TOXICOLOGICAL PROPERTIES OF THE ORGANO-PHOSPHORUS INSECTICIDE DIMETHOATE

BY

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The results are presented of extensive toxicological studies on the systemic organophosphate insecticide dimethoate, and compared with published results from other laboratories. It behaves as a typical indirect anticholinesterase, by conversion in the liver to at least four short-lived active metabolites, whose hydrolysis products are rapidly excreted, mainly in the urine. The acute oral toxicity of dimethoate is low in mammals but higher in avians. Dermal absorption is notably slow and dermal toxicity correspondingly low. Cumulative dosing of rats and guinea-pigs gave no cholinesterase inhibition at 0.7 and 4 mg./kg./day respectively. Dietary feeding to growing rats caused no cholinesterase inhibition at 0.5 mg./kg./day and no other effect at 10 times this dose. The main plant metabolite is identical with one formed in the liver, and comparative feeding tests with normal dimethoate and that partly metabolized in vegetation showed that residue analysis determined total hazard. Tests on humans, some with ^{32}P -labelled material, confirmed that metabolism and urinary excretion are very rapid, that skin absorption is very slow, and that at least 2.5 mg., and probably up to 18 mg., could be ingested daily for at least three weeks without cholinesterase inhibition or other effects. Vapour hazards proved negligible. Oral toxicity was not potentiated by any of 17 other insecticides. The earliest detectable effect of dimethoate poisoning was always erythrocyte cholinesterase inhibition. Symptoms of poisoning could be effectively treated by atropine but not by oxime therapy. No known cases of occupational poisoning have occurred during five years' commercial usage of dimethoate.

Introduction

In the early days of the development of organo-phosphorus insecticides most of the effective materials were also hazardous to mammals including man, *i.e.* they were not selectively toxic to insects; examples include parathion*, T.E.P.P., H.E.T.P., demeton, and schradan. Malathion was the first compound of this type to be manufactured whose selective action gave a low mammalian toxicity; this was followed by others, such as chlorothion, trichlorphon, and diazinon, and the experimental compound, acethion (O'Brien, Thorn, and Fisher, 1958; O'Brien, 1959a). These, however, have all been limited in their usefulness because they are not appreciably systemic in action, and where systemic activity was desired, earlier, less selective compounds, such as schradan or demeton, still had to be used. Further research by industry produced a series of

materials, of which dimethoate† and demeton-methyl most successfully combined selective toxicity to insects with systemic action. Dimethoate, the less toxic of the two, has been found to be of great agricultural value as a systemic insecticide (Geering, 1959) and may also have useful veterinary applications (Hewitt, Brebbia, and Waletzky, 1958a; Hewitt, Emro, Entwistle, Pankavich, Thorson, Wallace, and Waletzky, 1958b; Drummond, 1959; Marquardt and Lovelace, 1961; Dorrough and Arthur, 1961; Cheng, Frear, and Enos, 1961, 1962; Stones, 1961). It combines contact and systemic action, is useful against a wide range of insects, and also disappears at a rapid rate from the treated food crop.

In view of the potentialities of dimethoate, very detailed toxicological and biochemical studies have been carried out in these and other laboratories in

*The systematic names of pesticides referred to by common names are given as an Appendix (p. 63).

†In the United Kingdom and Italy dimethoate is available as 'Rogor': 'Rogor' is a registered trademark of Soc. Montecatini (Milan).

order to assess its hazards in use and the safe rates of intake in food-stuffs. Our aim here is to summarize this work and to support our estimate of hazards by the health records of those handling the compound.

Dimethoate is O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate:—

Three grades of material were studied: a pure, odourless grade obtained by repeated recrystallization from anhydrous ether; a laboratory grade, which is a white crystalline solid with a marked 'thiol' odour; and technical dimethoate, about 93% pure, which varies from off-white crystals to a grey semi-crystalline material, and in which the main impurity is O,O:S-trimethyl phosphorodithioate: $(\text{CH}_3\text{O})_2\text{PS}\cdot\text{S}\cdot\text{CH}_3$. Other workers have usually accepted specimens corresponding to our laboratory grade material as 'pure'. In fact pure dimethoate cannot be distinguished from laboratory grade materials by chemical tests, but it has no odour and is somewhat less toxic. It slowly reverts to laboratory grade on exposure to moist air.

Some important physical and chemical properties are summarized in Table 1. The compound is

TABLE 1
SOME PHYSICO-CHEMICAL PROPERTIES OF DIMETHOATE*

Melting point	49-51°C.
Vapour pressure	Below 10^{-3} mm. Hg†
Solubilities at 20-25°C., % w/v:	
Water	2.5
Chloroform	> 75
Methyl isobutyl ketone	50
Methyl naphthalene	30
Diethyl ether	11
Xylene	2.1
Partition coefficients:	
Chloroform/water	32.5
80-100° light petroleum/water	0.042
Olive oil/water	1.2
Hydrolysis rates in aqueous solvents, $\frac{1}{2}$ -lives:	
Water, 21°C.	Very long
Buffer, pH 4, 21°C.	> 100 hours
pH 7, 20°C.	120 hours
70°C.	6½ hours
pH 9, 21°C.	140 hours
pH 13, 21°C.	1 minute

*Properties were determined on pure or laboratory grade dimethoate.

†Accurate values could not be obtained because traces of more volatile decomposition products were slowly formed.

References: West *et al.*, 1961; Sauti and Pietri-Tonelli, 1959b; Fontanelli and Lanforti, 1959; American Cyanamid Co., 1957; Chilwell and Beecham, 1960.

soluble in a wide range of solvents, including water, is extractable from water by chloroform, and is unstable in alkaline solution but is fairly stable in water and acid solution at room temperature. Hydrolysis is catalysed by heavy metal ions, such as Cu^{++} , Fe^{+++} , and Mn^{++} . Hydrolysis can occur at the P-O-C, P-S, and amide links of the molecule:—

The product of P-S hydrolysis, N-methylmercaptoacetamide, readily oxidizes in air to the disulphide: $\text{CH}_3\text{NH}\cdot\text{CO}\cdot\text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_2\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}_3$, which is the degradation product usually found.

