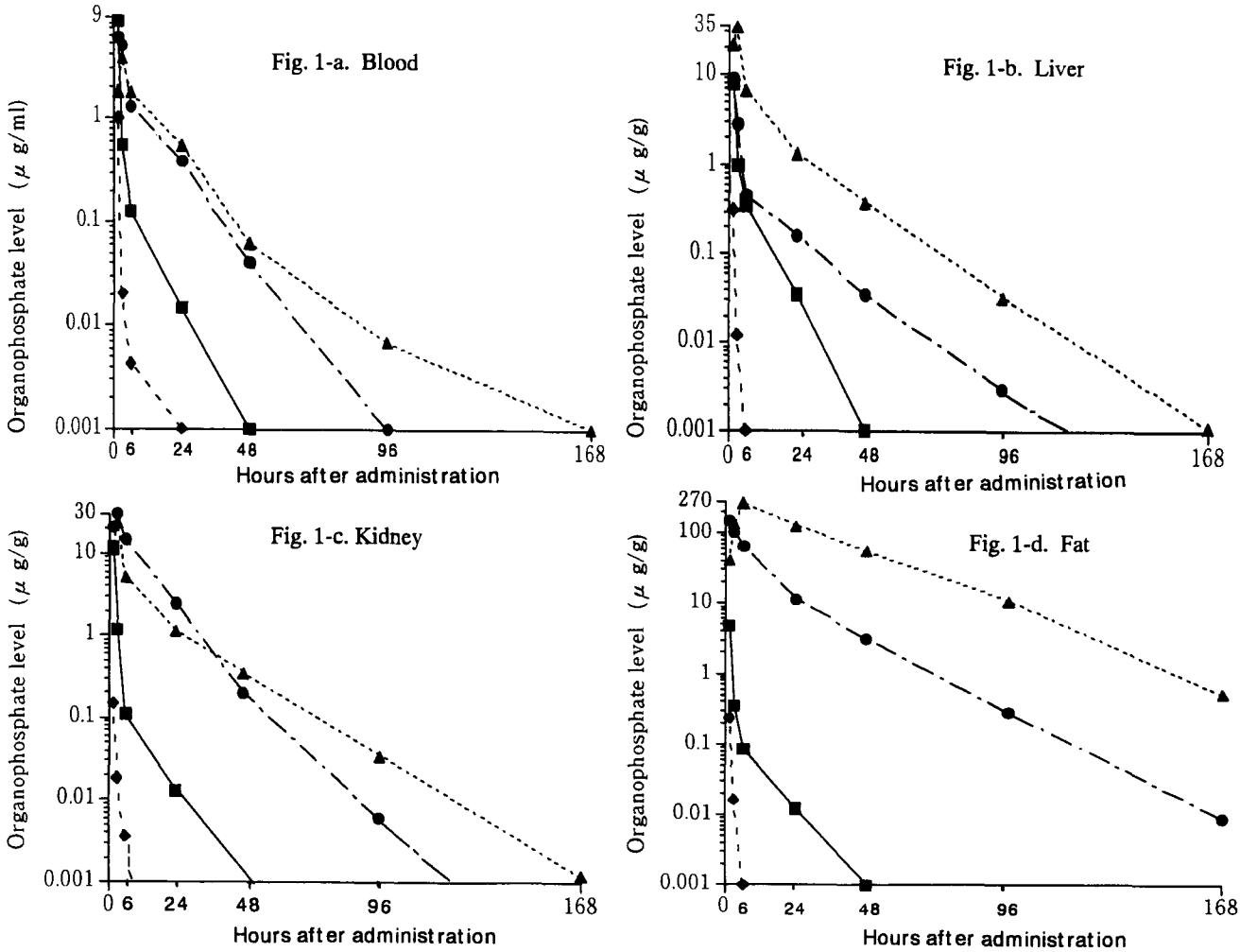


Fig. 1. Disappearance patterns of organophosphates in tissues of poisoned rats 7-week-old (means of 5 rats)

from the fat tissue, was very slow, showing transfer to the fat tissue. The fenitrothion-administration group an intermediate disappearance pattern between the trichlorfon-and dichlofenthion-administration groups.

The biological half-life was calculated by obtaining a regression line by the least squares method (Table 4). The biological half-lives of dichlorvos and trichlorfon were short in the trichlorfon-administration group. The biological half-life of fenitrothion was long (about 10 hours), and that of dichlofenthion was longest in the fat tissue.

Changes in ChE activity in the serum, erythrocytes, and the brain after administration of these organophoshporus compounds are shown in Fig. 2. In the trichlorfon group showing rapid disappearance from the body, ChE activity recovered rapidly. In the dichlofenthion group, recovery of ChE activity was slow. The fenitrothion group showed even slower recovery of ChE activity than the dichlofenthion group.

