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STUDIES ON THE MAMMALIAN TOXICITY OF FENTHION
(Dimethyl 4-methylthio-3-tolyl phosphorothionate)

by

Jean I. Francis and J. M. Barnes¹
Toxicology Research Unit, Medical Research Council Laboratories,
Woodmansterne Road, Carshalton, Surrey

Fenthion (dimethyl 4-methylthio-3-tolyl phosphorothionate)² is an organophosphorus insecticide developed by Schrader (1960) which has been found to be of value as a residual spray for the control of anopheline mosquitos acting as vectors of malaria. Preliminary studies carried out under the auspices of WHO have demonstrated the persistence of fenthion as a deposit on different types of surface. In the same investigations it was found that some of the residents in sprayed dwellings in Nigeria showed a significant fall in the activity of their whole blood cholinesterase (Elliott & Barnes, 1962).

Further work in Nigeria confirmed these observations on the residents of sprayed premises and demonstrated that the fall in cholinesterase was confined to the activity of the plasma enzyme (Taylor, 1961).

Published data on the toxicity of fenthion to laboratory animals is very limited; the LD₅₀ of a single dose by mouth to rats is reported as 250 mg/kg (Schrader, 1960). In an unpublished report, Dubois & Leighton (1959) record the approximate LD₅₀ to mice, rats and guinea pigs and their data will be compared in the appropriate place with the findings recorded in this paper. Dubois & Leighton also record the poor anticholinesterase inhibitory activity of fenthion (I) and assume that this will be metabolized to the corresponding phosphate (VI) in the mammal. However, in considering the

¹ Investigation carried out with the support of and on behalf of the World Health Organization.

² Other designations that have been used for this compound include Baytex; Bayer 29493.

toxicity of fenthion, account must be taken of oxidation taking place also at the thioether linkage to give the corresponding sulphoxide (II) and sulphone (IV). Schrader (personal communication) states that oxidation will take place preferentially here and only after this has happened will the oxidation of the thionophosphate to the phosphates (III and V) take place. For these reasons some comparative tests were made between fenthion itself and the corresponding sulphoxides and sulphone. The formulae are represented in Table 1. Compound VI can be prepared in the laboratory and will be oxidized in the body to Compound III but Schrader believes it improbable that any conversion from I to VI takes place in mammalian tissues.

The observations on laboratory animals are described in this paper and the findings discussed in relation to the possible health hazard that may confront those who have to apply fenthion by the conventional methods as a residual spray in domestic and other premises.

Materials and methods

Technical fenthion (96.5 per cent. purity) supplied through WHO was used in the majority of experiments. Samples of pure materials (Compounds I-VI, Table 1) were a gift from Dr G. Schrader.

The animals used and the diets they received were as follows:

Rats	M & F	150-250 g	Albino, Porton strain	Diet 41B (Bruce & Parkes, 1957)
Mice	M & F	18-25 g	Swiss TO strain, NIMR, Mill Hill	Diet 41B do.
Guinea pigs	M	600-900 g	Mixed breed	Diet SGI pellets (Short & Gammage, 1959)
Rabbits	M	2-2.8 kg	Mixed breed	Diet SGI pellets do.
Hens	F	500 g & 2 kg	RIR x Light Sussex	Grower pellets

Oral dosing was by stomach tube using solutions in arachis oil except where otherwise stated. For prolonged administration a solution in arachis oil was blended with diet 41B powder (10 ml/kg) and fed in jars.

Percutaneous application was of the undiluted material to the clipped skin over the back. Rats were held in small wire cages for six hours after skin application to prevent licking and a cardboard collar fitted to the rabbits for the same purpose. All these test animals were housed singly during the course of the experiments.

Cholinesterase activity of tissues and blood was determined by the method of Aldridge & Barnes (1961) and the inhibitory activity of the compounds was measured by the method of Aldridge (1950), using solutions in dimethylformamide. Animals were killed with coal gas.

The LD₅₀ values and their fiducial limits were determined by the method of Weil (1952).