The P-S group in dimethoate is readily oxidized by such agents as hydrogen peroxide, potassium permanganate, and iodine to a P-O group:—

The product (dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolate), which we shall call the thiolate analogue, is a toxic metabolite (see below) and has been isolated (Santi and Pietri-Tonelli, 1959 a, b; Fontanelli and Lanforti, 1959; Dauterman, Viado, Casida, and O'Brien, 1960). Dauterman *et al.* (1960) also showed that oxidation occurs slowly in air, and Santi and Pietri-Tonelli (1959 a, b) that the oxidation is accelerated by some metallic ions.

The O-methyl groups of dimethoate may undergo exchange reactions with alcohols, giving rise to changes in toxicological properties:—

Such reactions have been observed in 2-methoxy- and 2-ethoxy-ethanol (Casida and Sanderson, 1961, 1963) and in isopropanol. Any changes in other solvents are slow and are probably due to contact with moist air (see later).

Analytical methods have been devised for dimethoate in technical and formulated material (Dupée, Gardner, and Newton, 1960; Fusco, Marchese, Placucci, and Losco, 1960) and in crops (Chilwell and Beecham, 1960; Chilwell and Hartley, 1961; Bazzi, 1959; Enos and Frear, 1961; Giang and Schechter, 1963).

Materials and Methods

Chemicals.—Pure, laboratory grade, technical grade, and ^{32}P -labelled laboratory grade dimethoate (as available from the Radiochemical Centre, Amersham) was

used, and also formulations of technical grade dimethoate containing 32 or 40% w/v active ingredient in cyclohexanone or 2-methoxyethanol, with added surface active agents. The technical formulations in 2-methoxyethanol were freshly prepared and used before changes in toxicity due to exchange reactions had taken place, unless otherwise stated. A 20% wettable powder in china clay was also examined. Chemicals used in

potentiation tests, as listed later, were either commercial formulations or technical grades.

Animals.—Rats used in this work were Wistar or Glaxo-Wistar albinos, mainly 150 to 200 g., unless otherwise stated. Mice and guinea-pigs were albinos bred from M.R.C. stock. Other species were normal commercial hybrids, apart from trapped wild birds.

Administration.—Oral dosing was by stomach tube, under ether anaesthesia except in birds and the rabbit. Dermal toxicity was examined by the method described by Edson and Noakes (1960). In acute tests animals were normally observed for seven days. LD_{50} values were computed by the method of Weil (1952), except

where the group size was less than four; in these cases approximate values were based on general observation. In feeding trials dimethoate was mixed into a paste of measured amounts of water and M.R.C. diet 41 b meal, made up freshly for each group daily.

Biochemical Techniques.—Methods for manometric measurement of cholinesterase and anticholinesterase activities, and preparation and incubation with tissue slices, were as described by Fenwick, Barron, and Watson (1957) and Edson, Sanderson, Watson, and Noakes (1962). Some direct activities were confirmed by miosis (Sanderson, 1957). Toxicity tests were often accompanied by estimations of cholinesterase inhibition, which are a sensitive test of subacute poisoning by organophosphates, and pre-mortem blood samples were obtained by cardiac puncture under ether anaesthesia.

^{32}P Estimations.—These were done by standard techniques.

Chromatography.—Column chromatography was carried out essentially as described by Heath, Lane, and Park (1955). The column was first eluted with a 10:7:3 v/v mixture of carbon tetrachloride, trichloroethylene, and chloroform, saturated with water, replaced after a suitable period by chloroform saturated with water. Paper partition chromatography was by simple downward development on untreated Whatman No. 1 paper strips by either chloroform or carbon tetrachloride.

RESULTS

Throughout, comparisons are made between the pure or laboratory grades and the technical material. Results on the three grades are therefore given together.

Toxicity Tests

Signs of Poisoning.—Signs of poisoning were typical of those induced by inhibitors of acetylcholinesterase: fibrillations, salivation, lacrimation, urinary incontinence, diarrhoea and sometimes dyspnoea. The onset of symptoms was delayed 0.5 to 2 hours, the longer the purer the material. (This is typical of an indirect cholinesterase inhibitor, *i.e.* one which is inactive until metabolized.) With pure dimethoate, narcosis often preceded cholinergic effects. Deaths usually occurred 3 to 30 hours after dosing, while recovery from cholinergic effects usually took two to four days.

Male rats surviving 300 mg./kg. of oral laboratory grade dimethoate showed marked loss of weight after three days, which was largely regained after 10 to 20 days. There were no further ill-effects in five months and no macroscopic pathology after this period.

Hens surviving single or several weekly oral doses of 20 to 50 mg./kg. laboratory grade dimethoate recovered within three days of the last dose and then remained completely normal for eight months. They showed no evidence of neuromyopathy. This finding

has been confirmed using slightly higher doses than in our tests, with atropine protection, in the laboratories of Soc. Montecatini (personal communications).

Pathological Findings.—No specific macroscopic pathology attributable to dimethoate was seen *post mortem* in any test. Acute deaths showed the general congestion and liquid gut content usually found with anticholinesterase poisoning.

Oral Toxicity.—Table 2 shows the acute oral LD₅₀ values to various species obtained with different preparations of dimethoate in this laboratory. With many species, several LD₅₀ values were determined on a given grade of material, up to about 30 for rats given laboratory, technical, or formulated grades. In these instances, the overall range of LD₅₀ values obtained is shown in Table 2. This presentation tends to obscure the consistent finding in comparative experiments that female rats were a little less susceptible than males to dimethoate poisoning. Otherwise, there was no significant difference in susceptibility to laboratory grade material between normal rats, weanling and aged males, and pregnant females. The rat occupied a fairly central position in the range of species variation in susceptibility.

