Dermal Absorption of Fenitrothion* in Rat

by

J. D. KOHLI, M. Z. HASAN, and B. N. GUPTA

Industrial Toxicology Research Centre
Post Box 80
Lucknow, India

Exploiting the principle of selective toxicity, attempts have often been made to synthesize insecticidal compounds combining low mammalian toxicity with high insecticidal activity. NISHIZAWA et al. (1961a) investigated many organophosphorus insecticides and claimed for sumithion+ comparatively low mammalian toxicity and superior insecticidal activity compared to the other isomers, homologues and analogues. The generic name for the compound, as approved by British Standards Institution and recommended by International Organisation for standardization, is fenitrothion.

Fenitrothion is one tenth as toxic to rats (NISHIZAWA et al. 1961b) and one fiftieth as toxic to mice (MIYAMOTO et al. 1963a) as methyl parathion, the oral LD_{50} of fenitrothion being 788 - 870 mg/kg for mice and 200 - 283 mg/kg for male rats. Toxicity studies carried out by different routes show that oral LD_{50} is one fourth of dermal LD_{50} in mouse (IKEDA, 1960; UEDA, 1961; MIYAMOTO et al. 1963a) and one third in the male rat (IKEDA, 1960; S.C.C., 1960; NISHIZAWA et al., 1961b; C.T.B., 1966).

An insecticide may gain entry into the system through any of the major portals; namely oral, respiratory and dermal routes. Under normal conditions of manufacture, handling and spraying of insecticides large quantities of the material come in contact with the skin of the workers and remain on body surfaces for long periods. This may cause local skin lesions and also systemic manifestations after getting absorbed into the system. Since, the most likely route of entry of the compound in case of occupational exposures is through the skin, it was considered desirable to reassess the degree of absorption of fenitrothion from the skin. The present study was undertaken to determine (1) the rate of absorption of fenitrothion from the

* 0,0 -dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate.

+ Trade name given to fenitrothion by Sumitomo Company Limited.

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skin following single dermal application, (2) effect of repeated dermal applications of fenitrothion on the degree of absorption and (3) inhibition of cholinesterase activity in the above two cases.

Methods and Materials

Young adult rats of either sex of ITRC strain weighing 160 - 180 grams were used in this study. The skin over the back between the shoulders and hind quarters was shorn with electric clippers. 0.1 ml of fenitrothion (95% pure technical grade, density 1.32) was painted as such over an area of 2 x 2 cm. The rats were housed in separate cages during the period of experiment. The animals were divided in five groups of 8 animals each. After 2, 4, 8, 24 and 48 hours, each group of rats was anaesthetized with ether and blood was taken in a heparinized syringe from the inferior venacava.

For studying the effect of repeated applications twenty four animals were divided into four equal groups. One group served as controls. The animals of other groups received one, two and three dermal applications of fenitrothion respectively over as many areas of 2x2 cm between the shoulders and the hind quarters. 0.1 ml fenitrothion was applied in each application. Eight hours after application, rats were anaesthetized and heparinized blood was taken by venacaval puncture.

Determination of cholinesterase activity in whole blood

Cholenesterase activity was determined by the method of HESTRIN (1949) as follows : 0.1 ml fresh blood was taken in tubes containing 2 ml of phosphate buffer (pH = 7.2) and incubated at 37°C for 5 minutes. 2.0 ml of buffered acetylcholine (1 : 9) was added in the experimental tubes. The reaction was stopped by adding 4.0 ml of alkaline hydroxylamine (1 : 1) to each tube, exactly after 10 minutes. 2.0 ml of buffered acetylcholine was added in the control tubes just after the stoppage of the reaction. One minute later 2.0 ml of hydrochloric acid (1:2) and 2.0 ml of ferric chloride were added to all the tubes, the solutions were filtered and unreacted acetylcholine was estimated by taking absorption of these solutions at 540 mu in Klett Summerson Colorimeter.

Estimation of fenitrothion in blood plasma

One ml plasma was taken in a separatory funnel containing 1 ml of 5 N Hydrochloric acid and 5 ml of water. Fenitrothion was successively extracted with

10, 5, 5 and 3 ml of chloroform. Emulsion formed during extraction was removed by centrifugation. The total chloroform extract was dried over anhydrous sodium sulphate and then transferred to a 25 ml volumetric flask. Volume was made upto the mark and absorbance of this solution was taken at 270 mμ in a Unicam model SP500 spectrophotometer using chloroform blank. The amount of fenitrothion was determined from the standard curve prepared by plotting concentration of fenitrothion against absorbance at 270 mμ using the above method. The recovery of the method was 95.6 ± 0.4 per cent and sensitivity 2.5 μg/ml.

Results

The estimation of fenitrothion in the blood plasma after dermal application of the compound showed that the compound was readily absorbed through the rat skin and significant amounts of the compound (33 ± 6 microgram per ml of plasma) were present in the blood two hours after dermal application. The blood levels increased with time and reached maximum after eight hours (43 ± 4.3 microgram per ml plasma) and then decreased to 27 ± 4.8 microgram per ml after 48 hours (Fig.1).