Results

1. General picture of the toxic reaction

(a) Oral administration. The approximate LD₅₀ for different species given a single dose of fenthion by mouth are given in Table 2. Guinea pigs tested in these laboratories proved refractory to the toxic effects of fenthion in doses up to 1000 mg/kg and no increase in toxicity was noted when the solvent used by Dubois & Leighton was substituted for arachis oil after a dose of 800 mg/kg. There was, however, an average depression of the activity of plasma and red cell cholinesterase of 78 and 51 per cent. respectively in three guinea pigs killed 48 hours after a dose of 800 mg/kg of fenthion by mouth. The guinea pigs responded in the expected manner to parathion which proved lethal at 30 mg/kg. The hen was exceptionally sensitive to fenthion. Male rats are considerably more sensitive than female rats when fenthion was given by mouth.

The signs of poisoning were typically those produced by a cholinergic poison but in the male rat given an LD₅₀ dose fasciculations did not appear until 40-60 minutes after dosing; for female rats the interval was 60-90 minutes. Salivation, tremors, gross weakness and chromodacryorrhoea followed. Deaths first occurred 6-8 hours after dosing and might not take place for up to 72 hours in the case of male rats. Survivors remained hyperexcitable and irritable for 6-8 days. The smallest single oral dose producing signs of poisoning in rats

was 25 mg/kg. Male and female mice behaved alike. Fasciculations appeared 45-60 minutes after dosing followed by salivation and the secretion of a milky fluid round the eyes. Deaths first took place 1-1/2 to 4 hours after dosing and no males died later than 24 hours but a few females died between 30-48 hours. Survivors were lively and apparently normal within 48 hours. Rabbits were not visibly affected until the dose approached the LD_{50} . A general restlessness followed by excessive salivation and rapid noisy breathing preceded weakness and muscle fasciculations which usually appeared about 1-1/2 hours after dosing. In animals fatally poisoned the fasciculations disappeared within a further four hours, while salivation continued. At 24 hours the animals were weak and showed evidence of diarrhoea and incontinence of urine. Deaths occurred between 24-48 hours after dosing. Hens developed excessive salivation about two hours after dosing, followed by diarrhoea and the characteristic cholinergic spasticity. Deaths occurred 24-48 hours after dosing.

Two hens which just survived a dose of 25 mg/kg after showing severe cholinergic poisoning with a depression of their plasma cholinesterase of 92-95 per cent. at 24 hours, made a complete recovery and no delayed neurotoxic effects were seen.

The effects of repeated oral dosing were studied on male rats when discrete daily doses were given for five successive days. Groups of six rats each survived 10 and 25 mg/kg/day. Only one of four survived five daily doses of 50 mg/kg and none daily doses of 100 mg/kg. Thus the LD_{50} dose, if given in fractions on successive days, may be more dangerous than if received as a single dose. As a single dose 10 mg/kg produced no fasciculations whereas 25 mg/kg did produce obvious signs of poisoning. A group of female rats were given 200 mg/kg fenthion as a single oral dose (approximately one-third LD_{50}) and pairs killed 1, 2, 3, 4, 8 and 14 days after and the activity of brain acetylcholinesterase determined. Table 3 shows that the depression was maximal at 48 hours and recovery was incomplete even after 14 days. The largest dose producing no significant depression of the brain cholinesterase at 48 hours was 5 mg/kg. More prolonged

administration of fenthion to rats was achieved by incorporating it in their diets. Groups of 12 male and 12 female rats were each given diets containing 250, 20, 10 and 5 p.p.m. fenthion and 6 male and 6 female remained as controls. Weekly records of food intake and weight changes were made and after each week 2 males and 2 females were killed, together with one pair of controls and the brain cholinesterase activity measured. After four weeks the survivors were returned to a normal diet and the recovery followed by killing pairs at 1-6 week intervals. The findings are recorded in Table 4.

Differences in the sensitivity of male and female rats were not evident when fenthion was administered over long periods in this way. Mild and transient signs of poisoning were only seen in the rats on 250 p.p.m. despite the fact that their brain cholinesterase levels were reduced by over 80 per cent by the second week. Recovery of the activity of brain cholinesterase was comparatively slow.

