1. Introduction

Dichlorvos (2, 2-dichlorovinyl phosphate, DDVP) is an organophosphorus insecticide widely used all over the world. Since its commercial introduction in 1961, DDVP has enjoyed extensive use in many countries and has produced important benefits by controlling internal and external parasites in livestock and domestic animals and by controlling insects in houses and fields (ATSDR, 1997). Its annual worldwide sales in 2003 were about 40 million US dollars (Phillips McDougall, 2004). However, the International Agency for Research on Cancer (IARC) regarded DDVP as a possible carcinogen for humans (Group 2B) in 1991, and the U.S. Environmental Protection Agency (EPA) also considered it a probable carcinogen (Group B2) in 1995. Subsequently, in 1995, the U.S. EPA proposed cancellation of DDVP for stored agricultural commodity uses, all home uses, and for many commercial, institutional and industrial area uses; it also proposed strict regulation of retained uses such as application to livestock, animal premises and greenhouses (ATSDR, 1997). In the United Kingdom, the Department for Environment, Food and Rural Affairs (DEFRA) has suspended the sale of a range of products containing DDVP in 2002 because of the carcinogenicity concern (DEFRA, 2002).

From the viewpoint of setting regulations based on the toxicities other than carcinogenicity, the inhibition of acetylcholinesterase is considered to be observable at lower doses than other toxic effects. The reproductive toxicity is not taken into account (ATSDR, 1997; Hummel, 2000). However, in our previous study on the semen indices of indoor pesticide sprayers who often used fenitrothion, DDVP, chlorpyrifos and permethrin, the percentage of slow progressive or non-progressive motile sperm in their busy summer was significantly higher in the sprayers than in the controls (Kamijima et al., 2004). Among the above pesticides, only DDVP was suggested to have any debilitating effects on the male reproductive system in some animal experiments (Krause and Homola, 1974; Krause et al., 1976; Akbarsha et al., 2000), whereas conflicting study results had also been reported (Krause, 1977; NTP, 1989; Dikshith et al., 1976). Since public concern still remains about the possible male reproductive toxicity of DDVP, its comprehensive evaluation is needed, particularly about testicular histopathology and sperm motility.

The present study aims to provide information on the relationship between exposure markers of DDVP and indices of the male reproductive system in the rat under different dose levels. The extrapolation of the present rodent study results to human exposure settings could give a more precise estimate of the risk of testicular toxicity due to DDVP exposure alone, and could provide an answer to the public and occupational health concern.

2. Materials and methods

2.1. Chemicals

DDVP (at least 99.0% pure) was purchased from Kanto Chemical Corporation, Japan. Hanks's solution was obtained from Invitrogen, CA. All staining solutions for testicular histopathology and sperm morphology were purchased from Wako, Japan.

2.2. Animals and treatment

A total of 34 male, 9-week-old Wistar rats were purchased from Shizuoka Laboratory Animal Center, Japan. They were housed and acclimatized to new surroundings for a week under a controlled temperature of 23-25 oC and relative humidity at 57-60%, and then randomly divided into 4 groups (n=8 or 9). Food and water were provided ad libitum.

The rats were subcutaneously injected in the back of the neck with 0, 1, 2, or 4 mg/kg of DDVP which was dissolved in saline. Administration volume was 3 ml/kg. By subcutaneous injection, the absorbed chemical enters the systemic circulation directly, not via the portal system, as in inhalation and dermal exposure. The maximum dose was set to be 4 mg/kg because some rats died during our 9-week pilot study by the daily subcutaneous treatment with 5 mg/kg DDVP, which was beforehand considered tolerable based on the acute oral LD50 (80 mg/kg) (Hummel, 2000) and on the oral dose (10 mg/kg) tolerable for 48 days (Akbarsha et al., 2000). One more rat was assigned for each of the 2 and 4 mg/kg groups in animals died in the higher-dosed groups. The above treatments were performed 6 days a week for 9 weeks to cover the entire process of spermatogenesis. On the following day after the last injection, rats were weighed, and sacrificed by collecting the blood into heparinised tubes through the abdominal aorta under pentobarbital anesthesia. The epididymis, testis, prostate, seminal vesicle, liver, kidney and adrenal gland were dissected out and weighed. The present study experiments conformed to Japanese law concerning the protection and control of animals and the Guide to Animal Experimentation of Nagoya University School of Medicine.