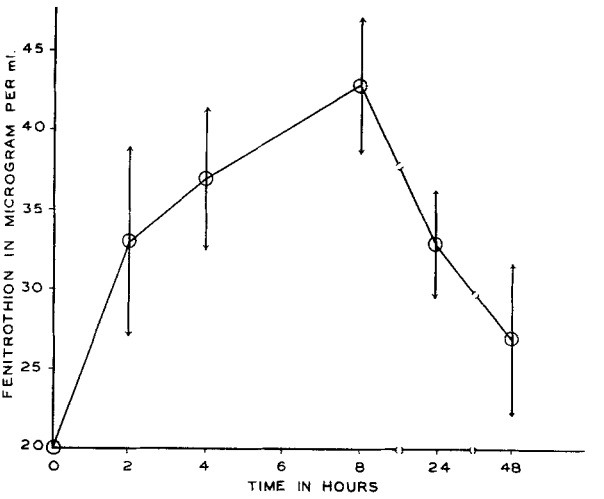

Fig. 1. Concentration of fenitrothion in plasma at different intervals after painting 0.1 ml of fenitrothion on 2 x 2 cm of skin. The values are mean ± standard errors of the values of six animals.

The degree of cholinesterase inhibition was followed at similar intervals as fenitrothion absorption. Fig. 2 shows acetylcholine concentration in the blood of rats after painting 0.1 ml of fenitrothion on 2 x 2 cm of the skin. There was a fair degree of parallelism between the degree of absorption of fenitrothion and cholinesterase inhibition. As the concentration of fenitrothion in plasma increased, there was increasing inhibition of cholinesterase activity.

In order to see the effect of increase in the exposed area 0.5 ml of fenitrothion was painted on 4 x 4 cm of the skin and painting was repeated after 24 hours. Blood samples taken after 48 hours showed complete inhibition of cholinesterase activity in the whole blood and the concentration of fenitrothion in the plasma was 250 µg/ml. At the moment of taking the blood these animals were in a moribund state and showed fine to coarse tremors.

To find the total absorption of fenitrothion, the unabsorbed compound was extracted by chloroform from the skin and estimated. It was found that 55±4.5 per cent of the compound remained unabsorbed even after 24 hours of dermal application of fenitrothion. In an other experiment repeated dermal application of the compound was found to increase the fenitrothion concentration and inhibition of cholinesterase activity in blood (Table 1).

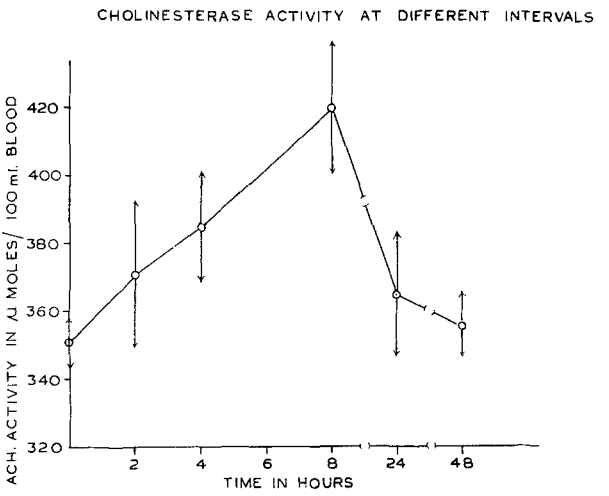

Fig. 2. Cholinesterase activity at different intervals calculated as micromoles of unreacted acetyl choline per 100 ml of blood in the blood of rats after painting 0.1 ml of fenitrothion on 2 x 2 cm of the skin. The values are mean ± standard errors of the values of six animals.

TABLE 1

Concentration of fenitrothion in plasma and cholinesterase activity in blood after eight hours of dermal application of fenitrothion (0.1 ml on 2 x 2 cm)

No. of dermal applications	No. of animals	Fenitrothion in µg/ml of plasma±S.E.	Cholinesterase activity expressed as µ moles of unreacted Ach./100 ml of blood ± S.E.
Nil	6	0	351 ± 7.4
One	6	43 ± 4.3	420 ± 29.4
Two	6	50.4 ± 2.7	497 ± 17.5
Three	6	59.4 ± 2.4	576 ± 7.7

Discussion

As is well known several organophosphate compounds are converted to their oxygen analogues which are the actual offending agents, being more active cholinesterase inhibitors. Fenitrothion is also converted to sumioxon which is the more powerful cholinesterase inhibitor. However fenitrothion has comparatively lower toxicity because of 1) slower formation of sumioxon (NISHIZAWA et al. 1961b), 2) comparatively weaker cholinesterase inhibitory activity of sumioxon and 3) rapid detoxification (MIYAMOTO et al. 1963b) of the parent compound by conversion to other inactive derivatives.

There appeared to be rapid absorption of fenitrothion from the skin (significant amounts being detected within two hours), but the levels attained in the blood were rather low. This was partly due to only partial absorption of the material after dermal application as shown in the present study; 50 to 60 per cent of the material could be recovered from the sites of application as long as 24 hours after application. Low blood levels were further due to rapid equiliberation with the tissues and excretion in the urine (MIYAMOTO, 1964 and HOLLINGWORTH et al. 1967). 75 per cent of the radioactivity of the administered compound could be recovered in the urine within 24 hours.

Inspite of quick excretion of fenitrothion, however, large quantities of the compound can be absorbed by repeated dermal application which may cause extreme degree of cholinesterase inhibition leading to death. Thus there was 19.6% cholinesterase inhibition after a single application, the inhibition increased to 64.1 per cent following three applications and there

was total cholinesterase inhibition after 5 exposures. It would thus appear that hazard of dangerous effects increases with the degree and repetition of exposures to fenitrothion.

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