(b) Percutaneous toxicity

Undiluted fenthion was applied from a micrometer syringe fitted with a polythene-tipped needle to the recently clipped skin. No covering was applied nor was the insecticide washed off. For male and female rats the LD₅₀ for a single skin application was 500 mg/kg (limits about 480-520). After a single dose fasciculations appeared in 4-1/2 to 6-1/2 hours, gradually increasing while the rats became hypersensitive to stimuli. Deaths occurred 3-5 days after dosing. Survivors at the lower doses (200 and 400 mg/kg) continued to exhibit tremors and remained excitable for 7-14 days. No differences were noted in the behaviour of male and female rats given fenthion by the dermal route. For a dose applied on five successive days the LD₅₀ was calculated to be 73 mg/kg/day, i.e. a total of 365 mg/kg or less than the single dose LD₅₀. Deaths occurred from 4-9 days after the first doses and survivors recovered by the tenth to twelfth day. The maximum dose tolerated for five successive daily applications was 60 mg/kg. For comparison single doses of the sulfoxide and the sulfone were applied to the skin of male rats. The sulfoxide produced no signs of poisoning at doses of 800 mg/kg or below. At 1600 and 2000 mg/kg some fasciculations were produced; at 3000 mg/kg

one of four rats died. The sulfone produced fasciculations at 400 mg/kg which were more severe after 800 mg/kg. At 1600 mg/kg four out of four rats died 48 to 144 hours after dosing.

In further experiments a group of rats received successive doses of fenthion applied to the clipped skin five days a week. At intervals a pair of rats (one male and one female) were killed for the determination of blood and brain cholinesterase activity and in the survivors the daily dose was raised. The results are summarized in Table 5 and indicate that over 18 weeks the survivors had received just over 4000 mg/kg. Only one rat showed severe weakness and signs of general poisoning. The rest showed fasciculations and some weakness usually just after the dose had been raised. These doses had reduced the blood and brain cholinesterase activity very considerably. Those animals getting 20 mg/kg by the dermal route were receiving a dose not dissimilar to the group on a diet of 250 p.p.m. fenthion. In order to find the largest dose that could be applied on four successive days without reducing the blood cholinesterase, male rabbits were used and the plasma and red cell cholinesterase activity determined before and on the day following the fourth successive application of fenthion to the clipped skin. The results are given in Table 6.

2. Therapy

The possible value of the reversing agent pyridine-2-aldoxime methane sulfonate (P_2S) combined with atropine was tested on male rats given a single dose of fenthion by mouth equivalent to $3 \times LD_{50}$ (645 mg/kg). In all experiments P_2S was given subcutaneously in saline in doses of 50 mg/kg with or without atropine (17.4 mg/kg).

A single dose of P_2S with or without atropine given an hour after fenthion reduced the fasciculations temporarily but had no effect on mortality.

In another experiment P_2S and atropine were given hourly (10 a.m. to 5 p.m.) on the first and second day after the fenthion. Three of six animals so treated survived, though they were ill for seven days. Even on the second day the injections of P_2S invariably reduced the fasciculations and improved the condition of the rats.

In further experiments P_2S was given as the fasciculations reappeared at any time during the two days or four days following the fenthion. Two out of six survived after treatment for two days and four out of six when treatment was extended over four days. It is clear therefore that while atropine and P_2S are able to counteract the toxic effects of fenthion, a single dose of this insecticide is so slowly metabolized and excreted that poisoning persists for several days and treatment, to be effective, must be maintained for as long as new cholinesterase inhibitor is being produced from the fenthion that has been absorbed.

3. Mechanism of action

Fenthion itself produces no inhibition of cholinesterase so that it must be converted to an active inhibitor in vivo since a dose of fenthion produces a profound fall in cholinesterase activity. As mentioned in the introduction, the changes that take place in fenthion in the animal are probably not confined to the oxidation of $P=S \rightarrow P=O$. The long delay between dosing and the appearance of signs of poisoning which cannot be reduced even if fenthion is given intravenously indicate the probability of other changes. The greater sensitivity of the male vs female rat is also the reverse of that usually seen when the toxicities of $P=S$ compounds are compared in the sexes of this species.

Because of the routes of metabolism suggested by Schrader (see Table 1) an opportunity was taken to compare the toxicities, time of onset of symptoms and in vitro inhibitory activity against cholinesterase of fenthion and the five analogues given in Table 1.