Table 4 Biological half-lives of organophoshpates in tissues of rats poisoned with three organophosphates

(unit: hrs)

Tissues	Trichlorfon	Dichlorvos*	Fenitrothion	Dichlofenthion
Blood	3.18	0.763	7.79	10.6
Liver	3.55	0.318	9.36	11.8
Kidney	2.85	0.881	7.72	12.5
Fat	2.46	0.387	10.7	21.7

*: Dichlorvos detected in rats administered trichlorfon

2. Effects of age and obesity in dichlofenthion poisoning

Prior to evaluation of the effects of the organophosphorus compounds according to age in weeks and the presence or absence of obesity, the serum and brain ChE activities in the normal state were compared among immature (3 weeks of age), young (7 weeks), mature (40 weeks), aged (80 weeks), and aged obese (80 weeks) rats (Fig. 3). The brain ChE activity did not differ among the age groups or between the aged groups with and without obesity. The serum ChE activity differed among the age groups and was significantly increased in the obese group. Dichlofenthion (120mg/kg) was administered to these rats. Severe poisoning was observed in the immature and young groups, and 4 of the 40 young rats died. Changes in the serum and brain ChE activities in these rats are shown in Figs. 4 and 5. In the immature and young groups, the maximum inhibition of the serum and brain ChE activities was observed 6 hours after administration, but subsequent recovery was rapid. In the mature and aged groups, the maximum inhibition was observed after 48 hours, and subsequent recovery was slow. The obese aged group showed more marked inhibition of the serum ChE activity than the aged group without obesity during the 48-hour observation period.

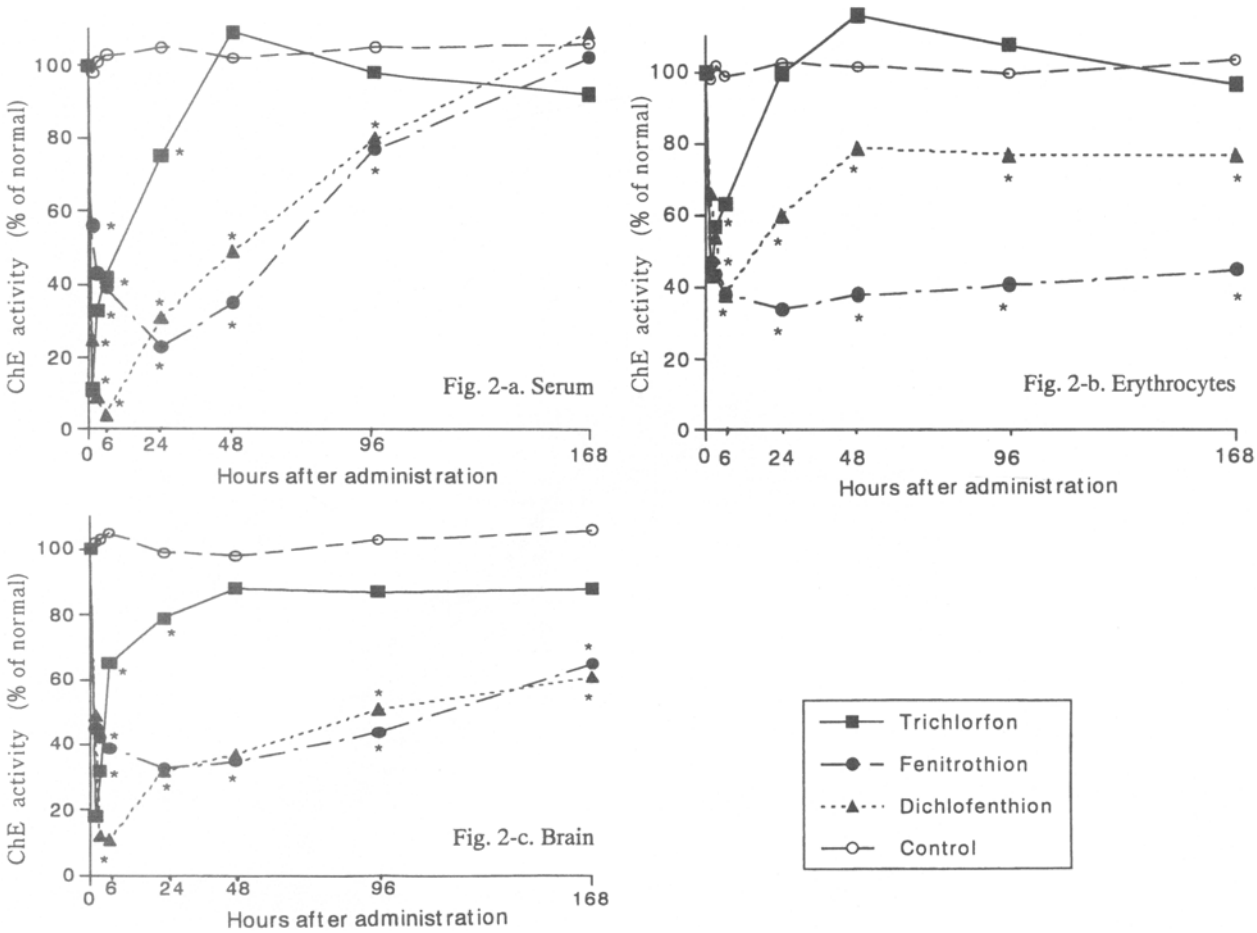


Fig. 2. Changes of ChE activity in tissues of rats 7-week- old after administration of organophosphates (* ; significant difference from control at $p < 0.05$) (means of 5 rats)