The results in Table 2 agree well with those of other workers (Santi and Pietri-Tonelli, 1959b; American Cyanamid Co., 1957, 1958; Edson and Noakes, 1960; Sherman and Ross, 1961; Soc. Montecatini, personal communications; West, Vidone, and Shaffer, 1961; Hewitt *et al.*, 1958a; Dauterman, Casida, Knaak, and Kowalczyk, 1959).

Other workers have examined the toxicity of dimethoate to larger animals, their results including the following acute oral LD₅₀ values: dogs >100 mg./kg. (Hewitt *et al.*, 1958b), horses >50 mg./kg. (Jackson *et al.* 1960), sheep 80 mg./kg. (Hewitt *et al.*, 1958a,b; Chamberlain, Gatterdam, and Hopkins,

TABLE 2
ACUTE ORAL LD₅₀ VALUES FOR DIMETHOATE (mg./kg.)

Species	Sex	Pure	Laboratory Grade	Technical	Liquid Formulations	Wetttable Powder
Rat	M	500-600	280-350	180-325	150-400	280
Rat	F	570-680	300-356	240-336	200-400	300
Mouse	F	60	—	60	—	—
Hamster	M	—	200	—	—	—
Guinea-pig	M/F	550	600	350-400	350-370	—
Rabbit	M/F	500	450	c.300	283	—
Hen	F	50	40	c. 30	25	—
Pheasant	M	15-20	15	20	25	—
Duck*	F	—	>40	—	—	—
Sparrow*	M/F	—	—	—	22	—
Blackbird*	M/F	—	—	—	26	—

*Group size less than four animals.

1961), and cattle 70 mg./kg. (Hewitt *et al.*, 1958a,b; Kaplanis, Robbins. Darrow, Hopkins, Monroe, and Treiber, 1959; Drummond, 1959; Dauterman *et al.*, 1959).

The effect of oral dosing on erythrocyte cholinesterase was studied. In rats given 300 mg./kg. of laboratory grade dimethoate, levels fell to a minimum of 15% of pre-exposure after three hours and had recovered to 85% after 14 days; plasma cholinesterase fell to 15% after 20 hours and rose to normal in 14 days. In a similar test at 190 mg./kg., erythrocyte and plasma cholinesterase activities followed a similar pattern, but brain cholinesterase fell to 37% of pre-exposure at seven days and only rose to 60% at 14 days. Maximum inhibition was attained much more slowly in the pheasant than in the rat or the guinea-pig.

Five human males ingested 0.25 mg./kg. without toxic effects or cholinesterase inhibition.

Toxicity of Stored Materials.—Tests were carried out on the effect of storage, at normal and raised temperatures, on the oral toxicity to rats of both solid and formulated dimethoate.

In contact with moist air, pure dimethoate gained toxicity and smell over a period of several weeks or months until it was comparable with laboratory grade; thereafter it behaved as laboratory grade. Material kept in sealed ampoules evacuated to 10^{-3} mm. Hg after flushing with nitrogen, and stored at 3°C., maintained its acute oral LD₅₀ to the rat at 600 to 700 mg./kg. for at least two years, after which time the odour was still only slight.

Laboratory grade material showed no appreciable change in toxicity for up to two years at room temperature, or several months at about 40°C., but decomposed faster above its melting point. The toxicity either decreased or occasionally increased slightly.

Work on liquid formulations of the technical grade showed that the toxicity increased on storage in some alcoholic solvents, particularly in 2-methoxy- and 2-ethoxyethanol; a smaller increase was also noted in isopropanol. After nine months' storage under tropical conditions of a trial formulation in 2-methoxyethanol, the acute oral LD₅₀ to rats fell to 15 mg./kg. Further investigation (Casida and Sanderson, 1961, 1963) indicated that the effect was due to the exchange reaction noted in the Introduction, and a reaction product with 2-methoxyethanol was isolated with an acute oral LD₅₀ to rats of about 1 mg./kg. Changes in other alcoholic solvents, such as propylene glycol, were much less, and in non-hydroxylic solvents the stability of solutions was comparable with that of undissolved

dimethoate; toxicity eventually fell very slowly due to hydrolysis of the active ingredient. Trial formulations in alcoholic solvents were therefore abandoned. A different liquid formulation using cyclohexanone as solvent was then developed for commercial use, and repeated batch tests on this formulation have demonstrated its toxicological stability under prolonged tropical storage. Wettable powder formulations with china clay have been produced with similar storage stability.

Dermal Toxicity.—A large number of tests on the dermal toxicity of dimethoate of various purities and as fresh liquid formulations showed little consistent variation of toxicity with composition. Acute dermal LD₅₀ values to rats, with 24-hour covered contact, fell within the range 700 to 1,150 mg./kg., except for a few samples in aromatic solvents such as xylene, some of which appeared to be up to twice as toxic dermally, possibly because the absorption rate was increased by solution of the skin lipids. Wetting agents in formulations did not appear to influence toxicity. Wettable powders had 24-hour contact dermal LD₅₀ values to rats of about 500 mg./kg.

