5.2.2 EYE IRRITATION/CORROSION

Species/strain:	New Zealand white rabbit
Results:	Highly corrosive **[X]**; Corrosive [ ]; Highly irritating [ ]; Irritating [ ]; Moderate irritating [ ]; Slightly irritating [ ]; Not irritating [ ]
Classification:	Irritating [ ]; Not irritating [ ]; Risk of serious damage to eyes [ ]
Method:	3 rabbits/unwashed group (2 males and 1 female, the same rabbits were used for the skin irritation test), 3 rabbits/washed of (1 male and 2 females). 96 hour-observation period, application: 0.1 g/rabbit; In the case of washed group, the treated eyes were flushed for 1 minute with ca. 300 ml water 30 seconds after application.
GLP:	Yes **[X]** No [ ] ? [ ]
Test substance: purity	82.6 %
Remarks:	54.3 scores after 48 hrs, unwashed group (Extremely irritating)
Reference:	Unpublished company report (1988)

5.3 SKIN SENSITISATION

No studies located

5.4 REPEATED DOSE TOXICITY

(a)

Species/strain:	Rat (Wistar)
Sex:	Female [ ]; Male [ ]; Male/Female **[X]**; No data [ ]
Route of Administration:	oral (Diet)
Exposure period:	6 months
Frequency of treatment:	
Post exposure observation period:	
Dose:	0, 150, 500 or 1500 ppm
Control group:	Yes **[X]**; No [ ]; No data [ ]; Concurrent no treatment [ ]; Concurrent vehicle **[X]**; Historical [ ]
NOEL:	500 ppm (30.7 mg/kg/day)
LOEL:	
Results:	A transient excretion of glucose into urine was observed in the rats fed 1500 ppm. No other abnormalities were noted.
Method:	
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	Commercial, purity: 99.5 %
Reference:	Botyu-Kagaku 40, 38-48 (1975)

5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

(a)

Type :	Bacterial reverse mutation assay
System of testing:	
Species/strain:	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538 *E. coli* WP2 uvrA
Concentration:	78.12 - 2500 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without **[X]**; No data [ ]

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Subchronic Toxicity Studies of Sumithion, Sumioxon and *p*-Nitrocresol in Rats and 92 Week Feeding Study of Sumithion with Special Reference to Change of Cholinesterase Activity.

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7. スミチオン，スミオキソン，*p*-ニトロクレゾールのラットにおける亜慢性毒性およびスミチオン92週摂食によるコリンエステラーゼの変動

門田忠臣，鴻田弘行，宮本純之（住友化学工業株式会社農薬事業部研究部） 50. 1. 16. 受理

有機リン殺虫剤スミチオンおよびその代謝産物の亜慢性毒性を明らかにするため最高スミチオン150ppm，スミオキソン50ppm，*p*-ニトロクレゾール1,500ppmを含む飼料をそれぞれ6カ月間ラットに摂食させ，体重測定，摂食・摂水量測定，血液検査，臨床生化学検査，尿検査，臓器重量測定，病理組織学的検査を実施した．

コリンエステラーゼ活性を除き，各検査項目についてこれらの化合物の投与に起因すると思われる異常は見出されなかった．血漿，血球，脳コリンエステラーゼはスミチオン，スミオキソンの投与量に相関して阻害されており，スミチオンは最低の10ppmでも雌血漿コリンエステラーゼを有意に阻害したところから．さらに92週に及ぶ追加摂食実験を行ない経時的に血液コリンエステラーゼ活性を測定した．この条件下でスミチオンの無影響量は飼料中5ppm，体重換算0.27mg/kg/dayであった．

一方スミオキソンの無影響量は，6カ月摂食後で飼料中5ppmであった．

Introduction

Sumithion® or *O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate is an organophosphorus insecticide now widely used to control various plant pests and insects of medical importance. The metabolic studies in mammals[1-6] revealed that orally administered radioactive Sumithion was easily absorbed from the gastrointestinal tract and distributed into various tissues. Sumithion was confirmed to be oxidized into the active metabolite Sumioxon, *O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorate in the animal both in vivo and in vitro. Both compounds were decomposed in animal body and the radioactivity was eliminated rapidly and completely, majorly into urine. The major degradation products in urine were desmethylsumithion, desmethylsumioxon, dimethylphosphorothioic acid, dimethylphosphoric acid, *p*-nitrocresol (3-methyl-4-nitrophenol) and its conjugates. Plant metabolism proceeds in essentially the similar manner[7-9]; a trace amount of Sumioxon and *p*-nitrocresol, free and bound with glucose, were found.

Although it has already been demonstrated that residue of Sumithion and its metabolites in various harvested crops is generally low[7-13], it is necessary to assess the chronic toxicity of Sumithion and other possibly toxicologically significant metabolites in humans. In this study, therefore, Sumioxon and *p*-nitrocresol as well as Sumithion were fed to rats for consecutive 6 months to examine the subchronic effects on various physiological parameters. A supplementary study was also carried out with special reference to the change of cholinesterase activity

during 92 week feeding of Sumithion.

