Brit. J. Pharmacol. (1947), **2**, 234.

THE TOXICITY OF ALKYL FLUOROPHOSPHONATES IN MAN AND ANIMALS

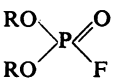
BY

B. A. KILBY AND M. KILBY

From the Physiological Laboratory, Cambridge

(Received March 13, 1947)

In May, 1940, we prepared the dimethyl and diethyl esters of fluorophosphonic acid and tested their effects on animals as lethal inhalants. When, about a year later, McCombie and Saunders prepared the di*iso*propyl ester, we found it had an even more powerful effect than the dimethyl and diethyl compounds.



$R = CH_3$, C_2H_5 or $(CH_3)_2CH$.

dialkyl fluorophosphonate

The present paper deals with this early and first systematic study of the toxicity of the fluorophosphonates which are now widely used for physiological experiments and clinical trials. When the toxic action was discovered it was not possible for us to publish the results, which were, however, circulated as a report to the Ministry of Supply. (Adrian, Kilby, and Kilby, 1940.)

The preparation of the fluorophosphonates and this study of their toxic properties was prompted by an observation of Lange and Krueger (1932). They had prepared the dimethyl and diethyl compounds and had stated at the end of their communication that inhalation for a few minutes might lead to difficulty in breathing, to disturbance of vision, hypersensitivity to light, and even to loss of consciousness.

METHODS

The dimethyl and diethyl fluorophosphonates used in these experiments were prepared by the method described by Lange and Krueger, and the di*iso*propyl ester was kindly supplied by Dr. H. McCombie, Dr. B. C. Saunders and their research team, who made it by the method announced in a preliminary communication. (McCombie and Saunders, 1946.)

The action and toxicity of fluorophosphonates were studied in man by inhalation and in animals by inhalation as well as by intravenous or subcutaneous injection.

Inhalation in man

An approximately cubical steel-framed glass chamber of 10 cu.m. capacity was used. The desired concentration of ester vapour was obtained by dissolving the calculated

weight of the compound in about 20 c.c. of ether and spraying it into the chamber by means of an atomizer worked by compressed air, mixing being achieved by three electric fans. After about 30 sec., the subjects, one to four in number, who had been waiting in an air-lock, walked into the chamber and quickly shut the door behind them. At the end of the exposure, the subjects left through the air-lock, and the chamber was cleared by a large suction fan. In the calculation of the concentration (e.g. 1 in 100,000) it is assumed that the ester is completely volatilized and that the gram-molecular weight occupies a volume of 22.4 litres.

Inhalation in animals

Static method.—A wood-framed glass chamber of 1.78 cu.m. capacity was used. A batch of animals (e.g., 3 rabbits, 4 guinea-pigs, 6 rats, and 10 mice) was placed inside in wire cages, the chamber sealed and the calculated amount of fluorophosphonate dissolved in 20 c.c. of ether sprayed into it. The exposure was timed from when about half of the material had been dispersed, an operation which usually took less than a minute. Mixing was achieved by an electric fan in the chamber. At the end of the exposure, an exhausting fan was switched on, the chamber opened and the cages removed by two operators wearing service respirators. The animals were removed to another room and kept under observation. Post-mortem examinations were made on animals that died.

Constant-flow technique.—An apparatus was constructed containing an all-glass exposure chamber of about a litre capacity fitted with ground glass joints, in which either one rat or four mice could be placed. A constant stream of air, at a known rate of flow, was passed through this chamber, either by using compressed air which was passed through a gas-meter in series with the chamber or, in other experiments, by suction, measured amounts of water being run out of a 20-litre aspirator. All or part of the air stream could be passed through a bubbler containing di*iso*propyl fluorophosphonate, which was weighed before and after the experiment to determine the amount of ester volatilized. The rate of volatilization was also controlled by varying the temperature of the water-bath in which the bubbler was immersed. An identical bubbler containing sulphuric acid was fitted in parallel, and in an exposure pure air was passed through the chamber until conditions were steady and the animal calm, and then by means of a three-way tap the air stream was caused to pass through the ester for the desired time. In this manner it was possible to make 1-min. exposures, because the desired concentration was rapidly attained at the beginning of the exposure, and the ester quickly swept out at the end. When only a part of the total air stream was passed through the bubblers, flow-meters were included in the circuit so that the air streams could be adjusted to give approximately the desired concentration of ester. The two air streams were allowed to mix in a suitable compartment before entering the exposure chamber. The bubblers, mixers and exposure chamber were connected by ground glass joints. When necessary, the gases leaving the exposure chamber were passed through towers containing activated charcoal or through service respirator canisters in order to remove the ester vapours.