By varying the time of contact, it was shown that dermal absorption of dimethoate from commercial formulations was slow. Thus a commercial formulation with a dermal LD₅₀ value to the rat with 24-hour contact of 700 mg./kg. caused no toxic effects at 800 mg./kg. when the rats were washed after two hours. Another test confirmed this and also showed that toxic effects were considerably reduced by decontamination at four or eight hours rather than 24 hours. Similarly, cholinesterase inhibition was negligible after two hours' contact with 50 mg./kg. but was marked after 24 hours' contact with 30 mg./kg., whereas 30 minutes' contact with 150 mg./kg. caused no inhibition. Absorption from wettable powder formulations was not significantly faster than from liquid formulations. The slowness of absorption was confirmed by using ³²P-labelled dimethoate. Propylene glycol solutions and liquid formulations were absorbed by the rat at comparable rates, 3 to 4% of an applied dose being taken up after one hour's contact and 10 to 25% after four hours, while absorption was still incomplete after 24 hours' contact. It seemed that part of the absorbed dimethoate formed a slowly-dispersing pool in the skin at the site of contact.

These findings are in good agreement with those of other workers (West *et al.*, 1961; American Cyanamid Co., 1957; Soc. Montecatini, personal communications; Edson and Noakes, 1960).

On the basis of these results, a series of tests involving the skin application of a commercial 32% liquid formulation of dimethoate to human subjects was undertaken. In a number of subjects, two-hour dermal contact with up to 2.5 ml. of the formulation, or completely immersing one hand and then bandaging for two hours before decontamination, caused no inhibition of blood cholinesterase.

The absence of skin irritancy of the liquid formulation was confirmed by two-hour contact patch tests on 50 subjects.

Decontamination from human skin was followed by using ^{32}P -labelled dimethoate as a liquid commercial-type formulation. Surface contamination was almost completely removed by washing with water alone after up to two hours' contact. After five-and-a-half hours, washing with water left 5 to 13% unremoved; but even this was largely removed by vigorous washing with soap and water. After five-and-a-half hours' dermal contact in humans, most of the applied dimethoate was therefore still present on the skin surface, unabsorbed.

Parenteral Toxicity.—Table 3 shows the range of acute LD_{50} values in various species obtained with different preparations of dimethoate in this laboratory. Seume and O'Brien (1960) and Krueger,

were temporarily removed during the spraying, and precautions were taken to avoid ingestion or dermal contact with sprayed surfaces. The temperature was maintained at 30°C. during the day (with artificial sunlight) and 21°C. at night. No blood cholinesterase inhibition or toxic effects appeared during 28 days' exposure. These results indicated that no adverse effects were likely to arise from prolonged vapour inhalation under these conditions. Other tests involving 48-hour exposure of aphids to dimethoate vapour approaching saturation failed to detect appreciable vapour toxicity.

In a separate experiment, two male rats were exposed for seven hours to the saturated vapour at 21°C. from molten technical dimethoate. They showed no toxic effects. After the test plasma cholinesterase was 92%, and erythrocyte 76% of normal.

Cumulative Toxicity.—Groups of 10 male rats were given 24 intraperitoneal injections in 34 days of laboratory grade dimethoate. The highest non-lethal daily dose was 40 mg./kg., and the highest daily dose not showing toxic effects was 3 mg./kg. Erythrocyte cholinesterase was inhibited more than plasma. No significant inhibition of erythrocyte, brain, or plasma cholinesterase was observed at 0.7 mg./kg. At higher doses, inhibition underwent little further change after the first five injections. There was no macroscopic pathology at any dose.

In a similar test involving daily subcutaneous injections to male guinea-pigs for three weeks, there were no toxic effects at the highest dosage rate, 16 mg./kg./day, and the highest dose showing no significant blood cholinesterase inhibition was 4 mg./kg./day.

A group of guinea-pigs given 40 mg./kg./day orally for three weeks showed some weight loss and weakness but no cholinergic effects. Erythrocyte cholinesterase fell to 19 to 23% of normal, and there was also slight inhibition of plasma cholinesterase.

Twenty adult human subjects each ingested daily 2.5 mg. (about 0.04 mg./kg.) of fresh aqueous laboratory grade dimethoate for four weeks. They showed complete absence of toxic effects and blood cholinesterase inhibition during this period. This dose corresponded to the daily consumption of 1 kg. food containing 2.5 p.p.m. dimethoate (see later).

Two human subjects similarly ingested daily 9 and 18 mg. (about 0.13 and 0.26 mg./kg.), respectively, for 21 days, with complete absence of blood cholinesterase inhibition.

Soc. Montecatini (personal communications) describe similar oral tests on rats and mice. In rats

TABLE 3

ACUTE INJECTION LD_{50} VALUES FOR DIMETHOATE (mg./kg.)

Route	Species	Sex	Laboratory Grade	Technical	Fresh liquid Formulations
Intraperitoneal	Rat	M	175-325	—	100-220
	Rat	F	350	—	200
	Mouse	M/F	—	—	45-50
Subcutaneous	Rat	M	350	—	—
	Mouse	M/F	120	60	112
	Hamster	M	200	60	170
Intravenous	Rat	M	450	—	—

O'Brien, and Dauterman (1960) report mouse intraperitoneal LD_{50} values in propylene glycol of 140 to 205 mg./kg. Other results by Soc. Montecatini (personal communications) are in close agreement with those above.

Vapour Toxicity.—In order to assess the effects of prolonged inhalation of vapour from dimethoate and possible plant metabolites (see later), a cage of 12 male rats was kept in a miniature greenhouse (0.6 m.³), containing growing bean plants, with minimum ventilation. The plants and sides of the chamber were sprayed daily with 5 ml. of 0.5% aqueous dimethoate formulation. The caged rats

the highest non-lethal dose was 20 mg./kg./day, whereas the mouse was less susceptible.