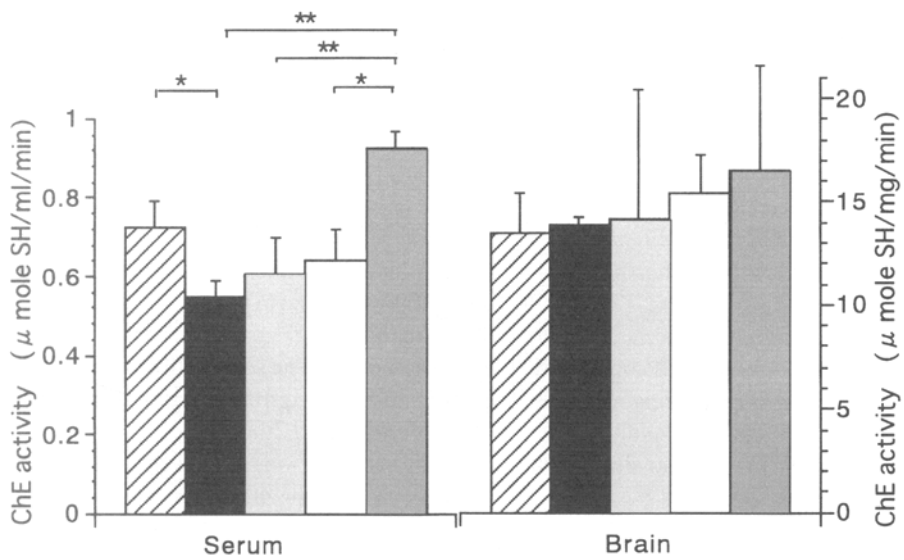


Fig. 3. Comparison of ChE activity in serum and brain of normal rat at various ages (mean ± SD of 7 rats)
(* and ** : significant difference at $p < 0.05$ and 0.01)

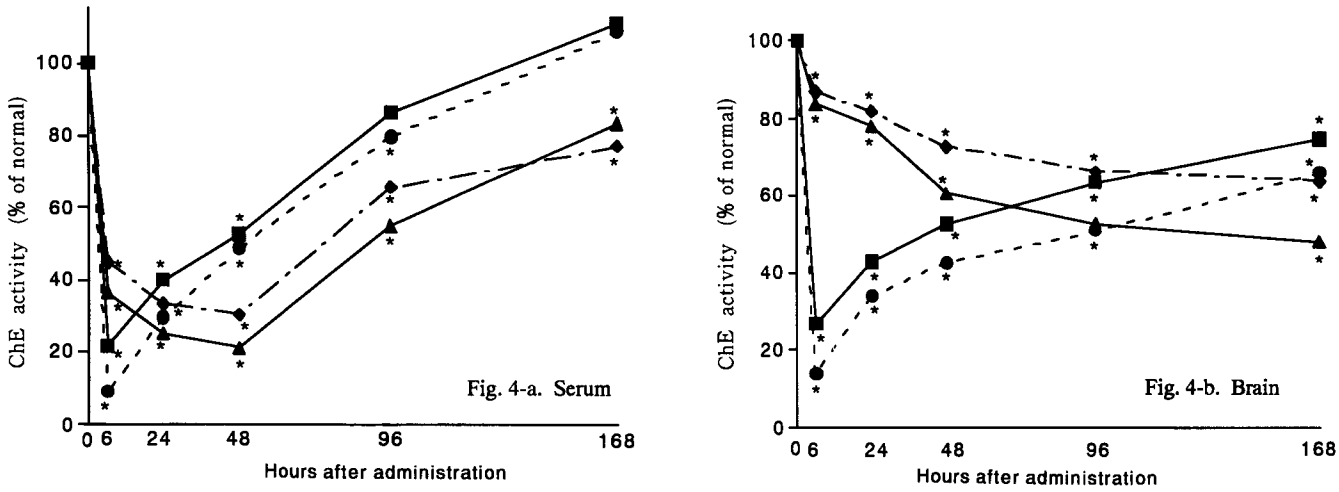
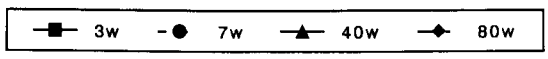


Fig. 4. Changes of ChE activity in tissues of rats at various ages after poisoning with dichlofenthion
(* : significant difference from normal rats of same age at $p<0.05$) (means of 5 rats)