2.3. Sperm motility analysis

Sperm were collected as quickly as possible after a rat was sacrificed. The cauda epididymidis was cut by knife to release sperm in 2 ml of Hanks's solution at 37 oC. Sperm in the cauda epididymidis were manually counted under microscopic observation on a warming plate (Tokai Hit Co., Japan), and sperm motility was categorized into "motile" or "non-motile."

2.4. Sperm morphology analysis

The sperm solution was smeared on glass slides, fixed in methanol and dried. The slides were stained later according to Bryan's method (1970). A total of 200 intact sperm were examined for morphological abnormality under the microscope. Abnormal heads were classified as straight, banana-shaped, and other unclassified abnormalities according to the method of Mori et al. (1991).

2.5. Epididymal sperm count

Residual cauda epididymidis were minced with scissors and filtered through gauze. Filtered samples were diluted with saline containing 0.5% formalin. This solution was infused into a Neubauer-type hemocytometer (Erma, Japan) for microscopic observation. The data were expressed as the total number of sperm per cauda epididymal tissue weight. Every sample was evaluated without information about its treatment.

2.6. Histopathological examinations

The right testes were fixed in Bouin's solution and embedded in glycol methacrylate (GMA) (Technovit 7100, Heraeus Kulzer Co., Germany). Tissue sections of the testis were stained with

periodic acid Schiff's reagent (PAS) and hematoxylin. The histopathological examination was carried out to evaluate possible changes in the seminiferous tubule level (e.g., atrophy), seminiferous epithelium level (e.g., disorganization, depletion), germ cell level (e.g., degeneration, retention, vacuolation), and Leydig cell level (e.g., degeneration, vacuolation). Three categories of stages (stages I-VI, stages VII-VIII, stages IX-XIV) were all evaluated in every rat without information about its treatment.

2.7. Round spermatid cell counts

Spermatogenic cells at stage VII were counted to evaluate the cellularity of the seminiferous epithelium. Three sections of stage VII in each rat were randomly photographed (Fujix Digital Camera HC-300Z/CL, Olympus Japan Co.). Only round spermatids and Sertoli cells were counted, since alterations in spermatogonia and spermatocytes also result in change in the round spermatids after treatment for 9 weeks.

2.8. Cholinesterase (ChE) activity

Blood samples were immediately centrifuged after their collection and maintained at –4 oC. Plasma and red cell ChE activities were then measured with the modified Voss and Sachsse method (1970) within the same day.

2.9. Testosterone assay

Plasma testosterone levels were measured with radioimmunoassay (RIA) by Mitsubishi Chemical BCL Co., Japan. Plasma samples were stored at –80 oC until the analyses.

2.10. Urinary dimethyl phosphate (DMP) concentration

Urinary DMP concentrations were measured by gas chromatography/mass spectrometry equipped with electron ionization (GC/MS-EI), PerkinElmer TurboMass Systems (Wellesley, MA) with the modification of a previously-described method (Hardt and Angerer, 2000). Standard curves for measuring DMP in the urine proved to be linear for concentrations ranging from 0.01 to 50 mg/l with a correlation coefficient of 0.999. The intra- and inter-assay coefficients of variation for the GC/MS assay were less than 15% at concentrations of 0.05 mg/l. The detection limit of DMP was 0.3 μg/l.