Dietary Toxicity.—Groups of 20 semi-adult male rats were maintained for six to 12 months on a diet containing various concentrations of laboratory grade dimethoate. At 800 p.p.m. severe toxic effects (cholinergic effects, weakness, apathy, and weight loss) developed within a few days and seemed likely to be lethal, so the chemical was withdrawn after one week, and recovery was complete in 10 to 14 days. Less severe toxic effects and a reduced rate of weight gain were induced by 200 p.p.m. but not by 50 p.p.m. or below. Marked inhibition of erythrocyte cholinesterase occurred at 50 p.p.m., but at 10 p.p.m. and below neither erythrocyte nor plasma cholinesterase showed significant inhibition throughout the test. At the end of the trial there was no macroscopic or microscopic pathology in any group attributable to the chemical. Cholinesterase inhibition in the higher groups was far more marked in erythrocytes and, to a lesser extent, in brain than in any other tissue. From this test the maximum intake which was without effect was 10 p.p.m., corresponding to 0.5 to 0.8 mg./kg./day.

In order to study less widely separated dosage levels, further groups of 20 weanling male rats were similarly tested for five-and-a-half months at dose levels of 5, 10, and 20 p.p.m. dimethoate. None of these groups showed toxic effects or reduction of rate of weight gain. Plasma cholinesterase was unaffected by 20 p.p.m. Erythrocyte cholinesterase was moderately inhibited by 20 p.p.m., and in this

test slightly so at 10 p.p.m. but not significantly at 5 p.p.m. The maximum ineffective intake rate thus became 5 p.p.m. (0.3 to 0.6 mg./kg./day). The difference from the previous test at 10 p.p.m. was probably due to the use of younger rats, whose food intake per unit body weight was higher.

A similar test was then performed, using the commercial liquid formulation of dimethoate; this lasted 12 weeks. Both male and female groups of weanling rats were used and showed no significant differences in response. Results were very similar to those with laboratory grade material, the maximum ineffective intake rate again being 5 p.p.m. (0.4 to 0.6 mg./kg./day). No pathological lesions were found.

The results of these three tests are summarized in Table 4.

These results are in close agreement both with our cumulative tests and with the dietary results of Soc. Montecatini (personal communications). However, West *et al.* (1961) and the American Cyanamid Co. (1957) found the dietary toxicity to be only one-seventh of ours, for some unknown reason.

Groups of guinea-pigs were fed for three weeks on lettuce and brassica leaves treated with dimethoate before harvest to leave analysed residues up to 189 p.p.m. in the highest dosage group. Treated vegetation was renewed weekly. Toxic effects were completely absent, and cholinesterase inhibitions were in excellent agreement with parallel groups given daily oral doses of the same quantity of laboratory grade dimethoate (see under Cumulative Toxicity, p. 57). There was no macroscopic pathology. This test showed both that vegetation

TABLE 4
SUMMARY OF RAT DIETARY TESTS ON DIMETHOATE

Material Rat sex Starting weight (g.) Duration of test	Test 1		Test 2		Test 3			
	Lab. grade M 177-200 12 months		Lab. grade M 80-87 5½ months		Fresh liquid formulation M/F 60-80 12 weeks			
Terminal R.B.C. and plasma cholinesterase, % of control:	R.B.C.	Plasma	R.B.C.	Plasma	Males R.B.C.	Plasma	Females R.B.C.	Plasma
0.5 p.p.m.	82	111	—	—	—	—	—	—
2	82	117	—	—	—	—	—	—
2.5	—	—	—	—	101	124	115	113
5	—	—	80	105	80	95	91	99
10	73	114	60	90	69	101	73	111
20	—	—	51	104	35	111	45	96
40	—	—	—	—	14	61	15	65
50	35	83 (6m.)	—	—	—	—	—	—
200	0	28 (6m.)	—	—	—	—	—	—
800	7	36 (1wk.)	—	—	—	—	—	—
Highest dose rate without reduced weight gain (p.p.m.)	50		> 20		> 40			
Highest dose rate without toxic effects (p.p.m.)	50		> 20		> 40			
Highest dose rate without cholinesterase inhibition (p.p.m.) (mg./kg./day)	10 0.5-0.8		5 0.3-0.6		5 0.4-0.6			

with the maximum attainable residue of dimethoate caused no toxic effects, and that the residues, including possible plant metabolites, behaved in exactly the same way as unmetabolized dimethoate (Chilwell and Beecham, 1960).

Birds have been studied by other workers. Sherman and Ross (1961) found that chicks fed dimethoate for one week showed toxic effects at 88 but not at 44 p.p.m. in the diet. Alessandrini (1960) found that 500 to 1,000 p.p.m. of dimethoate

however, E.P.N., tri-o-cresyl phosphate, and tri-o-cresyl phosphorothionate did potentiate dimethoate (Seume and O'Brien, 1960).

Casida and Sanderson (1961, 1963) have shown also that the difference in toxicity between pure dimethoate and other grades (Table 2) is due to a trace impurity, present in the other grades, which potentiates the toxicity of pure dimethoate. The impurity responsible is thought to be produced by partial hydrolysis according to the equation:—

caused 40% mortality when fed to quail for 70 days. Sherman, Ross, Sanchez, and Chang (1963) found that over 59 weeks 30 p.p.m. of dimethoate in the drinking water of laying hens caused inhibition of plasma cholinesterase and some reduced appetite but no egg abnormalities; recovery was rapid.

Rainbow trout showed LC_{50} concentrations to aqueous dimethoate formulation of 58 and 27 p.p.m. for 24 and 48 hours, respectively; 5 p.p.m. was considered a safe maximum initial concentration for infinite exposure, and 1 p.p.m. was suggested as an adequately safe value (Alabaster, personal communication). Under tropical conditions, 6 p.p.m. had a very slight effect on padi fish but not on tilapia (Prose, personal communication). Dimethoate thus proved far safer to fish than most other commercial insecticides (Edson, 1958).

Potentiation.—The acute oral LD_{50} values of mixtures of dimethoate, either laboratory grade or liquid formulation, and other insecticides were determined and compared with those calculated from the separate toxicities of the materials. They demonstrated that none of the following compounds potentiated the toxicity of dimethoate: azinphos-methyl, carbophenothion, carbaryl, D.D.T., demeton, demeton-methyl, diazinon, dimefox, dioxathion, E.P.N., 'Isolan', malathion, mevinphos, parathion, parathion-methyl, prothoate, or schradan.