RESULTS

Inhalation in man

The inhalation of air containing low concentrations of dimethyl, diethyl, or di*iso*propyl fluorophosphonate leads to effects qualitatively similar for all three esters, but varying in intensity with the nature of the ester dispersed, its concentration, and the time of exposure. Within a minute or so a feeling of tightness of the throat is noticed and respiration becomes slightly more laboured.

A few minutes later the pupils constrict to pin-point size and remain so for a long period; this causes the subject to experience the sensation that the room has dimmed, as though the sky had suddenly become heavily overcast. After a few hours, reading becomes almost impossible unless the book is held only a few inches from the eyes; at a distance of about 18 in. print appears blurred. This effect must be due to a strong constriction of the ciliary muscles. The di*iso*propyl ester has the most powerful action of the three compounds. When two subjects were exposed for 3 min. to a nominal concentration of 1 in 100,000 (82 mg./cu.m.) of this material, a tightness of the throat and slight difficulty in inspiration occurred during the exposure, and about 10 min. later the pupils constricted to pin-point size and remained so for days. The miosis subsided after about a week in the younger subject (28 years) and after two or three days in the elder (60 years). After about 24 hours there was eye-ache and headache which persisted for a day or two.

Inhalation in animals

Animals were exposed for longer periods and to higher concentrations than those used with man, and severe symptoms were produced, death frequently resulting. The effects were approximately the same in all species examined and with each of the three compounds. There was excessive salivation, nasal discharge, lacrimation and frequently pupil constriction, respiratory distress accompanied by intense gasping movements, and, in severely affected animals, convulsions leading to death, which usually occurred within about half an hour of the beginning of the 10-min. exposure, and sometimes actually during the exposure. The rapidity of action of these materials as lethal inhalants is noteworthy and the majority of animals that survived half an hour usually made a complete recovery, although a few deaths occurred up to 24 hours.

The mortalities produced in rabbits, guinea-pigs, rats, and mice by 10-min. exposures to various nominal concentrations of the three compounds are shown in Table I. For each experiment 3 rabbits, 4 guinea-pigs, 4 or 6 rats, and 4 or 10 mice were exposed in a static chamber. Of the four animal species, it will be seen that rabbits are the least and mice the most susceptible.

TABLE I

DEATHS RESULTING FROM 10-MIN. EXPOSURE OF SMALL ANIMALS TO VARIOUS CONCENTRATIONS OF FLUOROPHOSPHONIC ESTERS

Compound:	Methyl ester			Ethyl ester			*Iso*propyl ester	
Concentration:	1/5,000	1/10,000	1/20,000	1/5,000	1/10,000	1/20,000	1/10,000	1/20,000
Rabbits	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3
Guinea-pigs ..	3/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
Rats	4/4	4/6	1/4	4/4	0/6	1/4	6/6	4/6
Mice	4/4	10/10	4/4	4/4	10/10	0/4	10/10	3/10

A more detailed study was made of the toxicity of the di*iso*propyl ester for rats and mice; the concentration necessary to kill 50 per cent of the animals exposed (LC50) was determined for each of five constant exposure times (1, 2, 5, 10, and 30 min.) by the constant-flow technique. A convenient measure of the toxicity of a lethal inhalant is obtained by the product of the LC50 and the time of exposure in minutes (*t*). This value is referred to in the present paper as the LC*t*50. Ideally it should be a constant and independent of the exposure time, but if detoxification takes place during exposure LC*t*50 should increase greatly during long exposure to a low concentration; this is observed, for instance, when hydrogen cyanide is used as a lethal inhalant. The small changes observed in the values of LC*t*50 in our experiments with fluorophosphonates, however, were probably not statistically significant, but a minimum value of the LC*t*50 at 5–10 min. might be indicated. With the static method an increase in LC*t*50 with longer exposures may indicate that the concentration of toxic agent is falling off, but this effect can be eliminated by the constant-flow technique, in which a constant concentration is maintained by replacement. The LC*t*50 values for rats using 10- and 30-min. exposures were determined by both static and constant-flow methods, and almost identical values were obtained, indicating that no appreciable decay of concentration occurred in the static method up to 30 min., so that the nominal concentrations employed in the static method used to obtain the data in Table I are probably fairly close to the true concentration.