2.11. Statistical analysis

Dunnett's multiple comparisons were made between the exposure groups and the control following one-way analysis of variance (ANOVA), when the values were considered to be distributed normally. For the DMP concentration, the Kruskal-Wallis test was used to detect differences between the groups, and Steel's test was used for multiple comparison of the treated groups and the control group. Linear regression analysis was performed for sperm motility vs. blood ChE activities or urinary DMP concentration. The DMP value was transformed by root conversion because the standard variation increased in proportion to the mean value. A p-value less than 0.05 was considered statistically significant. JMP ver. 4 (SAS Institute Inc.) was used to analyze the data.

3. Results

3.1. Animals

After about 4 weeks' administration, all rats among the 4 mg/kg group developed severe acute signs of intoxication following injection: tremor, increased salivation, cramps, paralysis of limbs, and reddish secretion from ocular and nasal mucosa. However, the severity of the symptoms differed between the rats and between the days. Some rats administered 2 mg/kg developed much milder acute signs than those of the 4 mg/kg group with inter-individual and inter-day differences. In contrast, the 0 and 1 mg/kg groups did not have any acute signs. The injected sites of the skin were not ulcerated on either rat.

3.2. Reproductive and other organ weight

The body weight at the end of the treatment decreased significantly only in the 4 mg/kg group (Table 1). The absolute weight of testes and epididymes did not change, but their relative weights increased significantly only at 4 mg/kg (Table 1). The absolute and relative weights of the prostate and the seminal vesicle did not change. As for the non-reproductive organs, the absolute weight of the liver decreased with statistical significance, and that of the left adrenal gland significantly increased in the 4 mg/kg group (Table 2). The other organ weights did not show any significant changes.

3.3. Histopathological findings

Histopathologically, a difference in the frequency of abnormality of the testis in all 3 categorized stages did not differ between the control and any treated groups, while two previous studies showed such changes (Krause et al., 1974, 1976). The number of round spermatids per Sertoli cell in Stage VII did not change either (Fig. 1 and Table 3). No vacuolation, exfoliation, degeneration or retention in the seminiferous tubules nor any histopathological changes in intertubular spaces were observed in this study.

3.4. Blood ChE activity and urinary DMP concentration

The ChE activities in erythrocyte and plasma are shown in Table 4. Those in erythrocyte decreased dose-dependently, showing significant 44-55% inhibition in the treated groups. ChE activity in plasma showed the same but more moderate tendency with significant decreases only at 2 and 4 mg/kg. DMP in the urine significantly increased dose-dependently with larger standard deviations in higher-dose groups (Fig. 2).

3.5. Sperm motility

Sperm motilities in the cauda epididymidis decreased significantly in the 1 and 4 mg/kg groups but without a clear dose-response relationship (Table 5). The regression of the motility on the urinary DMP concentration and erythrocyte ChE activity were statistically significant (Figs. 3A, 3B), but not on the plasma ChE (Fig. 3C).

3.6. Sperm morphology

Morphological analysis detected no significant change in sperm head in any treated groups (Table 5). As for morphological abnormalities other than straight and banana-shaped sperm, a twin-tailed sperm was found in only one rat of the 4 mg/kg group.

3.7. Testosterone concentration

Testosterone concentrations in the plasma were not significantly different in any DDVP-treated groups (data not shown).

4. Discussion

This study showed DDVP decreased sperm motility in the rat, whereas no significant changes were found in the histopathology of seminiferous tubules, sperm counts, sperm morphology, histopathology of seminiferous tubules, plasma testosterone concentrations or reproductive organ weights. The extent of the % decrease in sperm motility in the treated groups was at most about 14% of the control group. A dose-response relationship was evident between the sperm motility and the internal exposure dose, i.e., blood ChE or urinary DMP. These findings are virtually consistent with a study indicating that the duration time of sperm motility was shorter in rats treated with DDVP (Akbarsha et al., 2001), though the authors did not indicate the % sperm motility parameter. They mentioned that the decreased sperm motility might have resulted from the retention of cytoplasmic droplets in the cauda epididymis sperm. Alternatively, mitochondrial enzymatic activity might have been decreased due to DDVP exposure, which in turn decreased sperm motility as reported in an asthenozoospermia patient (Ruiz-Pesini et al., 1998). Sarin and Gill (1999) showed that the activity of cytochrome oxidase (COX) in rat brain exposed to DDVP decreased significantly. Additionally, parathion and methylparathion, other organophosphorus pesiticides, inhibited (Ca2+ + Mg2+)-ATPase on the erythrocyte membrane (Blasiak, 1995). Another example is alpha-chlorohydrin (ACH) which inhibits motility of mature sperm in the cauda epididymidis without a decrease in the sperm counts or testicular weights, and without any detectable damage to the epididymis or to the testis under lower doses (Jones, 1983). The mechanism of this inhibition is that ACH inhibits sperm glycolytic enzymes, which results in an inability to maintain continuous ATP synthesis in sperm (Mohri et al., 1975; Brown-Woodman et al., 1978). Thus, the mechanism of decreased sperm motility caused by DDVP exposure needs to be studied further.

When examining at the testis histopathologically in detail, the number of round spermatids per Sertoli cell in stage VII did not change with the treatment, indicating that DDVP did not have an antiandrogenic effect. This result was in accordance with the fact that plasma testosterone concentrations were not changed. Krause and Homola (1974) observed disturbed spermatogenesis and an increased number and hypertrophy of Leydig cells after DDVP treatment, used too few mice for statistical analysis. Krause et al. (1976) also suggested that spermatogenic cells and Leydig cells were slightly reduced in the testes of juvenile rats exposed to DDVP. However, another study by Krause in 1977 did not reveal any disturbances of the spermatogenetic epithelium. Furthermore, Dikshith et al. (1976) showed that dermal painting of DDVP did not affect the histopathology of skin and testis in rats. In their study, the testes of two rats which had died after 5 applications of DDVP showed complete necrosis of the majority of the seminiferous tubules, but there was no such testicular damage in the remaining animals with subsequent exposures up to 90 days. In 1989, the National Toxicology Program (NTP) in the U.S. showed no histopathological changes in the testes, epididymes and prostate of rats and mice orally exposed to DDVP for 2 years. Our study results were in accordance with these results that DDVP did not affect testicular histopathology.

As for sperm morphology, the appearance of twin-tailed sperm was reported in DDVP-treated mice (Wyrobek and Bruce, 1975). The report indicated that mouse treated intraperitoneally with 5 mg/kg DDVP for 5 days had about 2.5% abnormality, and even at about 1 mg/kg DDVP induced 1.25% abnormality. However, twin-tailed sperm were virtually absent in the present study. Taken together, although experiments with different animal species/strains, and different routes and doses might result in clearer effects, the results of the previous and our animal studies suggest that the

testicular toxicity of DDVP is much milder, and limited to decreased sperm motility or slight increase in morphologically-abnormal sperm than the results of testicular toxic pesticides such as dibromochloropropane (DBCP) (Kluwe, 1983).

The final goal of the present study is to estimate the risk of testicular dysfunction due to DDVP in human, though the observed effect in the present study was small and without a clear dose-response relationship. Since the average DMP concentration in the urine of workers occupationally exposed to DDVP was 0.09 mg/l (Saito et al., 1984), the decrease in their sperm motility is anticipated to be 0.22% on a calculation using the obtained regression formula. Even in a person with the maximum DMP excretion of 0.71 mg/l (Saito et al., 1984), the expected decrease in sperm motility would be at most 0.63%. Additionally, erythrocyte ChE inhibition observed in the present rat study was more than 40% in the 1 mg/kg group showing 12% decrease in sperm motility. This does not usually occur even in occupationally-exposed workers. Thus, the risk of testicular dysfunction due to DDVP exposure alone would be small under the usual occupational exposure without a reduction in ChE activities, and would be negligible in general environmental exposure.