Materials and Methods

Sumithion was a technical product of Sumitomo Chemical Co., Ltd (lot No. 417, purity 97.2%). Sumioxon (99% pure) and *p*-nitrocresol (99.5% pure) were prepared in this laboratory.

Six week old male and female Wistar strain rats were purchased from Nihon Dobutsu Co., Ltd. and were housed in individual cages and kept at 24±1°C and relative humidity of 60±10 % for 6 months. All animals had free access to food and water. The control group received basal diet (Nihon Crea CE-2) and the test groups received powdered diet containing the test material. The organization of the experiment is reproduced in Table 1. Behavioral changes and mortality were observed every day and body-weight, food and water consumption were recorded weekly. Urinary sugar (Benedict method), protein (sulfosalicylic acid method), bilirubin (ferric sulfate method), urobilinogen (Ehrich method) and occult blood (Benzidine method) were examined at 4th, 8th, 12th and 24th week (Shinotest Lab. Co.). At the termination of 6 month feeding, all the animals were anesthetized with ether and blood was withdrawn from abdominal aorta for hematological, clinical biochemical examinations including cholinesterase activity measurement. Red blood cells, white blood cells and thrombocytes were counted by a microcell counter (Toa Electric Co., Ltd. type II) and differential leucocyte counts were carried out as usual. Total blood hemoglobin content was measured by the standard technique involving cyanomethohemoglobin formation[14]. Hematocrit and sedimentation rate were determined by the standard technique. Clinical biochemistry examinations were carried out with sodium, potassium, chloride, total protein, albumin, alkaline phosphatase (ALP), GOT, GPT, blood urea nitrogen, glucose and bilirubin by an autoanalyzer (Technicon Co., SMA 60/12). Cholinesterase activity (ChE) was determined of plasma, red blood cells and brain by an electrometric method[15]. The enzyme preparations were reported elsewhere[16].

Immediately after blood sampling every tissue and organ were grossly observed and major organs were dissected out to weigh. Also the following tissues were subjected to histopathological examinations by fixation with 10% formalin, followed by double staining with Hematoxylin and Eosin ; brain, eye, spinal cord, peripheral nerve, heart, lung, spleen, bone marrow, lymph node, thymus, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, urinary bladder, testis or ovary, prostate or uterus, pituitary, thyroid, adrenal and bronchus.

A supplementary study was carried out with special reference to the change of cholinesterase activity of plasma and red blood cells during 92 week feeding of 2.5, 5 and 10 ppm of Sumithion. Each group was composed of 15 males and 15 females. At 2nd, 4th, 6th, 8th, 12th, 16th, 20th, 24th, 42nd, 68th and 92nd week of feeding, a small amount of blood was obtained from orbital

Table 1. Experimental designs of feeding test of Sumithion, Sumioxon and *p*-nitrocresol to rats.

Group	Compound	Dietary level ppm	Number of animals male	female
C	—	0	15	15
T-I	Sumithion	10	15	15
T-II	〃	30	15	15
T-III	〃	150	15	15
T-IV	Sumioxon	5	15	15
T-V	〃	15	15	15
T-VI	〃	50	15	15
T-VII	*p*-nitrocresol	150	15	15
T-VIII	〃	500	15	15
T-IX	〃	1500	15	15

plexus by using glass capillary under ether anesthetization. Brain cholinesterase activity was measured after 92 week feeding.

Results and Discussions

Behavior and mortality; There was no signs of toxicity during 6 month feeding of Sumithion, Sumioxon or *p*-nitrocresol. One death was observed in male fed 15 ppm Sumioxon during starvation period just prior to necropsy.

Body weight change; Table 2 shows mean body weight change during the feeding period. These compounds were found not to affect the body weight gain adversely except that a slight depression of body weight gain was observed during the initial feeding period of 150 ppm Sumithion in both males and females (1st to 2nd week). However, the body weight gain recovered to the normal level in 2 to 3 weeks.

Food and water consumption; The results of food and water consumption measurement revealed that only at high dosage of Sumithion the animals ingested a little more amount of food at the initial 2 weeks. There were no appreciable differences in water consumption among control and any test groups. Intake of the test compounds during the feeding period was calculated and the results are shown in Table 3.

Table 3. Intake of Sumithion, Sumioxon and *p*-nitrocresol during 6 month feeding period.

Compound	Dietary level ppm	Compound ingested mg/kg body weight/day male	female
Sumithion	10	0.59	0.64
	30	1.83	2.00
	150	9.16	11.2
Sumioxon	5	0.31	0.34
	15	0.91	0.99
	50	2.99	3.66
p-nitrocresol	150	9.23	10.1
	500	30.7	32.8
	1500	94.7	101.00

Table 2. Body weight gain during 6 month feeding of Sumithion, Sumioxon and *p*-nitrocresol to rats.