Other workers (Soc. Montecatini, personal communications; American Cyanamid Co., 1958; and West *et al.*, 1961) have confirmed most of these negatives, including E.P.N., and have also shown that amiton oxalate, dicapthion, ethion, and phorate are without potentiating action.

When tested intraperitoneally in the mouse,

i.e. the compound O-methyl S-(N-methylcarbamoyl-methyl) phosphorothioic acid.

Biochemical Studies *in Vitro*

Inhibition of Cholinesterase.—The concentration of pure dimethoate inhibiting 50% of rat brain cholinesterase (the I_{50}) was about $8.5 \times 10^{-3}\text{M}$. Less pure grades were slightly more powerful inhibitors, inhibition increasing with exposure to air, probably due to the formation of the thiolate analogue. For laboratory grade, the I_{50} was about $1 \times 10^{-3}\text{M}$. Inhibition increased proportionately with the time of contact between enzyme and inhibitor up to 30 minutes, and almost ceased after one hour, being independent of enzyme concentration. Erythrocyte cholinesterase inhibited by dimethoate could not be re-activated by washing with saline, *i.e.* inhibition was irreversible, as confirmed by Edson *et al.* (1962).

Activation by Tissue Preparations.—When incubated with liver slices, the anticholinesterase activity of dimethoate increased, though less readily than that of parathion. For example, incubation of 2 ml. of 10^{-4}M dimethoate with 100 mg. of rat liver slices for one hour at 37°C . caused a fourfold increase in activity. With rat liver, the degree of activation increased exponentially both with time and with the quantity of liver present, much more so with the former, indicating that a complex multi-stage process was probably involved. No activation was observed if oxygen was excluded from the system; and the product of activation was stable for seven days, but not 14, at room temperature in Krebs-Ringer phosphate buffer solution at pH 7.4 (Fenwick *et al.*, 1957).

These results are consistent with the assumption that activation consists of the oxidation of the P=S group to a P=O group, *i.e.* with the conversion of dimethoate to its thiolate analogue (see Introduction).

Mouse and guinea-pig liver slices were about as active as rat slices, whose activity did not vary with the sex or age of the rat; but pheasant liver slices were initially somewhat more active and showed greater variation with liver quantity. Rat kidney slices also showed a slight capacity to activate dimethoate.

There were indications of a direct inhibitor circulating in animal blood one to six hours after oral dosing with dimethoate.

In experiments by O'Brien (1959b) and Dahm, Kopecky, and Walker (1962) liver microsomal preparations did not activate dimethoate. Usually microsomal preparations are more effective than slices. The reason for the difference here is not known.

Metabolic Studies in Various Species

Storage and Excretion.—Tests with ^{32}P -labelled dimethoate showed that it was fairly rapidly absorbed from the gut. *In vitro* hydrolysis was not increased by the centrifuged stomach content of the rat but was accelerated by the alkalinity of the duodenum. *In vivo* little radioactivity appeared below the duodenum. Absorption was apparently at least 75% complete, and probably often more, in man, mouse, rat, and guinea-pig, possibly varying slightly with the rate of passage through the gut. Accordingly, there is only a small difference between the oral and intraperitoneal toxicity of dimethoate as already shown.

In the species examined, the ^{32}P after absorption was found in higher concentration in liver, kidney, urine, and bile than in other tissues; in the rat the concentration was roughly uniform in blood, muscle, brain, and testis after one hour. The concentration in fat was markedly less than in other tissues probably because, while dimethoate itself partitions approximately evenly between lipoids and water, its degradation products are preferentially water-soluble (see later).

Excretion of ^{32}P was rapid after oral dosing with ^{32}P -dimethoate. From the rat over half was excreted in the urine in 24 hours and 25% in the faeces; and nine days after dosing only 0.9 to 1.1% of the ^{32}P administered remained in the animals. From a pheasant, 70% was excreted in the droppings in 24 hours, and from human subjects 76 to 100% appeared in the urine in the same time.

Faecal excretion may represent excretion of

unabsorbed material or excretion of ^{32}P in bile. The latter was demonstrated in the guinea-pig. These results are in good agreement with those of Dauterman *et al.* (1959), Kaplanis *et al.* (1959) and Chamberlain *et al.* (1961). Cheng *et al.* (1961, 1962) and Dauterman *et al.* (1959) have shown that little ^{32}P from ^{32}P -dimethoate is excreted in milk, in which residues become negligible after the first day.

Metabolism.—The ^{32}P in all tissues and excreta always partitioned less in favour of chloroform from water than dimethoate itself, showing that degradation to materials more preferentially water-soluble had occurred. This degradation was further explored by separation of the ^{32}P -labelled materials formed.

The compounds present were first separated by continuous chloroform extraction from water at pH 7.0, when non-ionic compounds were extracted by chloroform and ionic compounds remained in the water. The latter, consisting of a mixture of the products formed by the hydrolysis of P-O-C, P-S-C, and amide links of the dimethoate before or after thiono-oxidation, were not further separated. In rat and human urine, 74 to 98% of the excreted ^{32}P was in this form. These ionic hydrolysis products are all known to be of low mammalian toxicity and without anticholinesterase action (Dauterman *et al.*, 1959; Ahmed, Casida and Nichols, 1958; Krueger *et al.*, 1960; Soc. Montecatini, personal communications; Kaplanis *et al.*, 1959; Chamberlain *et al.*, 1961).

The chloroform-soluble fractions of human and rat urine obtained after oral dosing with ^{32}P -labelled dimethoate, and also of labelled dimethoate which had been incubated with rat liver slices, were then examined by both column and paper chromatography. Four distinct labelled non-ionic metabolites with anticholinesterase action were isolated. Some of their properties are compared with those of pure dimethoate in Table 5, in which the numbering of the metabolites is arbitrary. Consistent results were obtained with a number of separate preparations.