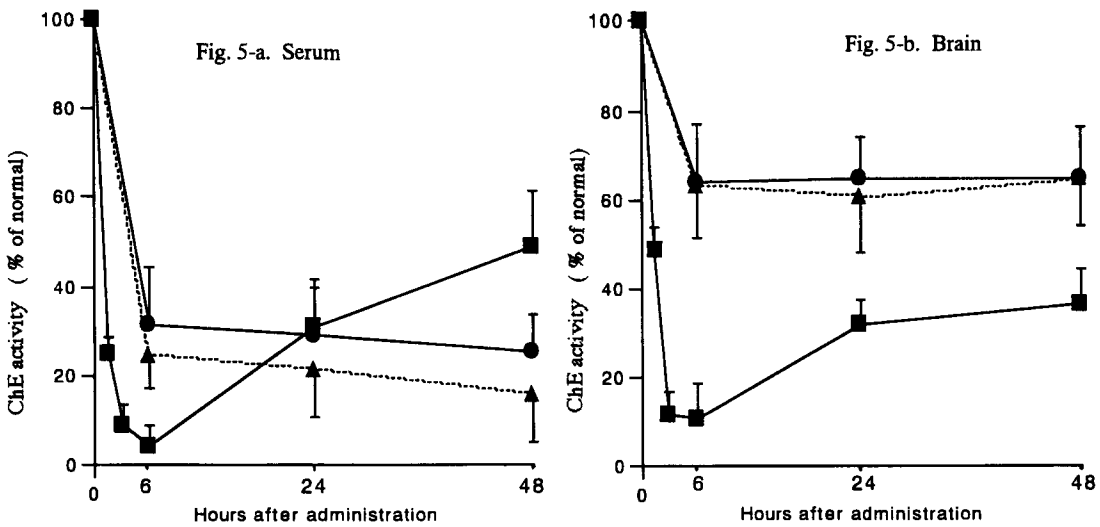
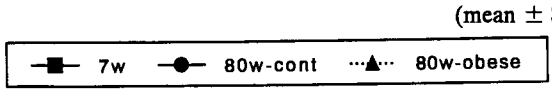


Fig. 5. Changes of ChE activity in serum and brain of rats poisoned with dichlofenthion
(mean ± SD of 5 rats)



Changes in the dichlofenthion concentration in the tissues after administration are shown in Figs. 6 and 7. In the immature and young groups, the concentrations in the serum and fat tissue reached a peak within 6 hours after administration, and subsequent disappearance was rapid. In the mature and aged groups, the peak concentration was observed later, and disappearance was slow, especially in the fat tissue. The obese aged group showed slightly higher dichlofenthion concentrations in the serum, liver,

Table 5 Biological half-lives of dichlofenthion in serum and fat of rats at various ages

(unit: hrs)

Tissues	3-week-old	7-week-old	40-week-old	80-week-old
Serum	11.9	11.4	9.6	10.1
Fat	19.6	61.4	61.4	84.9

Table 6 Comparison of dichlofenthion levels in fatty tissues at various sites of obese rats 80-week-old 6 hrs after administration of dichlofenthion

Site	Dichlofenthion level (μ g/g)*
Kidney	36.4 ± 18.4
Epididymal	21.4 ± 5.49
Femoral	32.7 ± 24.7
Shoulder	18.5 ± 14.5
Thoracic	36.2 ± 13.0

*: mean ± SD of 5 rats

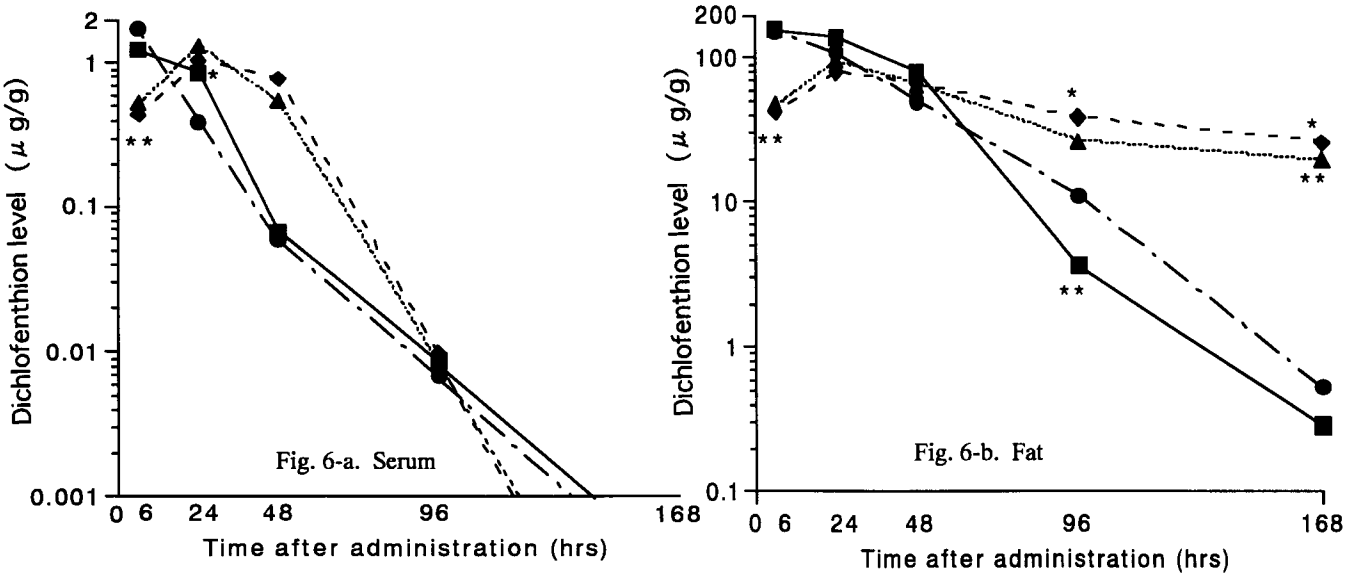
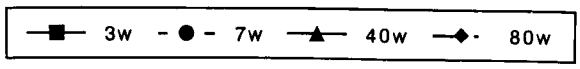