Sex	Experimental groups		Body weight[a], g Initial	Final	Gain
Male	control	0 ppm	188±6.13	419±11.6	231± 9.0
	Sumithion	10 ppm	194±4.50	438± 8.6	244±10.3
		30 ppm	178±3.48	434±13.9	256±15.5
		150 ppm	197±3.87	427±11.5	230±10.8
	Sumioxon	5 ppm	156±2.48**	408±13.9	252±14.2
		15 ppm	165±6.03*	410±12.2	245±13.2
		50 ppm	197±6.67	423±12.5	226±10.8
	p-nitrocresol	150 ppm	177±7.85	428±12.0	251±11.6
		500 ppm	185±7.75	430±16.5	245±14.2
		1500 ppm	181±5.30	447±18.7	266±18.3
Female	control	0 ppm	145±4.35	273± 8.4	128± 5.4
	Sumithion	10 ppm	155±3.86	279± 7.4	124± 6.7
		30 ppm	143±3.68	262± 5.3	119± 4.1
		150 ppm	141±5.31	252± 5.2*	111± 6.3
	Sumioxon	5 ppm	140±2.74	263± 6.7	123± 5.6
		15 ppm	153±2.83	270± 2.8	117± 3.5
		50 ppm	148±3.22	279± 7.7	131± 6.6
	p-nitrocresol	150 ppm	143±3.68	269± 5.2	126± 5.5
		500 ppm	142±2.79	273± 5.8	131± 4.9
		1500 ppm	145±4.08	268± 6.1	123± 5.5

[a] mean±standard error

* $p<0.05$, ** $p<0.01$

Table 4. Hematological studies in rats treated with Sumithion, Sumioxon and *p*-nitrocresol for 6 months.[a)]

Sex	Groups	Dietary level ppm	Red blood cell $\times 10^4$	Thrombocyte $\times 10^4$	Sedimentation rate mm/hr	Hematocrit %	Hemoglobin g/dl	White blood cell $\times 10^2$
Male	control	0	919±19.9	74.1±6.10	1.54±0.38	48.6±1.08	14.7±0.26	88.2±4.60
	Sumithion	10	918±12.2	76.5±3.59	2.42±0.50	46.0±0.61	15.2±0.22	92.7±4.91
		30	874±11.2	78.7±4.34	2.99±0.81	46.0±0.93	14.5±0.19	84.4±6.39
		150	904±14.7	68.0±4.05	1.25±0.27	46.3±0.69	14.6±0.19	91.5±4.39
	Sumioxon	5	940±10.8	79.9±6.70	2.55±0.35	46.1±0.48	14.1±0.17	97.6±3.80
		15	945±12.0	74.5±4.40	2.64±0.46	46.9±0.70	13.9±0.19*	90.6±5.20
		50	939±16.5	81.0±5.80	1.88±0.30	47.8±1.08	14.0±0.28	92.4±4.90
	p-nitrocresol	150	991±16.1**	71.8±5.00	1.49±0.42	47.3±0.97	14.0±0.24	80.9±6.00
		500	979±20.0	82.5±6.40	1.49±0.32	47.8±0.95	14.1±0.22	92.1±4.00
		1500	996±16.2**	78.1±4.10	1.37±0.40	47.1±0.72	14.0±0.21	90.3±3.70
Female	control	0	843±14.7	90.5±6.95	0.94±0.29	43.8±0.72	13.8±0.11	78.8±4.95
	Sumithion	10	885±15.8*	88.2±6.43	0.84±0.17	45.6±0.89	13.8±0.20	79.9±6.01
		30	836±13.8	82.3±4.85	0.99±0.33	42.8±0.72	13.7±0.14	70.5±5.17
		150	840±13.0	84.0±5.42	1.78±0.45	42.1±0.27*	13.9±0.11	70.5±5.17
	Sumioxon	5	826± 8.2	79.1±4.00	2.38±0.49*	43.5±0.73	13.5±0.13	66.1±4.40
		15	845± 9.8	82.7±5.01	1.98±0.31*	43.7±0.60	13.8±0.15	60.9±3.70**
		50	877± 6.6*	85.1±3.40	1.61±0.29	43.5±0.58	13.9±0.19	62.1±4.50*
	p-nitrocresol	150	827±13.3	79.8±4.00	1.41±0.30	43.1±0.46	13.9±0.19	53.1±3.90**
		500	821±14.1	79.9±3.31	2.33±0.50	42.8±0.50	13.7±0.09	49.3±3.31**
		1500	845±16.7	79.3±4.72	1.89±0.41	43.5±0.78	13.5±0.15	49.5±2.31**

a) mean ± standard error
* $p<0.05$, ** $p<0.01$

Hematology; Table 4 summarizes the results of the hematological examinations (Differential leucocyte counts were also carried out although the data are not submitted here). Although there were several significant differences in some cases indicated by asterisk, they were not attributable to the compounds, since some of them were not dose-related and others were seemingly within the range of physiological fluctuations.