The anticholinesterase activities of these materials were characterized not only by their I_{50} concentrations but also by the slopes of their inhibition-log concentration curves.

The chloroform-soluble fractions of urine samples consistently contained all four metabolites but no detectable dimethoate. Metabolites II and IV were present in the greatest amounts with less of metabolite I and only very little of metabolite III.

Incubation of labelled dimethoate with rat liver slices also produced metabolites II and IV, with a little metabolite III and about 10% ionic hydrolysis

TABLE 5
PROPERTIES OF ANTICHOLINESTERASE DIMETHOATE METABOLITES

Material	Partition Coefficient Chloroform/water	Paper Rf		Molar I_{50}	Change in % Cholinesterase Inhibition from Tenfold Concentration Change
		CCl ₄	CHCl ₃		
Pure dimethoate	32.5	0.66	0.95	8.5×10^{-3}	29
Metabolite I	5.4	0.6	0.9	4.7×10^{-4}	68
II	1.1	0.30	0.78	1.1×10^{-5}	54
III	0.33	0.03	0.27	$ca. 2 \times 10^{-5}$	—
IV	0.028	0.02	0.05	$ca. 1 \times 10^{-4}$	—

products; any metabolite I was masked on the chromatograms by the large excess of unchanged dimethoate. Control tests with no liver showed only a trace of metabolite II present as an impurity in the starting material. Some metabolite IV was formed when separated metabolite II was further incubated with liver slices. Incubation with rat kidney slices caused greatly increased hydrolysis of metabolite IV, but not of metabolites I or II, to ionic phosphates.

Metabolism in the pheasant was explored to investigate its 20-fold greater susceptibility to dimethoate compared with the rat. This could not be explained by the slightly greater initial activation brought about by pheasant liver slices already described. Metabolite IV was equally readily hydrolysed by kidney tissue of both species. However, pheasant droppings contained proportionately less metabolite IV and more metabolite II than rat or human urine, and pheasant liver did not detectably produce metabolite IV from metabolite II, as did rat liver. Also, as stated earlier, maximum cholinesterase inhibition was attained more slowly in the pheasant than in the rat. It thus seemed likely that the difference was due mainly to less ready conversion of metabolite II to the readily detoxified metabolite IV in the pheasant, so that active cholinesterase inhibitors accumulated, with the slightly faster initial activation to metabolite II in the liver a contributory factor.

From locusts poisoned with labelled dimethoate, almost all the ^{32}P was recovered as metabolite II, with a trace of metabolite III and a small amount of ionic phosphates. In this species therefore detoxification was very much slower even than in the pheasant. Variations in the rates of loss of active dimethoate metabolites therefore probably account for much of the selective toxicity of dimethoate to insects.

Since the potentiating impurity referred to by Casida and Sanderson (1961, 1963) showed similar species variation in effectiveness, it is possible that this might act on the system converting metabolite II to metabolite IV.

Work on the plant metabolism of dimethoate, described by Chilwell and Beecham (1960), showed

that metabolite II was formed, but its levels fell off with time as rapidly as those of unchanged dimethoate. The remaining metabolites were ionic phosphates. Thus plants produced no toxic metabolites that were not also produced by mammalian metabolism.

Comparison of Table 5 with the results of Santi and Pietri-Tonelli (1959b) identifies metabolite II as the thiolate analogue of dimethoate:—

Formation of this material has been confirmed by other workers (Dauterman *et al.*, 1959; Alessandrini, 1960; Chamberlain *et al.*, 1961; Kaplanis *et al.*, 1959; Roberts, Radeleff, and Kaplanis, 1958; Spencer, 1959; Santi and Pietri-Tonelli, 1959a; Santi and Giacomelli, 1962; Dauterman *et al.*, 1960; Krueger *et al.*, 1960; Bull, Lindquist and Hacskeylo, 1963; Soc. Montecatini, personal communications). The other three metabolites in Table 5 have not been identified.

Therapy

Sanderson and Edson (1959) have shown that repeated atropine injections are effective in the treatment of oral dimethoate poisoning. Repeated injections of cholinesterase-reactivating oximes, such as pralidoxime iodide (P-2-AM), had no beneficial effect; this was consistent with the enzyme inhibition being largely irreversible.

O'Brien (1961) found that SKF 525A (2-diethyl-aminoethyl 2:2-diphenylvalerate hydrochloride) protected mice against intraperitoneal dimethoate but was of little benefit in rats or house-flies. (SKF 525A inhibits the oxidation of many organophosphate insecticides to their active metabolites by liver *in vivo*.)

Discussion

Pure dimethoate is of low toxicity to mammals but is rather more toxic to birds. Commercial grade dimethoate, whether formulated for spraying or not, is somewhat more toxic to some mammals, but the LD₅₀ values after oral administration are still above 100 mg./kg. The toxicity is much the same however the compound is given, except that the dermal toxicity is low because the compound is only slowly absorbed through the skin. This has been demonstrated both in rats and in man. The compound is without irritant action on the skin, and its vapour toxicity is negligible.

Dimethoate is toxic by virtue of its oxidation to its phosphorothiolate analogue and related metabolites, in which a phosphoryl group (P=O) is substituted for the thionate group (P=S) in the original compound. The analogues are powerfully anticholinesterase, and correspondingly the signs of poisoning are typically cholinergic. The compound does not induce delayed ataxia in hens, and there are no pathological lesions in animals which have recovered from the acute or chronic effects of the chemical. The earliest sign of poisoning is always inhibition of erythrocyte cholinesterase.