Estimates of the LC50 and LC*t*50 values for rats and mice are shown in Table II. These estimates were obtained, by graphical interpolation, from the results of experiments in which at least 4 animals were used to determine each point on the mortality–concentration curves; in all, 197 rats and 390 mice were used.

TABLE II

LC50 AND LC*t*50 VALUES FOR RATS AND MICE EXPOSED TO DI*iso*PROPYL FLUOROPHOSPHONATE. CONSTANT-FLOW TECHNIQUE

Animal	Exposure time min.	Deaths within 2 hr.		Deaths within 48 hr.	
		LC50 mg./cu.m.	LC*t*50 mg./cu.m./min.	LC50 mg./cu.m.	LC*t*50 mg./cu.m./min.
Rats ..	1	4,200	4,200	4,200	4,200
	2	2,000	4,000	1,800	3,600
	5	700	3,500	570	2,850
	10	360	3,600	280	2,800
	30	180	5,400	150	4,500
Mice ..	1	5,000	5,000	4,000	4,000
	2	2,650	5,300	1,900	3,800
	5	750	3,750	540	2,700
	10	440	4,400	350	3,500
	30	185	5,550	150	4,500

A more detailed analysis of the survival period of these animals shows that 55 per cent of the rats and 53 per cent of the mice had died within the first 2 hours after exposure; during the next 48 hours a further 15 per cent of the rats and 22 per cent of the mice died; there were no further deaths among the rats and only 1 per cent among the mice, 30 per cent of the rats and 23 per cent of the mice surviving. The rapid lethal action of fluorophosphonates becomes even more evident if the deaths occurring during the first two hours are grouped in half-hour intervals; if for this calculation the deaths after 30-min. exposure are omitted, it is found that the majority of animals died within the first half-hour after exposure. Of the 108 rats dying within the first two hours, 95 died within the first, 10 within the second, and 3 within the third half-hour period after the beginning of exposure; the corresponding figures for 207 mice dying during the first two hours were 183, 10, 9, and 8 mice respectively for the four half-hour intervals.

Injection into animals

After injection of a solution of the di*iso*propyl ester in normal saline into the ear vein of rabbits there was excessive salivation, muscular twitchings, and loss of muscular co-ordination, sometimes urination and defaecation and usually convulsions prior to death. The pulse-rate was slowed like the respiratory-rate, but respiration ceased before the heart stopped beating. There was constriction of the pupils, commencing 2–5 min. after injection. The LD50 determined in a small number of rabbits was 0.5–0.75 mg./kg.

The LD50 for mice for subcutaneous injection of the di*iso*propyl ester dissolved in normal saline was 4 mg./kg., determined by graphical interpolation of the results of injections into batches of mice over a suitable dose range.

Atropine

Since it had been found (see Adrian, Feldberg and Kilby, 1946, 1947) that fluorophosphonates were extremely strong inhibitors of cholinesterase, experiments were made to determine whether atropine would be an effective antidote.

In rabbits intravenous injections of atropine were made either before or immediately after intravenous injection of a lethal dose of di*iso*propyl fluorophosphonate. When atropine was injected after the ester it was incapable of saving life, but when it was injected before the ester it appeared to reduce the death rate. For instance, when atropine (in doses between 4 and 50 mg./kg.) was injected immediately after a lethal dose of di*iso*propyl fluorophosphonate (1 mg./kg.) the sole effect was to alleviate the severity of the symptoms and postpone death for a short time. This effect of atropine occurred only if the atropine was given immediately after the fluorophosphonate; if given only a few minutes later atropine had no alleviating effects whatever. When, on the other hand, atropine (10 mg. per kg.) was given intravenously 10 min. before a lethal intravenous dose of fluorophosphonate (1 mg./kg.) the appearance of symptoms was not only delayed and their severity reduced, but the lives of some of the animals were actually saved; for instance, out of five rabbits so treated, three survived and the other two died only after 1½ to 3½ days, whereas all five control rabbits, given the fluorophosphonate without atropine, died within 45 min.

Discussion

The toxic effects of the fluorophosphonates resemble in many respects those of eserine and prostigmine and are probably due to the anti-cholinesterase activity which these esters have been shown to exhibit. (Adrian, Feldberg, and Kilby, 1946, 1947.) Parasympathomimetic effects are very pronounced: the eye effects, the excessive salivation and lacrimation, and the slowing of the heart may easily be explained as being due to accumulation of acetylcholine released from the parasympathetic endings. It is thus not surprising that these effects are alleviated by atropine. In addition, the fluorophosphonates appear to have a definite "nicotine-like" action on skeletal muscle and on the central nervous system. The excitatory effects of fluorophosphonates on these structures are less pronounced, and the paralysing effects predominate. The fluorophosphonates share this predominance of a paralysing action with other anti-cholinesterases, and the problem of why under certain conditions some inhibitors of cholinesterase are mainly excitatory and others mainly depressant has never been satisfactorily explained. (For review see Feldberg, 1945.)

Death from fluorophosphonate poisoning probably results from respiratory failure, partly owing to obstruction of bronchioles, but mainly to paralysis of the respiratory centre; in this way, too, the fluorophosphonates resemble other anti-cholinesterases. However, no detailed analysis has been made of the exact nature of the cause of death.

Summary

1. Inhalation of fluorophosphonates in man and animals leads to respiratory distress, pupillary constriction, and spasm of accommodation. In animals, inhalation in higher concentrations than those used in man causes in addition excessive salivation, lacrimation, convulsions, and death, probably owing to respiratory failure. Similar effects are observed in animals on intravenous injection of fluorophosphonates.

2. The percentage of deaths among small animals after 10 min. exposure to various concentrations of dimethyl, diethyl, and di*iso*propyl fluorophosphonates is recorded. The concentration of di*iso*propyl fluorophosphonate which will kill 50 per cent of rats or mice exposed (LC50) was found for 1, 2, 5, 10, and 30 min. exposures. The product of the LC50 and the time of exposure in minutes (the LC*t*50) was found to vary between 2,700 and 4,500 mg./cu.m./min. The LC*t*50 showed a minimum for 5 to 10 min. exposures.

3. Death, if it occurred, usually took place within the first half-hour after exposure.

4. The LD50 of di*iso*propyl fluorophosphonate is about 4 mg./kg. for subcutaneous injection in mice and 0.5–0.75 mg./kg. for intravenous injection in rabbits.

5. Atropine given before the fluorophosphonate alleviated the severity of symptoms and reduced the death rate, but had little effect if given afterwards.

We are indebted to Professor E. D. Adrian and Dr. W. Feldberg for much helpful advice and criticism during this work, and to the Chief Scientist, Ministry of Supply, for permission to publish these results.

REFERENCES

Adrian, E. D., Feldberg, W., and Kilby, B. A. (1946). *Nature, Lond.*, **158**, 625.
Adrian, E. D., Feldberg, W., and Kilby, B. A. (1947). *Brit. J. Pharmacol.*, **2**, 56.
Adrian, E. D., Kilby, B. A., and Kilby, M. (1940). Report on dimethyl and diethyl fluorophosphonates to Ministry of Supply (July 4).
Adrian, E. D., Kilby, B. A., and Kilby, M. (1942). Report on physiological examination of di*iso*propyl fluorophosphonate (Part I) to Ministry of Supply (Aug. 12).
Barrett, A. A., Feldberg, W., Kilby, B. A., and Kilby, M. (1942). Report on physiological examination of di*iso*propyl fluorophosphonate (Part II) to Ministry of Supply (Nov. 10).
Feldberg, W. (1945). *Physiol. Rev.*, **25**, 596.
Lange, W., and Krueger, G. V. (1932). *Ber. dtsch. chem. Ges.*, **65**, 1598.
McCombie, H., and Saunders, B. C. (1946). *Nature, Lond.*, **157**, 287.