Urinalysis; At the latter half of the feeding period urinary protein was found in all rats examined of both control and each of the test groups. However, the degree was not dependent on the administration of the compounds. A transient excretion of sugar into urine was observed in the rats fed 1500 ppm *p*-nitrocresol at 4th and 8th week, but it disappeared thereafter. No other abnormalities were noted.

Clinical biochemistry and cholinesterase activity; The results of blood biochemistry and cholinesterase activity after 6 month feeding are shown in Table 5. Although there were some significant differences in several of the parameters tested, these differences (excluding cholinesterases) were neither so large nor dose-dependent and, therefore, are presumably not attributable to the feeding of these chemicals. On the other hand, plasma, red blood cell and brain cholinesterase activity of the animals given Sumithion or Sumioxon showed a dose-related decrease. Female plasma cholinesterase was more susceptible to Sumithion than the male enzyme and even at the lowest dosage (10 ppm) the activity of the former was significantly lower than the control, whereas the latter showed no inhibition. Sumioxon at 5 and 15 ppm caused no significant inhibition of plasma enzyme. No reduction in red blood cell cholinesterase activity was observed by Sumithion at 10 ppm (male and female) and 30 ppm (male) or by Sumioxon at 5 and 15 ppm (male and female). The inhibition of brain cholinesterase activity was more remarkably observed in females than males; in females Sumithion at 150 and 30 ppm and Sumioxon at 50 and 15 ppm were inhibitory, while in males only Sumithion at 150 ppm was inhibitory.

Organ weight; The weight of 9 major organs was determined and their ratios to body weight were calculated. Table 6 shows the ratios. Although the weight and the ratio of several organs in a certain treated groups were significantly deviated from those of the control, they were regarded as not dependent on the compounds administered.

Histopathology; Histopathological examinations were made of 24 tissues of all rats given the highest dosage of Sumithion, Sumioxon and *p*-nitrocresol in comparison with the control. The results are summarized in Table 7. Pneumonia or abscess in lung (excluding *p*-nitrocresol group), vacuolar degeneration in the parenchymal cells as well as a slight bile duct proliferation and infiltration of oval cells in liver and a slight magnification of the renal tubule and cell infiltration in interstitial connective tissues in kidney were findings common to both control and treated groups and these changes were not due to the administration of the compounds. No other signs of abnormalities were observed in any tissues examined which might lead to the formation of hyperplasia.

Thus, no adverse effects were observed in all the parameters examined except cholinesterase inhibition by 6 month feeding of up to 150 ppm Sumithion, 50 ppm Sumioxon and 1500 ppm *p*-nitrocresol to rats. The findings were in well accord with 2 year chronic feeding study of Sumithion in beagle dogs[17], where 200 ppm of Sumithion gave no other untoward reactions than inhibition of cholinesterases. Sumioxon at 5 ppm was not inhibitory on plasma, red blood cell and brain cholinesterase, while Sumithion at the lowest level (10 ppm) inhibited the female plasma cholinesterase. Therefore, a supplementary feeding experiment of Sumithion to rats was undertaken to establish no effect level in terms of the effect on cholinesterases. In this study each 15 male and 15 female rats were kept on diet containing 0, 2.5, 5 and 10 ppm of Sumithion and blood was taken periodically and plasma and red blood cell cholinesterases were determined by delta pH method. At the termination of the feeding the animals were sacrificed and brain cholinesterase activity was determined. Some of the test animals were dead in the latter half of

Table 5. Blood biochemical studies in rats treated with Sumithion, Sumioxon and *p*-nitrocresol for 6 months.[a)]

Sex	Groups	Dietary level ppm	Sodium Meq/l	Potassium Meq/l	Chlorine Meq/l	Glucose mg/dl	Blood urea-N mg/dl	Total protein g/dl	Albumin g/dl
Male	control	0	140±0.81	8.84±0.32	101±1.36	93.8±3.09	16.7±0.96	7.21±0.13	2.48±0.09
	Sumithion	10	139±0.30*	9.50±0.27	101±0.73	107 ±5.43	15.0±0.56	6.89±0.10	2.48±0.08
		30	139±0.71	9.47±0.20	103±0.40	112 ±6.53*	16.1±0.57	6.77±0.10*	2.45±0.11
		150	140±0.52	9.58±0.31	103±0.64	98.2±4.97	20.1±2.58	6.78±0.09*	2.46±0.07
	Sumioxon	5	142±0.45*	7.56±0.20**	101±0.50	113 ±3.79**	16.8±0.65	6.73±0.10*	2.44±0.08
		15	142±0.40	9.00±0.40	101±0.72	101 ±7.16	15.9±0.73	6.88±0.16	2.56±0.07
		50	140±0.70	9.08±0.24	102±0.69	112 ±7.26*	17.0±1.04	6.63±0.06**	2.51±0.12
	p-nitrocresol	150	143±1.07	8.95±0.35	103±0.71	92.2±8.28	16.4±0.61	7.02±0.12	2.39±0.15
		500	142±0.79	8.89±0.26	109±6.13	107 ±9.14	14.8±0.96	7.07±0.06	2.58±0.09
		1500	142±0.79*	8.76±0.32	102±0.58	98.3±5.82	15.9±0.87	6.90±0.13	2.47±0.11
Female	control	0	146±0.62	11.6±0.59	108±0.54	90.8±5.98	15.3±0.47	7.96±0.12	3.69±0.09
	Sumithion	10	151±0.88	8.88±0.30	107±0.56	73.8±3.96*	17.9±0.64	7.98±0.13	3.47±0.13
		30	146±0.65	11.2±0.61	106±0.69*	71.2±3.33*	17.1±0.65	7.83±0.12	3.65±0.16
		150	149±0.65*	10.8±0.34	108±0.70	74.6±3.00**	20.7±0.79**	7.71±0.14	3.50±0.14
	Sumioxon	5	142±0.47**	7.66±0.25**	102±0.56**	81.7±8.15	15.9±1.03	7.89±0.11	3.41±0.15
		15	144±1.01	9.26±0.39	104±1.39	83.9±4.56	16.6±0.77	7.56±0.13*	3.21±0.10*
		50	143±0.56**	7.47±0.25**	103±0.37	81.6±3.15	16.7±0.79	7.57±0.13*	3.31±0.02*
	p-nitrocresol	150	135±1.28**	7.90±0.28**	101±0.70**	79.0±6.36	14.4±1.12	7.01±0.08**	2.96±0.14*
		500	140±1.11**	8.22±0.37**	103±0.53**	81.0±4.17	13.5±0.82	7.32±0.07**	3.45±0.15
		1500	142±0.57**	8.72±0.30**	104±0.71**	75.4±4.67	15.0±1.18	7.93±0.10	3.53±0.14

[a)] mean ± standard error

* $p < 0.05$, ** $p < 0.01$

Table 5. (Continued)

Sex	Groups	Dietary level ppm	Bililubin mg/dl	ALP K-A u	GOT Karmen u	GPT Karmen u	Cholinesterase[b] plasma ⊿PH	Cholinesterase[b] red blood call ⊿pH	Cholinesterase[b] brain ⊿PH
Male	control	0	0.26±0.02	31.2±3.92	228±7.49	77.0±2.09	0.89±0.14	0.36±0.04	0.98±0.05
	Sumithion	10	0.28±0.01	22.1±1.36	199±10.7*	74.5±3.04	0.84±0.07	0.35±0.02	1.09±0.07
		30	0.22±0.01	18.1±1.04**	206±5.43	72.3±0.88	0.60±0.05	0.30±0.05	0.88±0.03
		150	0.25±0.02	28.3±1.86	187±10.8**	71.1±1.89	0.51±0.03*	0.11±0.04**	0.46±0.10**
	Sumioxon	5	0.30±0.01**	21.8±1.63*	203±12.4	63.0±3.06**	0.66±0.06	0.42±0.02	1.16±0.06*
		15	0.29±0.01	25.1±3.13	190±9.32**	68.3±3.06**	0.59±0.08	0.36±0.03	0.93±0.04
		50	0.27±0.02	24.4±2.27	196±14.3	70.2±1.28*	0.60±0.06	0.24±0.02*	0.96±0.04
	p-nitrocresol	150	0.35±0.02**	21.0±2.05*	187±8.45**	75.3±3.36	—	—	—
		500	0.33±0.02**	19.7±2.02*	193±4.29**	67.0±1.63**	—	—	—
		1500	0.32±0.01**	20.5±1.52*	192±9.80**	66.4±1.33**	—	—	—
Female	control	0	0.33±0.02	17.1±1.93	226±9.85	31.1±1.32	1.84±0.31	0.38±0.04	0.95±0.04
	Sumithion	10	0.39±0.02	15.8±1.22	251±10.4	23.9±1.79	0.83±0.09**	0.32±0.02	0.95±0.04
		30	0.33±0.02	12.4±0.73	240±16.3	23.7±1.75	0.92±0.17**	0.27±0.02*	0.66±0.02**
		150	0.30±0.00**	15.3±1.24	226±9.85	19.7±0.82**	0.45±0.04**	0.13±0.02**	0.29±0.01**
	Sumioxon	5	0.33±0.02	16.1±1.58	231±11.4	24.1±2.11*	1.60±0.09	0.40±0.02	0.92±0.06
		15	0.33±0.02	13.3±1.43	239±9.71	20.3±2.24	1.24±0.19	0.35±0.05	0.83±0.03*
		50	0.40±0.01**	17.0±1.86	254±14.9	26.8±2.44	0.78±0.12*	0.15±0.01**	0.72±0.02**
	p-nitrocresol	150	0.31±0.01	9.69±1.49**	182±13.8*	19.8±3.83*	—	—	—
		500	0.33±0.02	13.9±1.74	192±10.0*	19.4±1.73**	—	—	—
		1500	0.34±0.02	13.0±1.02	228±8.80	20.8±1.76**	—	—	—