The LD_{50} of each determined in earlier experiments was given to rats in the same volume of 90 per cent. glycerol formol, 10 per cent. ethanol (the best common solvent for the compounds). The first onset of fasciculations was noted. The results are presented in Table 7. The delay in the onset of fasciculations does support the idea that in Compounds I and IV conversion to the sulfoxide or sulfone must take place. However, despite its relatively high toxicity and speed of onset of poisoning it will be seen that VI has a very poor activity against cholinesterase in vitro thus suggesting that some further metabolic transformation is necessary before the true poison is produced.

Many of the metabolic transformations of drugs and poisons take place in the liver and some of the reactions catalysed by a system in the liver cell microsomes are inhibited by the compound SKF 525. Six rats were given 50 mg/kg SKF 525 intraperitoneally one hour before 396 mg/kg (LD_{90}) of fenthion was given by mouth. Whereas six rats receiving such a dose of fenthion alone all showed severe fasciculations within an hour, of those pre-treated with SKF 525 only one showed any fasciculations within 2-1/2 hours of receiving the fenthion. This was the only rat of the six so treated that died and at 48 hours the survivors were much improved, whereas all those receiving fenthion alone died within 24-48 hours. Murphy, Anderson & Dubois (1959) have shown that tri-ortho-cresylphosphate much increases the toxicity of malathion by inhibiting an esterase that normally destroys malathion in the rat. Rats dosed 24 hours earlier with 50 mg/kg TOCP responded as untreated rats to the LD_{10} , LD_{50} and LD_{90} of fenthion given by mouth.

Discussion

Fenthion is an organophosphorus insecticide of intermediate toxicity to mammals though it displays considerable differences in its toxicity to various species. Fowls, on the other hand, are very sensitive to fenthion.

If the behaviour of the rat is considered in some detail fenthion is seen to differ from a number of other well-known phosphorothionates of the same general type acting as anticholinesterases after administration. The signs of poisoning from a single oral dose develop rather slowly but persist for several days. There is little difference if the fenthion is injected, even by the intravenous route. Furthermore, male rats are more sensitive than female rats, whereas for most phosphorothionates the converse is true. Male rats are, however, more sensitive than females to Schradan when the oxidation of a methyl amino group is necessary for the production of the toxic anticholinesterase. These observations on rats suggest that fenthion is not converted to the toxic anticholinesterase by a simple oxidation $P=S \rightarrow P=O$. It has been stated (see introduction) that the sulfoxide and sulfone of the thio ether are produced before the $P=S \rightarrow P=O$ oxidation takes place. It has been found that both these compounds when given orally to rats are more toxic than fenthion itself and

their effects come on more rapidly than after fenthion. However, neither compound is an active anticholinesterase, so that presumably the usual $P=S \rightarrow P=O$ oxidation must also take place before the cholinesterase is inhibited. However, even the sulfoxide of the phosphorothiolate (III) with an LD_{50} of 30 mg/kg has a rather poor activity as an inhibitor (Table 7) and this suggests that yet a further change takes place before the final toxic product is released.

The apparent complexity of the metabolic transformation needed in order to convert fenthion to its final toxic product makes it easier to understand the differences among species in their sensitivity to this compound. On the other hand, it makes it difficult to predict whether man will behave like the sensitive fowl or the insensitive guinea pig.

The other unusual reaction to fenthion is the very prolonged effect from a single dose. This suggests that a large part of the compound must be stored soon after administration and then slowly released to be metabolized. That there is a continuous production of new anticholinesterase is suggested by the response to P_2S even four days after dosing when fasciculations are still reversed and reappear in an hour or so. If the prolonged effects were due to a persistent inhibition of cholinesterase from one wave of inhibitor, fasciculations would not reappear after successive doses of P_2S . From the practical point of view the use of P_2S in therapy of any case of accidental poisoning would need to be repeated and prolonged. Fortunately the low toxicity of this oxime means that this would be safe to recommend.

Clearly much further work on the absorption, distribution and metabolism of fenthion in different animal species is required before a full understanding of its toxicity and the potential hazard it presents can be appreciated.

If oxidation to the sulfoxide and sulfone takes place on sprayed surfaces, products more toxic to mammals by mouth are produced but neither penetrate the skin as readily as fenthion itself. Unfortunately it is not known by what route the insecticide deposits are absorbed by the residents of the sprayed houses. The prolonged action of a single dose of fenthion would suggest that repeated small doses absorbed in such premises are more likely to cause a cumulative effect than would be the case with the organophosphorus compound with a more transient action.