Fig. 6. Disappearance patterns of dichlofenthion in tissues of poisoned rats at various ages
(*, ** : significant difference from 7-week-old rats at $p<0.05$, 0.01) (means of 5 rats)



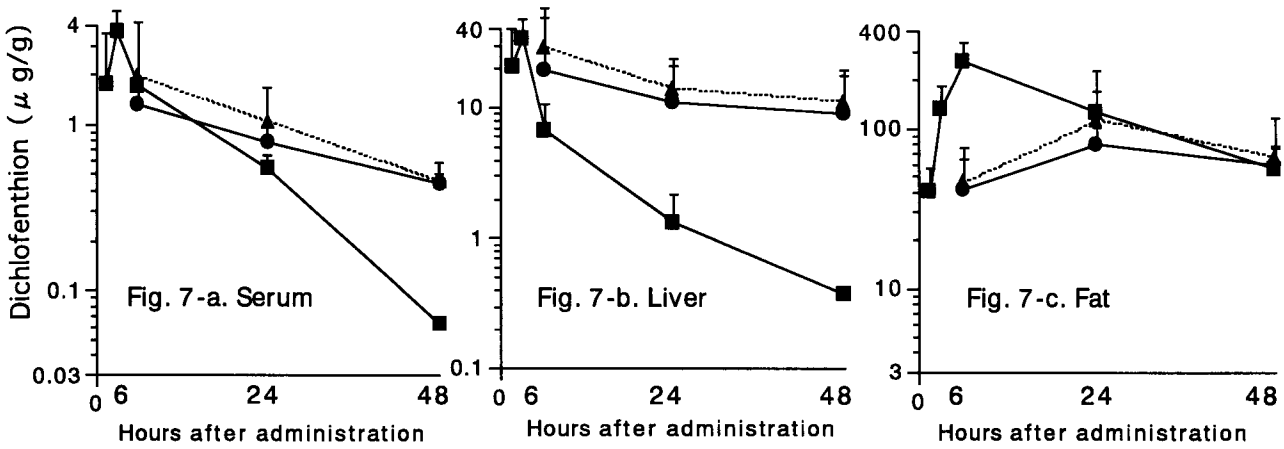
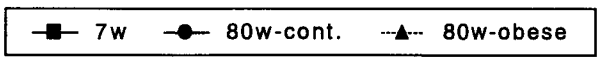


Fig. 7. Disappearance patterns of dichlofenthion in tissues of poisoned rats (mean ± SD of 4 rats)



and fat tissue than the aged group without obesity.

The biological half-life calculated from the concentration in the serum and fat tissue in each age group is shown in Table 5. The half-life of dichlofenthion in the serum did not differ, but that in the fat tissue markedly differed among the age groups. Table 6 shows dichlofenthion distribution in the fat tissues in the entire body. The dichlofenthion concentration did not significantly differ among the fat tissues in the perirenal, periepididymal, femoral, scapular, and costal areas.

Discussion

1. Retention in the body according to compound solubility

The solubility markedly differed in the organophosphorus compounds used in this study. The difference in water solubility between trichlorfon and dichlofenthion was 6×10^5 (Table 1). Therefore, the amounts of the compounds remaining in the body were compared based on changes in the concentrations in the rat serum, liver, kidneys, and fat tissue (Fig. 1).

Highly water-soluble trichlorfon is rapidly converted into dichlorvos *in vivo* even in a relatively weak alkaline state and is activated by potent ChE inhibitors, causing toxic symptoms [12]. In the trichlorfon-administration group, both trichlorfon and dichlorvos were measured. Trichlorfon was rapidly converted into dichlorvos in the rat body. Both trichlorfon and dichlorvos were detected in the blood, liver, kidneys, and fat tissue, but they rapidly disappeared after administration due to high water solubility (trichlorfon, 15.4%; dichlorvos, 1.0%), decreasing below

the detection limit (0.001 μg/g) within 48 hours. On the other hand, dichlofenthion, which was the most fat-soluble (water solubility, 0.245 ppm), disappeared very slowly from the body. In particular, the dichlofenthion concentration in the fat tissue reached a peak (264 ppm) 6 hours after administration and was 0.524 ppm even after 7 days, showing transfer to the fat tissue. The water solubility of fenitrothion (30.0 ppm) was between that of trichlorfon and that of dichlofenthion, and its disappearance pattern in the rat body was also intermediate between the two compounds.