[b] ⊿PH for 60 min., at 37.5°C

Table 6. Ratio of organ weight to body weight of rats treated with Sumithion, Sumioxon and *p*-nitrocresol for 6 months. [a)]

Sex	Groups	Dietary level ppm	Lung %	Liver %	Kidney %	Spleen %	Heart %
Male	control	0	0.69±0.06	2.31±0.08	0.59±0.01	0.24±0.02	0.30±0.01
	Sumithion	10	0.54±0.03*	2.39±0.04	0.64±0.02	0.21±0.02	0.31±0.01
		30	0.56±0.05	2.38±0.08	0.64±0.01*	0.19±0.01*	0.32±0.01
		150	0.59±0.04	2.36±0.05	0.62±0.01	0.19±0.01*	0.30±0.01
	Sumioxon	5	0.61±0.05	2.34±0.06	0.60±0.02	0.20±0.02	0.30±0.01
		15	0.65±0.04	2.37±0.05	0.63±0.01	0.24±0.02	0.32±0.01
		50	0.62±0.06	2.49±0.06	0.64±0.03	0.20±0.01*	0.32±0.01
	p-nitrocresol	150	0.67±0.09	2.12±0.04	0.60±0.02	0.22±0.02	0.28±0.01**
		500	0.68±0.06	2.28±0.05	0.62±0.02	0.21±0.01	0.31±0.01
		1500	0.55±0.02*	2.25±0.05	0.60±0.02	0.20±0.01	0.31±0.01
Female	control	0	0.65±0.05	2.63±0.08	0.66±0.02	0.23±0.01	0.32±0.01
	Sumithion	10	0.67±0.04	2.34±0.06**	0.63±0.01	0.24±0.02	0.34±0.01
		30	0.66±0.03	2.43±0.05*	0.60±0.01**	0.22±0.01	0.34±0.01
		150	0.68±0.03	2.62±0.05	0.70±0.02	0.26±0.02	0.36±0.01**
	Sumioxon	5	0.68±0.03	2.37±0.03**	0.61±0.01*	0.26±0.03	0.35±0.01*
		15	0.58±0.02	2.43±0.04*	0.62±0.01	0.23±0.01	0.34±0.01
		50	0.67±0.03	2.34±0.06**	0.62±0.01	0.23±0.01	0.34±0.01
	p-nitrocresol	150	0.64±0.04	2.49±0.03	0.60±0.03	0.24±0.02	0.35±0.01**
		500	0.60±0.03	2.36±0.03**	0.61±0.02	0.24±0.02	0.35±0.01*
		1500	0.68±0.04	2.40±0.04*	0.61±0.02	0.23±0.01	0.34±0.01

[a)] mean ± standard error
* $p<0.05$, ** $p<0.01$

Table 6. (Continued)

Sex	Groups	Dietary level ppm	Adrenal mg %	Testis %	Ovary mg %	Brain %
Male	control	0	11.8±0.83	0.73±0.02	/	0.45±0.01
	Sumithion	10	11.8±0.55	0.72±0.02	/	0.46±0.01
		30	10.8±0.77	0.74±0.03	/	0.45±0.01
		150	10.4±0.72	0.70±0.02	/	0.45±0.01
	Sumioxon	5	13.2±1.21	0.74±0.02	/	0.46±0.02
		15	13.1±1.14	0.75±0.02	/	0.48±0.01
		50	12.0±0.25	0.72±0.02	/	0.45±0.02
	p-nitrocresol	150	12.4±0.70	0.74±0.02	/	0.43±0.01
		500	12.2±0.81	0.73±0.02	/	0.45±0.02
		1500	10.4±0.66	0.72±0.03	/	0.43±0.02
Female	control	0	20.6±1.11	/	28.0±3.12	0.66±0.02
	Sumithion	10	21.3±1.23	/	31.0±3.60	0.62±0.02
		30	19.8±0.96	/	26.5±1.89	0.64±0.04
		150	22.5±1.19	/	35.4±3.62	0.72±0.02*
	Sumioxon	5	22.9±1.19	/	29.7±3.30	0.66±0.02
		15	22.3±1.23	/	27.0±2.78	0.65±0.01
		50	20.2±1.04	/	31.7±3.36	0.66±0.02
	p-nitrocresol	150	21.4±1.00	/	27.8±2.93	0.65±0.02
		500	22.7±1.11	/	28.0±2.27	0.65±0.01
		1500	20.9±0.92	/	26.9±2.37	0.64±0.01

Table 7. Histopathological findings of rats treated with Sumithion, Sumioxon and *p*-nitrocresol for 6 months.

Tissues	control		Sumithion 150ppm		Sumioxon 50ppm		*p*-nitrocresol 1500ppm	
	male	female	male	female	male	female	male	female
Brain	—	—	—	—	—	—	—	—
Eye	—	—	—	—	—	—	—	—
Spinal cord	—	—	—	—	—	—	—	—
Peripheral nerve	—	—	—	—	—	—	—	—
Bronchus	—	—	—	—	—	—	—	—
Lung*	±	±	±	±	±	—	—	—
Heart	—	—	—	—	—	—	—	—
Spleen	—	—	—	—	—	—	—	—
Bone marrow	—	—	—	—	—	—	—	—
Lymph node	—	—	—	—	—	—	—	—
Thymus	—	—	—	—	—	—	—	—
Esophagus	—	—	—	—	—	—	—	—
Stomach	—	—	—	—	—	—	—	—
Small intestine	—	—	—	—	—	—	—	—
Large intestine	—	—	—	—	—	—	—	—
Liver	—	—	—	—	—	—	—	—
Pancreas	—	—	—	—	—	—	—	—
Kidney	—	—	—	—	—	—	—	—
Urinary bladder	—	—	—	—	—	—	—	—
Testis	—	/	—	/	—	/	—	/
Prostate	—	/	—	/	—	/	—	/
Ovary	/	—	/	—	/	—	/	—
Uterus	/	—	/	—	/	—	/	—
Pituitary	—	—	—	—	—	—	—	—
Thyroid	—	—	—	—	—	—	—	—
Adrenal	—	—	—	—	—	—	—	—

* Pneumonia or abscess

Table 8. Mortality of rats treated with Sumithion for 92 weeks.

Sex	Dose ppm	No. of rat	Number of dead animals 0～12 week	12～24	24～48	48～60	60～72	72～84	84～92	Mortality	%
Male	0	15	0	1	2	0	1	3	0	7/15	46.7
	2.5	15	3	0	0	0	1	2	2	8/15	53.3
	5	15	0	0	2	2	1	0	3	8/15	53.3
	10	15	0	0	3	0	1	3	0	7/15	46.7
Female	0	15	1	1	3	0	1	1	0	7/15	46.7
	2.5	15	2	0	0	1	2	1	2	8/15	53.3
	5	15	0	0	1	0	4	1	1	7/15	46.7
	10	15	1	0	0	1	1	2	2	7/15	46.7

the feeding period, as shown in Table 8, mainly due to pneumonia, which indicates that Sumithion had no adverse effects on survival of the animals. Fig. 1 shows change of plasma cholinesterase activity. The enzyme was not inhibited by 2.5 ppm Sumithion throughout the 92 week experimental period. At 5 ppm the cholinesterase activity was slightly depressed at the early period of feeding, but it tended to recover on longer feeding. The tendency was similar to that reported by other workers[18]. Sumithion at 10 ppm was significantly inhibitory, just like the