TABLE 1. FENTHION AND ITS OXIDATION PRODUCTS

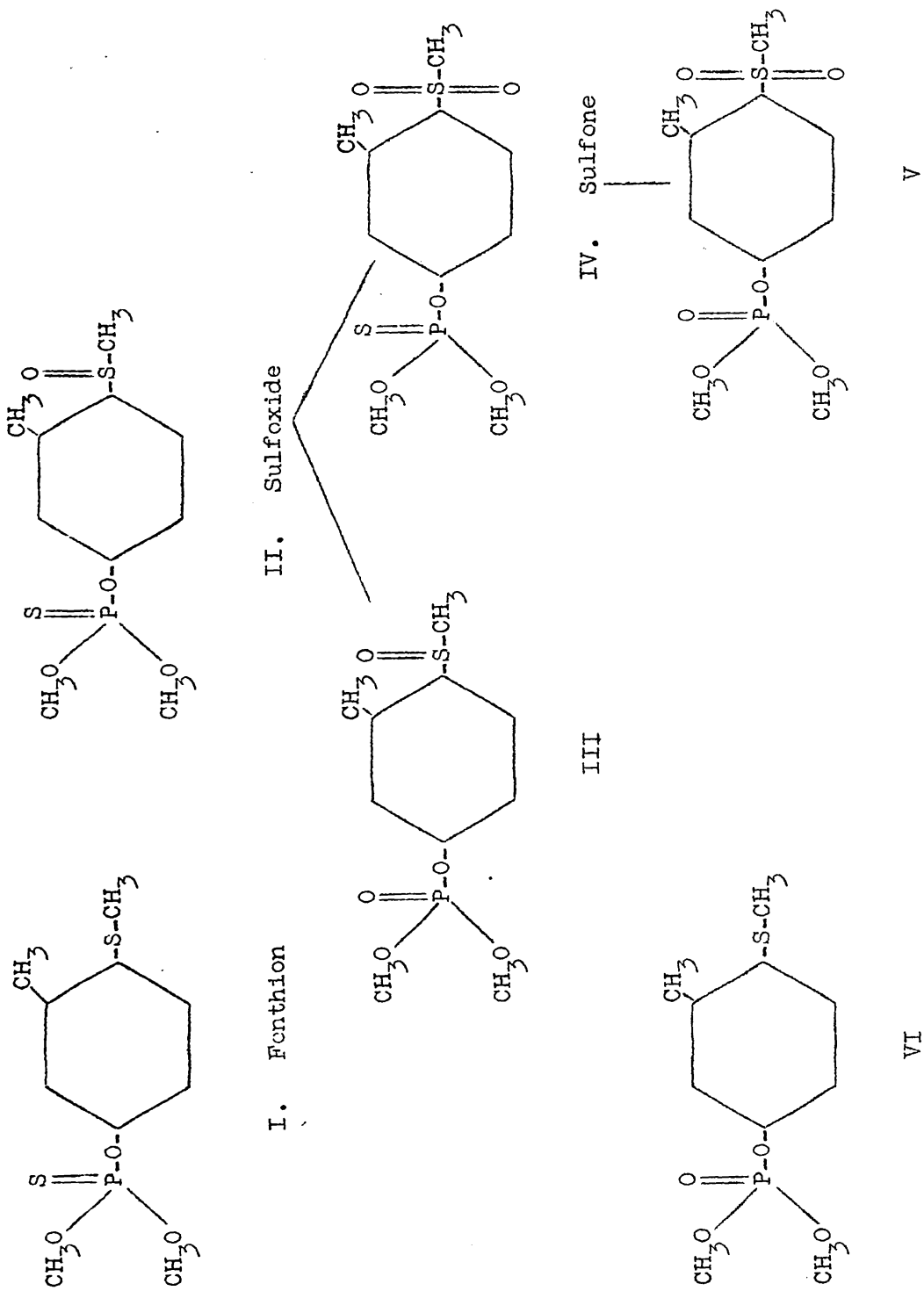


TABLE 2. THE APPROXIMATE LD₅₀ FOR FENTHION (TECHNICAL 96.5 PER CENT.)
 GIVEN AS A SINGLE ORAL DOSE TO ANIMALS SUBSEQUENTLY OBSERVED
 FOR 7-10 DAYS