The biological half-life (Table 4) of dichlorvos in the trichlorfon group was within 1 hour in each tissue. The biological half-life of trichlorfon itself was also short and was similar among the tissues (about 4 hours). On the other hand, highly fat-soluble fenitrothion and dichlofenthion showed longer biological half-lives. The half-life of dichlofenthion in the fat tissue was the longest (21 hours), showing retention in the fat tissue. The ratio of the concentration in the fat tissue to that in the blood (fat/blood ratio) was 0.57 for trichlorfon but 26.2 for fenitrothion and 141 for dichlofenthion, confirming transfer of dichlofenthion to the fat tissue.

These results suggest that the retention of organophosphorus compounds is closely associated with water solubility or fat solubility. With higher fat solubility, disappearance from the body requires more time due to transfer to the fat tissue.

Chiou et al. [11] compared water solubilities of numerous chemical substances and reported a marked negative correlation between the biological concentration coefficient and water solubility of the compound in aquatic organisms. In mammals also, the solubility of chemical compounds appears to be related to transfer to the fat tissue. The water solubilities [11] of trichlorfon and dichlorvos are relatively high (15.4% and 1.0%, respectively). On the other hand, the water solubility of the highly fat-soluble organophosphate (leptphos) is 0.0047 ppm, which is even higher than that of p, p'-DDT (0.031ppm) or 4, 4'-PCB (0.0636 ppm), organochlorine compounds. Among other organophosphorus compounds, the water solubility is 24.0 ppm for parathion, 55.0 ppm for fenthion (Baycid), and 145 ppm for malathion. These values are close to the water solubility of fenitrothion (30.0 ppm), suggesting similar retention in the body.

Compared with organochlorine compounds, organophoshphorus compounds are generally considered to be rapidly metabolized, with little remaining in the body. However, in this study, the solubility differed among the organophosphorus compounds, and transfer to the fat tissue was marked for highly fat-soluble compounds. Therefore, the retention of organophosphorus compounds should be evaluated in terms of the solubility of each compound.

2. Differences in the recovery pattern of ChE activity among compounds

The recovery patterns of ChE activity in rat serum, erythrocytes, and the brain after administration differed among the organophosphorus compounds (Fig. 2). In the trichlorfon group showing rapid disappearance from the body, ChE activity was markedly inhibited 90 minutes after administration but recovered rapidly, being in the normal range after 48 hours. On the other hand, in the group that received dichlofenthion, which is highly fat-soluble and markedly remains in the body, the peak inhibition of ChE activity was observed 6 hours after administration, and ChE activity recovered to normal in the serum but not in the erythrocytes and the brain even after 7 days. This may be because dichlofenthion retained at a high concentration in the fat tissue was gradually transferred to the blood, and disappearance from the blood was prolonged. In addition, this small amount of dichlofenthion may have caused delay of recovery of ChE activity. In the fenitrothion group, the peak inhibition of ChE activity was observed after 24 hours, and subsequent recovery was slow, especially in the erythrocytes and brain. Though fenitrothion is less fat-soluble than dichlofenthion and more rapidly disappeared from the body, recovery of ChE activity was slow. This may have been associated with differences in the aging rate of phosphorylated ChE [7]. ChE phosphorylated by oxon-type fenitrothion shows rapid aging and does not recover spontaneously. Therefore, the enzyme activity may remain low until new ChE is produced. In fenitrothion poisoning, PAM, an antidote, generally has no marked effects [16]. This may also associated with this aging rate. Therefore, in fenitrothion poisoning, rapid aging of phosphorylated ChE together with relatively slow disappearance of fenitrothion from the body might have resulted in slow recovery of ChE activity in the brain and erythrocytes.

These results suggested that the recovery course of ChE activity inhibited by organophosphorus compounds was markedly affected by retention of organophosphorus compounds and the aging rate of phosphorylated ChE.

3. Effects of rat age in weeks on dichlofenthion poisoning

Dichlofenthion, which was the most fat-soluble, was administered to immature (3 weeks of age), young (7 weeks), mature (40 weeks), and aged (80 weeks) rats, and its disappearance from the serum and fat tissue was compared (Fig. 6). The immature and young groups showed similar courses with a peak concentration 6 hours after administration. In the mature and aged groups, the peak concentration was observed after 24 hours, and subsequent disappearance was slow. In particular, in the fat tissue of the mature and aged rats, the remaining concentration was 20 ppm even after 7 days, suggesting subsequent gradual transfer to the blood. Based on these changes, the biological half-life of dichlofenthion was compared among the age groups (Table 5). The half-life of dichlofenthion did not differ in the serum but markedly differed in the fat tissue among the age groups. The half-life in the fat tissue was 19.6 hours in the immature group but 84.9 hours (3.5 days) in the aged group, indicating marked retention of the compound in the fat tissue with aging.