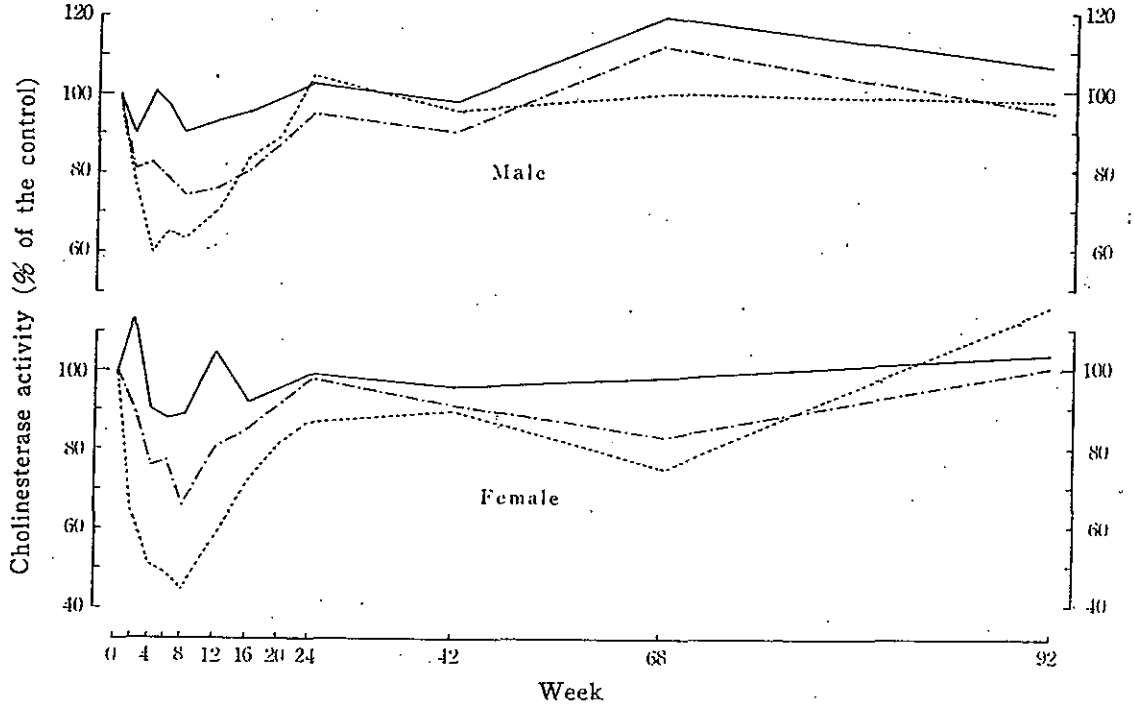

Fig. 1. Changes of plasma cholinesterase activity in rats treated with Sumithion for 92 weeks.
—— 2.5 ppm —·—·— 5.0 ppm ----- 10 ppm

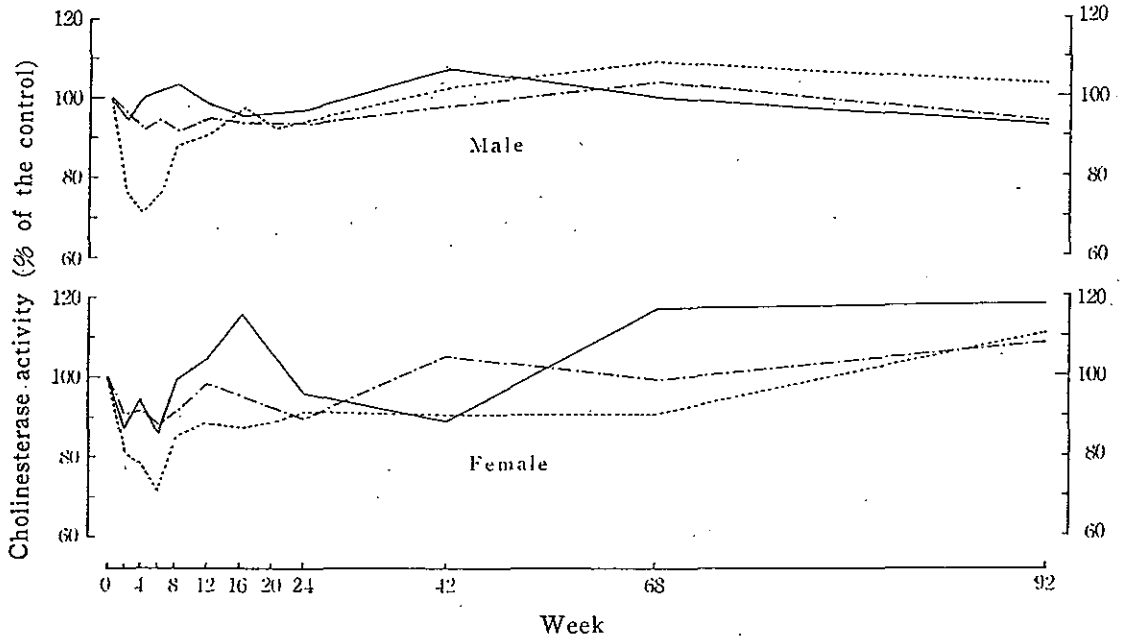

Fig. 2. Changes of red cell cholinesterase activity in rats treated with Sumithion for 92 weeks.
—— 2.5 ppm —·—·— 5.0 ppm ----- 10 ppm

Table 9. Brain cholinesterase activity of rats treated with Sumithion for 92 weeks.

Dietary level ppm	Male		Female	
	ΔPH[a)]	%	ΔPH[a)]	%
0	0.918±0.041	100	0.905±0.016	100
2.5	0.911±0.024	99.3	0.906±0.078	100
5	0.880±0.020	95.9	0.896±0.026	99.0
10	0.860±0.034	93.7	0.865±0.016	95.6

a) ΔPH for 60min. at 37.5°C
Mean ± standard error is given. (n=15)

previous trial.

Red blood cell cholinesterase was again less susceptible, as shown in Fig. 2, to Sumithion than the plasma enzyme and even at 10 ppm the inhibition was not so significant. The activity of brain cholinesterase of the treated rats was comparable to the control, as shown in Table 9. Histopathological examination of the animals sacrificed at the termination of the feeding revealed no adverse effects which were due to administration of Sumithion.

Based on the supplementary experiment, no effect level of Sumithion with respects to cholinesterase inhibition can be determined to be 5 ppm in food or 0.27 mg/kg body weight/day in male or 0.28 mg/kg/day in female, which is rather close to the no effect level in dog (5 ppm in food or 0.15 mg/kg/day)[17] or in human (0.2 mg/kg/day by subacute oral administration to volunteers)[19].

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