Acknowledgements

This work was partially supported by Health and Labor Sciences Research Grants (Research on Risk of Chemical Substances) from the Ministry of Health, Labor and Welfare, Japan, and the Uehara Memorial Foundation. This study does not represent the official views of any organization in Japan. The authors thank Ms. Yoshiko Nagai, Mr. Yasuhito Goto and Mr. Yasuyuki Nishizato for their assistance in the pilot study, and Dr. Osamu Yamanoshita and Ms. Maiko Miyata for advice on the testosterone assay.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (1997) Toxicological profile for Dichlorvos. Atlanta, GA: Public Health Service, U.S. Department of Health and Human Services.

Akbarsha, M.A., Latha, P.N., Murugaian, P. (2000) Retention of cytoplasmic droplet by rat cauda epididymal spermatozoa after treatment with cytotoxic and xenobiotic agents. J. Reprod. Fertil. 120, 385-90.

Blasiak, J. (1995) Inhibition of erythrocyte membrane (Ca2+ + Mg2+)-ATPase by the organophosphorus insecticides parathion and methylparathion. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 110, 119-25.

Brown-Woodman, P.D., Mohri, H., Mohri, T., Suter, D., White, I.G. (1978) Mode of action of alpha-chlorohydrin as a male anti-fertility agent. Inhibition of the metabolism of ram spermatozoa by alpha-chlorohydrin and location of block in glycolysis. Biochem. J. 170, 23-37.

Bryan, J.H. (1970) An eosin-fast green-naphthol yellow mixture for differential staining of cytologic components in mammalian spermatozoa. Stain. Technol. 45, 231-6.

Department for Environment, Food and Rural Affairs (DEFRA), United Kingdom (2002) News Release 157/02.

Dikshith, T.S., Datta, K.K., Chandra, P. (1976) 90 day dermal toxicity of DDVP in male rats. Bull. Environ. Contam. Toxicol. 15, 574-80.

Hardt, J., Angerer, J. (2000) Determination of dialkyl phosphates in human urine using gas chromatography-mass spectrometry. J. Anal. Toxicol. 24, 678-84.

Hummel, S.V. (2000) Human health risk assessment dichlorvos (DDVP): Environmental Protection Agency (EPA), Office of Pesticide Programs, Health Effects Division.

Jones, A.R. (1983) Antifertility actions of alpha-chlorohydrin in the male. Aust. J. Biol. Sci. 36, 333-50.

Kamijima, M., Hibi, H., Gotoh, M., Taki, K., Saito, I., Wang, H., Itohara, S., Yamada, T., Ichihara, G., Shibata, E., Nakajima, T., Takeuchi, Y. (2004) A survey of semen indices in insecticide sprayers. J. Occup. Health. 46, 109-18, 2004.

Kluwe, W.M., Gupta, B.N., Lamb, J.Ct. (1983) The comparative effects of 1,2-dibromo-3-chloropropane (DBCP) and its metabolites, 3-chloro-1,2-propaneoxide (epichlorohydrin), 3-chloro-1,2-propanediol (alphachlorohydrin), and oxalic acid, on the urogenital system of male rats. Toxicol. Appl. Pharmacol. 70. 67-86.

Krause, W. (1977) Influence of DDT, DDVP and malathion on FSH, LH and testosterone serum levels and testosterone concentration in testis. Bull. Environ. Contam. Toxicol. 18. 231-42.

Krause, W., Hamm, K., Weissmuller, J. (1976) Damage to spermatogenesis in juvenile rat treated with DDVP and malathion. Bull. Environ. Contam. Toxicol. 15. 458-62.

Krause, W., Homola, S. (1974) Alterations of the seminiferous epithelium and the Leydig cells of the mouse testis after the application of dichlorvos (DDVP). Bull. Environ. Contam. Toxicol. 11. 429-33.

Mohri, H., Suter, D.A., Brown-Woodman, P.D., White, I.G., Ridley, D.D. (1975) Identification of the biochemical lesion produced by alpha-chlorohydrin in spermatozoa. Nature 255. 75-7.

Mori, K., Kaido, M., Fujishiro, K., Inoue, N., Koide, O., Hori, H., Tanaka, I. (1991) Dose dependent effects of inhaled ethylene oxide on spermatogenesis in rats. Br. J. Ind. Med. 48. 270-4.

National Toxicology Program (NTP) (1989) Toxicology and carcinogenesis studies of dichlorvos in F344/N rats and B6C3F1 mice. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institute of Health. Report No.: NTP TR 342.

Phillips McDougall (2004) Products Section-2003 Market. In: Phillips McDougall-AgriService. Phillips McDougall, Pathhead, November issue, pp.137.

Ruiz-Pesini, E., Diez, C., Lapena, A.C., Perez-Martos, A., Montoya, J., Alvarez, E., Arenas, J., Lopez-Perez, M.J. (1998) Correlation of sperm motility with mitochondrial enzymatic activities. Clin. Chem. 44. 1616-20.

Saito, I., Hisanaga, N., Takeuchi, Y., Ono, Y., Iwata, M., Masuda, K., Gotoh, M., Matsumoto, T., Fukaya, Y., Okutani, H., Seki, T., Koike, Y. (1984) Assessment of the exposure of pest control operators to organophosphorus pesticides. Organophosphorus pesticides in blood and alkyl phosphate metabolites in urine. Sangyo Igaku (Jap. J. Ind. Health) 26. 15-21.

Sarin, S., Gill, K.D. (1999) Dichlorvos induced alterations in glucose homeostasis: possible implications on the state of neuronal function in rats. Mol. Cell. Biochem. 199. 87-92.

Voss, G., Sachsse, K. (1970) Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine-DTNB procedure. Toxicol. Appl. Pharmacol. 16. 764-72.

Wyrobek, A.J., Bruce, W.R. (1975) Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. U S A 72. 4425-9.

Table 1

Body and reproductive organ weights in rats exposed to DDVP

	0 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Number of rats	8	8	9	9
Body weight (g)	426±24	423±14	413±8.5	369±8.5 *
Absolute organ weight (g)				
Epididymes (left)	0.58±0.02	0.56±0.07	0.57±0.03	0.57±0.05
Testes (left)	1.62±0.07	1.63±0.11	1.64±0.15	1.64±0.17
Prostate	0.70±0.09	0.80±0.13	0.63±0.24	0.58±0.15
Seminal vesicle	1.96±0.19	2.17±0.21	2.19±0.31	1.88±0.16
Relative organ weight (g/kg body weight)				
Epididymes (left)	1.37±0.11	1.33±0.10	1.39±0.11	1.54±0.16 *
Testes (left)	3.81±0.26	3.85±0.22	4.01±0.34	4.45±0.42 *
Prostate	1.71±0.21	1.83±0.41	1.81±0.30	1.50±0.38
Seminal vesicle	4.77±0.48	4.98±0.53	5.35±0.68	5.07±0.31

Values are means±SD. * indicates significant difference at p <0.05 when compared with control group.

Table 2

Weight of adrenal gland, liver, kidney in rats exposed to DDVP

	0 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Number of rats	8	8	9	9
Absolute organ weight (g)				
Adrenal gland (left)	0.034±0.004	0.036±0.009	0.035±0.012	0.044±0.007 *
Liver	12.76±1.73	12.71±0.85	11.69±1.52	10.83±1.89 *
Kidney (left)	1.28±0.13	1.32±0.10	1.34±0.04	1.23±0.12
Relative organ weight (g/kg body weight)				
Adrenal gland (left)	0.09±0.02	0.08±0.02	0.09±0.01	0.11±0.03
Liver	29.92±2.84	30.25±1.64	28.36±2.73	29.06±3.42
Kidney (left)	3.06±0.28	3.16±0.17	3.19±0.21	3.25±0.15

Values are means±SD. * indicates significant difference at p <0.05 when compared with control group.

Table 3

Spermatogenic cell count in stage VII seminiferous tubule of rats exposed to DDVP

	0 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Number of rats	8	8	9	9
Round spermatids/ Sertoli cell	14.2±3.1	15.9±4.7	13.4±1.6	13.5±2.1

Values are means±SD. No significant difference was presented at p <0.05 when compared with control group.

Table 4

Cholinesterase activities in erythrocyte and in plasma

	0 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Number of rats	8	8	9	9
Erythrocyte (μmol/ml/min)	1.66±0.20	0.92±0.13 *	0.77±0.17 *	0.76±0.16 *
Plasma (μmol/ml/min)	0.82±0.12	0.74±0.13	0.62±0.11 *	0.58±0.10 *

Values are means±SD. * indicates significant difference at p <0.05 when compared with control group.

Table 5

Sperm count, head abnormality and motility

	0 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Number of rats	8	8	9	9
Sperm count (x 10^6/g cauda)	314 ± 113	315 ± 93	352 ± 148	349 ± 79
Sperm head abnormality (%)	2.0 ± 1.1	2.9 ± 1.8	3.1 ± 1.4	2.2 ± 1.1
Sperm motility (%)	87.1±2.4	76.8±4.4*	79.2±8.6	74.6±2.2*

Values are means±SD. * indicates significant difference at p <0.05 when compared with control group.

Figure legends

Fig. 1 Stage VII seminiferous tubules of rats subcutaneously injected with DDVP at (A) 0 mg/kg and (B) 4 mg/kg for 9 weeks. No abnormality was found.

Fig. 2 Urinary dimethyl phosphate (DMP) concentration in rats subcutaneously injected with DDVP. 24-hour urine was collected after the last DDVP treatment. Each bar represents the mean±SD. * indicates significant difference at $p < 0.01$ when compared with the control group.

Fig. 3 Regression of sperm motility on internal doses. All data on both exposure and control groups were plotted in the figure (n=34). A: Regression of % sperm motility on erythrocyte cholinesterase (E-ChE) activity was significant (p= 0.028). Motility=9.744x(E-ChE)+69.394. R2=0.2463. B: Regression of % sperm motility on root-transformed dimethyl phosphate (DMP) concentration was significant (p= 0.043). Motility=–0.7481x(DMP)1/2 +82.424. R2=0.1215. C: Regression of % sperm motility on plasma cholinesterase (P-ChE) activity was not significant.

Fig.1

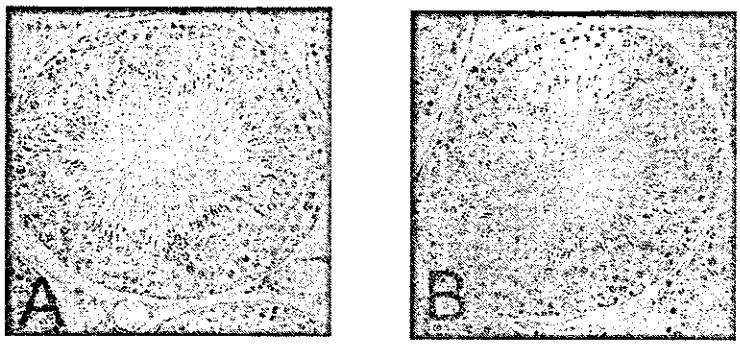

Fig.2

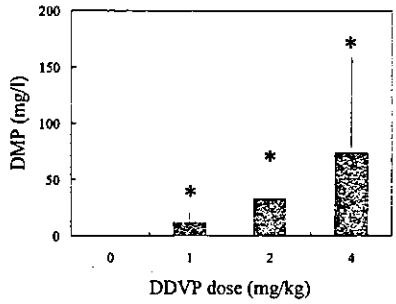

Fig.3