Species	Sex	LD ₅₀ mg/kg	Dubois & Leighton
Rat	M	215 (108-250)	190
	F	615 (548-687)	310
Mouse	M	150 (126-178)	
	F	190 (145-277)	
Guinea pig	M	>1000	260
Hen		30-40	
Rabbit	M	150-175	

TABLE 3. BRAIN CHOLINESTERASE ACTIVITY IN FEMALE RATS RECEIVING A
 SINGLE DOSE OF FENTHION BY MOUTH. BRAIN HOMOGENATE
 WAS USED WITH ACETYLCHOLINE CHLORIDE AS SUBSTRATE

(a) Activity at different times after an oral dose of 200 mg/kg.

Days	Per cent. activity
1	47.6
2	24.5
3	31.0
4	39.0
8	51.6
14	73.5

(b) Activity at 48 hours after a single oral dose

Dose mg/kg	Per cent. activity
100	42.2
50	61.1
20	80.0
10	86.4
5	97.0
2.5	100

TABLE 4. THE EFFECT OF FEEDING FENTHION FOR FOUR WEEKS AT DIFFERENT CONCENTRATIONS IN THE DIET OF MALE AND FEMALE RATS, ON THE GROWTH, FOOD INTAKE AND BRAIN CHOLINESTERASE ACTIVITY, AND THE RECOVERY WITHDRAWAL

Average starting weights 225 g male and 185 g female.

Weight changes are weekly unless otherwise indicated.

For each group of animals receiving fenthion there was a group of controls whose weight gain and food intake is given in brackets.

Male rats					
Fenthion in diet	Week	Weight gain (g) <u>/C/</u>	Food intake (g) <u>/C/</u>	Calc. dose mg/kg	Brain ChE % activity
250 p.p.m.	1	+0.5 (+3)	102.5 (108.8)	85.4	22
	2	-7 (+13.7)	102.5 (101.8)	90.0	15.8
	3	-9.2 (+7.5)	119.0 (90.0)	110.0	17.0
	4	+5 (+6.3)	127.5 (103.0)	114.0	16.2
	2	+10.8 (+6.8) (in 2 weeks)	-	-	53.4
	4	+29.2 (+5) (in 2 weeks)	-	-	93.6
20 p.p.m.	1	-0.8 (+5.9)	135 (133.3)	10.3	79.2
	2	+3.8 (+15.5)	160 (136.0)	11.6	73.6
	3	+12.1 (+23.0)	176 (138.0)	11.6	65.7
	4	+12.1 (+5.0)	189 (125.0)	11.9	62.5

TABLE 4 (continued)

Male rats					
Fenthion in diet	Week	Weight gain (g) \overline{C}	Food intake (g) \overline{C}	Calc. dose mg/kg	Brain ChE % activity
Off diet	2	+7.9 (+10) (in 2 weeks)	-	-	70.5
	6	+18.4 (+10) (in 4 weeks)	-	-	83.0
10 p.p.m.	1	+0.8 (-8.3)	133 (133.3)	6.1	91
	2	+20.2 (+19.5)	160 (160.0)	6.4	83.0
	3	+25.8 (+36.8)	151 (172.5)	6.0	75.0
	4	+24.3 (+17.2)	139 (150.0)	4.8	74.0
Off diet	1	+17.6 (+22.5)	-	-	80.0
	4	+48.8 (+77.5) (in 3 weeks)	-	-	88.0
5 p.p.m.	1	+3.8 (+3.3)	127.5 (133.3)	2.9	94.0
	2	+27.0 (+28.9)	157.5 (160.0)	2.8	96.0
	3	+18.0 (+22.0)	170.0 (175.0)	3.2	91.2
	4	+13.5 (+11.7)	151.5 (138.3)	3.1	85.0
Off diet	1	+18.5 (+23.3)	-	-	86.3
	3	+30.0 (+30) (in 2 weeks)	-	-	90.0

TABLE 4 (continued)

Female rats					
Fenthion in diet	Week	Weight gain (g) $\overline{[C]}$	Food intake (g) $\overline{[C]}$	Calc. dose mg/kg	Brain ChE % activity
250 p.p.m.	1	-5.8 (+9.2)	105 (100)	143.6	23.3
	2	-0.5 (+1.1)	94.5 (107)	127.0	16.6
	3	+10.2 (+12.6)	115.5 (112.5)	152.6	14.3
	4	+0.75 (+8.3)	102.5 (108.3)	122.5	14.0
Off diet	2	+7.5 (+9.5) (in 2 weeks)	-	-	61
	4	+12.3 (+10) (in 2 weeks)	-	-	87
20 p.p.m.	1	+1.2 (+6.7)	111.2 (110.0)	10.5	80.2
	2	0 (+6.8)	143.0 (120.0)	14.2	58.0
	3	+11.6 (+8.0)	127.4 (122.5)	11.0	61.0
	4	+6.0 (+11.7)	174.6 (132.0)	11.9	59.8
Off diet	2	+3.0 (+0)	-	-	68.3
	6	+16.8 (+10) (in 4 weeks)	-	-	80.0
10 p.p.m.	1	-7 (+6.4)	130 (125.8)	6.2	96.0
	2	+11.5 (+7.0)	130 (126.0)	5.8	82.5
	3	-2.6 (-0.8)	125 (136.3)	5.7	81.0
	4	+5.5 (+3.8)	92.5 (141.7)	4.0	74.3
Off diet	1	+3.6 (+2.5)	-	-	82.0
	4	+15 (+3.5) (in 3 weeks)	-	-	83.3
5 p.p.m.	1	+9.5 (+7.4)	90.5 (108.3)	2.9	92.8
	2	+12.0 (+6.9)	107.0 (145.0)	2.8	91.4
	3	+3.0 (+12.2)	111.5 (132.5)	3.2	97.3
	4	+13.3 (+13.2)	117.5 (123.3)	3.1	88.8
Off diet	1	9.2 (+15)	-	-	93.0
	3	+7.5 (+15) (in 2 weeks)	-	-	94.0

TABLE 5. THE EFFECT OF REPEATED DAILY APPLICATIONS OF FENTHION TO THE CLIPPED SKIN OF RATS

Daily dose (mg/kg)	Total period (days)	Total dose	No. of rats	% normal activity ChE	
				Brain	RBC
20	19	380	12 (6M + 6F)	18	19.4
20	24	480	10 (5M + 5F)	24	20.3
20	25)	740	8 (4M + 4F)	15	22.5
40	6)				
20	25)	770	7 (3M + 4F)	11.8	17.3
40	6)				
60	5)				
20	25)	2 390	5 (2M + 3F)	11.2	
40	6)				
60	27				

TABLE 6. THE ACTIVITY OF RED CELL AND PLASMA CHOLINESTERASE IN MALE RABBITS DETERMINED 24 HOURS AFTER THE LAST OF FOUR SUCCESSIVE DAILY APPLICATIONS OF FENTHION TO THE CLIPPED SKIN. THE RESULT IS EXPRESSED AS A PERCENTAGE OF THE PRE-TREATMENT LEVEL OF EACH ANIMAL

Dose mg/kg	RBC	Plasma
5	76%	86%
2.5	75%	93%
1.5	100%	90%

TABLE 7. TOXICITY OF FENTHION AND ITS OXIDATION PRODUCTS

(Male rats given the dose of each compound by mouth as a solution in 99-per cent. glycerol formal/10 per cent. ethanol made up so that the volume of solvent was the same).

Cholinesterase inhibition measured against human red cells.

Compound	Dose mg/kg	Onset of Fascicu- lations (min)	Mortality	Conc. for 50% inhibition
P=S - SCH ₃ I	220	37 (30-46)	3/4	$> 5 \times 10^{-4} \text{ M}$
P=S - SOCH ₃ II	125	16 (14-19)	3/4	$4.5 \times 10^{-5} \text{ M}$
P=S - SO ₂ CH ₃ III	125	16 (11-27)	1/4	$4.7 \times 10^{-4} \text{ M}$
P=O - SCH ₃ IV	125	20 (19-21)	1/4	$2.65 \times 10^{-6} \text{ M}$
P=O - SOCH ₃ V	50	12 (11-12)	1/4	$4.8 \times 10^{-5} \text{ M}$
P=O - SO ₂ CH ₃ VI	30	9 (8-10)	2/4	$3.2 \times 10^{-5} \text{ M}$

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