The recovery course of ChE activity after dichlofenthion administration also differed among the age groups (Fig. 4). In the immature and young groups, the peak inhibition of serum ChE activity was observed 6 hours after administration, followed by rapid recovery. In the mature and aged groups, the peak inhibition was observed after 48 hours. The brain ChE activity was most markedly inhibited after 6 hours and gradually recovered in the immature and young groups. In the mature and aged groups, the inhibition rate was low, but no recovery was observed even after 7 days. These results suggest that dichlofenthion is markedly distributed in the fat tissue in mature and aged rats, and that, therefore, inhibition of the serum and brain ChE activity is slight, causing slight toxic symptoms. However, gradual transfer of dichlofenthion from the fat tissue may have resulted in slow disappearance from the body tissue and slow recovery of ChE activity. In the immature and young rats, toxic symptoms developed

early and were severe compared with mature and aged rats, which reflected the course of ChE activity.

As shown in Fig. 3, in normal rats that did not receive organophosphorus compounds, the brain ChE activity did not significantly differ, but the serum ChE activity differed among the age groups. The serum ChE activity was significantly high in the rats aged 3 weeks, lowest in the rats aged 7 weeks and increased again with age.

Serum ChE activity differs according to animal species, sex, and age. In humans, serum ChE activity is lower in females than in males and changes with the sexual cycle[17]. We have observed higher serum ChE activity in mice and humans than in dogs and rats and in female rats and mice than in male rats and mice. Rat serum ChE activity does not differ between males and females until the age of 5 weeks but significantly increases thereafter in females[18, 19]. Thus, the liver-derived ChE activity in the serum differs among species and between males and females. The high serum ChE activity in the immaure rats in this study may be associated with this sex difference. Male rats aged 3 weeks are sexually immature and have serum ChE activity similar to that in females. This may have caused a higher serum ChE activity in the immature males than in the other age groups. At the age of 7 weeks, sex differentiation is advanced, which may be associated with the low serum ChE activity in the males in this age group. Subsequent age-related changes may have gradually increased serum ChE activity.

Cytochrome p-450 in liver is closely involved in the development of toxicity and detoxication of organophosphorus compounds. Maeda et al.[20] and Kamataki[21] reported differences of sex-spcific forms of cytochrome P-450 between males and females, among species, and among strains. In the amount of sex-specific forms of cytochrome P-450, P-450 female is predominant in immature rats of the both sexes until 25 days after birth, but P-450 male becomes predominant in only male rats with sexual maturation[21].

These differences according to sex and week-age in serum ChE activity or cytochrome P-450 seemed to have caused differences in the acute toxicity of organophosphorus compounds in rats. In dichlofenthion poisoning in this study, acute toxicity symptoms were the most marked, and the death rate was high in the young rats (aged 7 weeks).

Comparison of the acute toxicity of organophosphorus compounds in humans is difficult because of the differences in the amounts of the compounds and treatments. However, we previously reported a higher death rate in males than in females with poisoning and slow recovery in aged patients[8]. These findings in humans may also be associated with differences in liver cytochrome P-450 and ChE activites according to age and sex.

4. Effects of obesity in dichlofenthion poisoning

Since highly fat-soluble dichlofenthion is markedly transferred to fat tissue, we observed the course of acute poisoning in obese rats with a large amount of fat tissue in the body. Dichlofenthion (120 mg/kg)was orally administered to aged rats with obesity resulting from abnormal enhancement of eating activity by injuring the satiety center of the ventromedial nucleus of the hypothalamus[13], and the course until 48 hours was compared with that in the other age groups (Fig. 5). Compared with the young group, the aged group and obese aged group showed slight toxic symptoms, slight inhibition of serum and brain ChE activities, and a slow course. The course was similar in the aged groups with and without obesity.

In humans with fatty liver, ChE production in the liver is enhanced, resulting in an increase in serum ChE activity[17]. In the obese rats in this study, autopsy showed marked fatty liver compared with the aged rats without obesity, and the serum ChE activity was 1.4-fold higher (Fig. 3). In obese rats, the serum ChE activity may be high originally due to fatty liver. Therefore, even when inhibition of serum ChE activity by dichlofenthion is more marked in obese aged rats than in aged rats without obesity, remaining ChE activity may be similar. This may explain the similar toxic symptoms in the aged groups with and without obesity.

Dichlofenthion disappearance from the serum, liver, and fat tissue was rapid in the young group but slow in the aged groups with or without obesity (Fig. 7), which was associated with slow recovery in ChE activity. In the aged groups with or without obesity, the peak concentration in the fat tissue was observed after 24 hours, and subsequent disappearance was delayed. However, differences between the aged groups with and without obesity were slight. In the rats aged 80 weeks, fat tissue such as that around the epididymis was increased compared with rats aged 7 weeks or 40 weeks (Table 2) and was close to that in the obese aged rats. The distribution of dichlofenthion in the fat tissues in the entire body was evaluated (Table 6). No significant differences were observed in the dichlofenthion concentration among the fat tissues in the perirenal, periepididymal, scapular, femoral, and costal areas. Highly fat-soluble dichlofenthion appeared to be widely distributed in the entire body in the obese rats.

The aged or obese rats with a large amount of fat tissue showed less marked inhibition in the serum and brain ChE activities and milder toxic symptoms than the young rats, probably due to the marked distribution of dichlofenthion in the fat tissues in the entire body. However, re-exposure to dichlofenthion, which was gradually transferred from the fat tissues in the entire body, appeared to have caused the delay in dichlofenthion disappearance from the body and recovery of ChE activity in the aged or obese rats.

These findings in rats demonstrate that the pattern of recovery and the degree of symptoms of acute organophosphorus poisoning differ with age and the organophosphate.

References

1) Levine RS, Doull J. Global estimates of acute pesticide morbidity and mortality. Rev Environ Contam Toxicol 1992; **129**: 29-50.
2) Hayes WJ. Pesticides Studies in Man. Baltimore, Maryland: Williams and Wilkins Company, 1982: 301-453.
3) Naito H. Poisoning of Industrial Products, Gases, Pesticides, Drugs and Natural Toxins–Cases, Pathogenesis and its Treatment. Tokyo: Nankoudou, 1991: 142-54.
4) Yamanaka S, Yoshida M, Yamamura Y, Nishimura M, Takaesu Y. A study on acute organophosphorus poisoning–Changes in the activity and isoenzyme patterns of serum cholinesterase in human poisoning. Jpn J Hyg 1993; **48**: 955-65.
5) Funk KA, Liu CH, Wilson BW, Higgins RJ. Avian embryonic brain reaggregate culture system, 1. Characterization for organophosphorus compound toxicity studies. Toxicol Appl Pharmacol 1994; **124**: 149-58.
6) Funk KA, Liu CH, Wilson BW, Higgins RJ. Avian embryonic brain

reaggregate culture system, 2. NTE activity discriminates between effects of a single neuropathic or nonneuropathic organophosphorus compound exposure. Toxicol Appl Pharmacol 1994; **124**: 159-63.
7) Yamanaka S. Problems and effects of therapeutic drugs against acute organophosphorus poisoning. Jpn J Toxicol 1988; **1**: 39-46.
8) Yamanaka S, Nishimura M, Yoshida M, Yamamura Y, Yamamoto Y. Differences of the post-poisoning course in acute organophosphorus toxicity. Jpn J Hyg 1989; **44**: 116.
9) Davies JE, Barquet A, Freed VH, Haque R, Morgade C, Sonneborn RE, Vaclavec C. Human pesticide poisoning by a fat-soluble organophosphate insecticide. Arch Environ Health 1975; **30**: 608-13.
10) Yoshida M, Shimada E, Yamanaka S, Aoyama H, Yamamura Y, Owada S. A case of acute poisoning with fenitrothion (Sumithion). Human Toxicol 1987; **6**: 403-6.
11) Chiou CT, Freed VH, Schmedding DW, Kohnert RL. Partition coefficient and bioaccumulation of selected organic chemicals. Environ Sci Technol 1977; **11**: 475-8.
12) Kudamatsu A. Physio-chemical property, action mechanism and metabolism of Dipterex. Noyaku Kenkyu 1969; **16**: 26-34.
13) Hales CN, Kennedy GC. Plasma glucose, fatty acid and insulin concentrations hypothalamic-hyperphagic rats. Biochem J 1964; **90**: 620-4.
14) Yamanaka S. Determination of organophosphorus compounds in blood by gaschromatography for diagnosis of acute poisoning. J Clin and Experi Medicine 1990; **155**: 120.
15) Voss G, Sachsse K. Red cell and plasma cholinesterase activity in microsamples of human and animal blood determined simultaneously by a modified acetylcholine/DTNB procedure. Toxicol Appl Pharmacol 1970; **16**: 764-72.
16) Yamanaka S, Ichioka K, Nishimura M. Effects of organophosphorus compounds and the efficacy of PAM on rat serum cholinesterase activity and isoenzyme. Jpn J Hyg 1981; **36**: 773-82.
17) Shibata H, Fujioka T. Enzyme, cholinesterase. Clinic Allround 1991; **40**: 1474-8.
18) Kondo T, Yoshida M, Kasahara K. Acute florosis in female rats, time course of inhibition and recovery of cholinesterase in serum and salivary glands. J Dental Health 1976; **26**: 187-92.
19) Schmidt E, Schmidt FW. Sex difference of plasma cholinesterase in the rat. Enzyme 1978; **23**: 52-5.
20) Maeda K, Kamataki T, Nagai T, Kato R. Postnatal development of constitutive forms of cytochrome P-450 in liver microsomes of male and female rats. Biochem Pharmacol 1984; **33**: 509-12.
21) Kamataki T. Differences of cytoshrome P-450 by sex, colonies and species. Charles Rivers Letter 1989; Vol. 2, No. 2: 1-7.

(Received Nov. 6, 1995/Accepted Jun. 5, 1996)