Cholinesterase is inhibited irreversibly *in vivo* and *in vitro*. Accordingly, the compound shows some cumulative toxicity in feeding trials; and symptoms after toxic doses may persist for two to four days. As is to be expected also, cholinesterase reactivating agents are without therapeutic effect, and therapy depends upon atropine and supportive treatment. There is, however, no accumulation of toxic material *in vivo*. From all species studied, including man, the ³²P content of a dose of ³²P-dimethoate is mostly degraded and excreted in one day, and only about 1% is retained in rats for nine days. There is therefore no hidden hazard due to storage in tissues of the type demonstrated with certain chlorinated hydrocarbon insecticides.

Metabolism is complex. In mammals it takes place mainly in the liver and consists, probably for the most part, of hydrolysis to harmless ionic products, but also of some oxidation to the thiolate analogue and to three other toxic metabolites. These three have not been reported before, though the possibility of other active metabolites has been suggested (O'Brien, 1960; Bull *et al.*, 1963). They appear to be much less stable *in vivo* than the thiolate analogue and also less toxic. It is open to speculation whether one or more of these metabolites might be formed by successive oxidation and removal of the N-methyl group after thiono-oxidation, as in substituted carbamates and phosphoramidates (Hodgson and Casida, 1961; Spencer, O'Brien, and

White, 1957), a reaction which Lock and Sagar (1960) suggest can occur more generally. In the pheasant, the thiolate analogue tends to accumulate, and it has been shown that its conversion to one of the other toxic metabolites (IV, Table 5) is much slower than in mammals. This probably explains the greater susceptibility of the pheasant. In the locust, the main product is the highly toxic thiolate analogue. These findings explain the great selectivity of the compound between mammals and insects. In plants, the thiolate analogue is produced but is unstable. It is the only toxic metabolite found, and as methods of analysis record it as dimethoate, it presents no additional hazard.

The action of dimethoate is not potentiated by a wide range of other insecticides when both are given orally.

The properties of dimethoate described above were investigated most intensively during the period 1958-59. Careful comparisons of the experimental data with data for pesticides presenting acknowledged hazards during field use suggested that dimethoate could be expected to be safe to handle in the field, even in the tropics, as long as caution and cleanliness were ensured when handling the concentrated material and during prolonged operations. During the first two years of commercial use in Britain, many hundreds of weekly cholinesterase estimations were carried out on the blood of reasonably experienced spray operators using dimethoate very intensively. The complete absence of cholinesterase inhibition in all samples examined proved very reassuring. Since then dimethoate has been widely used under temperate, subtropical, and tropical climatic and working conditions, without any known case of occupational poisoning. The notably low dermal toxicity and absorption rate of dimethoate is probably the factor most favourable to safe use, especially under tropical and subtropical working conditions. Similarly, no known instances have occurred of avian, fish or farmstock poisoning in Britain or elsewhere.

The safety of human consumers of food crops sprayed with dimethoate is materially aided by its comparatively low dietary toxicity and its fairly rapid disappearance from treated vegetation. In the rat, an intake rate of 0.5 mg./kg./day can be tolerated without cholinesterase depression, while an intake rate of 20 mg./kg./day is needed to cause clinical signs of toxicity. An intake rate for humans of 0.05 mg./kg./day should therefore be completely safe. This is equivalent to a 40 kg. child consuming 2 mg. dimethoate daily, *i.e.* 1 kg. food containing 2 p.p.m. dimethoate residues. Chilwell and Beecham (1960) have shown that the edible portion of a very wide range of crops always contained less than

2 p.p.m. dimethoate residues within one week of spraying. Subject to the observance of a minimum safety interval of one week between the last application of dimethoate and harvesting the crop, it may be safely presumed that dimethoate residue risks to food consumers are nil.

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Appendix

The systematic names of pesticides mentioned in the text are given below. Only the active ingredients are named.

acethion	S-carbethoxymethyl O,O-diethyl phosphorodithioate
amiton oxalate	2-(diethoxyphosphinylthio)-ethyl-diethylammonium hydrogen oxalate
azinphos-methyl	S-(3,4-dihydro-4-oxobenzo-[d][1,2,3]-triazin-3-ylmethyl) O,O-dimethyl phosphorodithioate
carbaryl	α -naphthyl N-methylcarbamate
carbophenothion	S-(4-chlorophenylthiomethyl) O,O-diethyl phosphorodithioate
chlorothion	3-chloro-4-nitrophenyl dimethyl phosphorothionate
D.D.T.	1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane
dicapthion	2-chloro-4-nitrophenyl dimethyl phosphorothionate
demeton	diethyl S-2-ethylthioethyl phosphorothiolate and diethyl 2-ethylthio-ethyl phosphorothionate
demeton-methyl	dimethyl homologues of demeton
diazinon	diethyl 4-(2-isopropyl-6-methylpyrimidinyl) phosphorothionate
dimefox	tetramethylphosphorodiamidic fluoride
dimethoate	O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate
dioxathion	2,3-bis(diethoxyphosphinothioylthio)-1,4-dioxane
E.P.N.	ethyl p-nitrophenyl phenylphosphonothionate
ethion	bis(diethoxyphosphinothioylthio)-methane
H.E.T.P.	see T.E.P.P.
Isolan	1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate

malathion	S-(1:2-dicarbethoxyethyl) O,O-dimethyl phosphorodithioate	phorate	O,O-diethyl S-(ethylthio)methyl phosphorodithioate
mevinphos	dimethyl 2-methoxycarbonyl-1-methylvinyl phosphate	prothoate	O,O-diethyl S-(N-isopropylcarbamoylmethyl) phosphorodithioate
parathion	diethyl <i>p</i> -nitrophenyl phosphorothionate	schradan	octamethylpyrophosphoramidate
parathion-methyl	dimethyl <i>p</i> -nitrophenyl phosphorothionate	T.E.P.P.	tetraethyl pyrophosphate
		trichlorphon	dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate