Table X. Ratio Between Amount of Bromide Secreted by Cows[a] in Milk and Amount of Bromide Ingested

Commodity		Mg. Br^-	Kg. Milk		Mg. of Br^- Secreted		
Descrip.	P.P.M. Br^-	Ingested per Day	Produced per Day	P.P.M. Br^- in Milk	in Milk per Day	Ratio Mg. of Br^- Secreted in Milk/Mg. of Br^- Intake	
Grain–CH_3Br	53	185	13.7	8	109	0.59	
	100	350	13.2	8	106	0.30	Av. 0.38
	220	770	13.2	15	198	0.26	
Grain–NaBr	50	175	13.2	2	26	0.15	
	100	350	13.2	4	53	0.15	Av. 0.18
	200	700	13.2	12	159	0.23	

[a] Four cows at Michigan State University, fed 3.5 kg. per day.

Correlation of Blood and Milk Bromide Levels

The correlations between blood and milk bromide levels where the cows were fed sodium bromide or grain fumigated with methyl bromide are plotted for comparison in Figure 1. Although the slopes of the regression curves are slightly different, they indicate similarity between the sources of bromide with respect to blood–milk bromide level relationships.

Conclusions

When dietary bromide is held at a steady concentration, an equilibrium is established with respect to milk bromide levels. The time of equilibration appears to be about 20 to 30 days.

Lactating cows fed a methyl bromide fumigated grain ration at the rate of 1 pound of ration to each 4 pounds of milk produced will secrete milk having increased levels of bromide as follows:

P.P.M. Total Bromide	
in Grain	in Milk
53	4 to 12
100	7 to 12
220	10 to 20

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SOLVENT EFFECTS ON TOXICITY

Reaction of Certain Phosphorothionate Insecticides with Alcohols and Potentiation by Breakdown Products

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MOST PESTICIDES are formulated with solvents, wetting agents, and other materials which increase the opportunity of the pesticide to contact the pest. Formulating agents are selected on the basis of their chemical, physical, and toxicological properties so as to achieve maximum pest control with minimal toxicity to other organisms. Careful toxicological studies are made on the pesticide before and immediately after formulation. As the formulated pesticide is often stored for long periods, and sometimes is exposed to elevated temperatures, the toxicological properties of the formulation must be rechecked on samples undergoing various storage conditions.

Organophosphorus insecticides often pose difficulties in formulation, particularly when a high degree of systemic activity is desired. The instability of certain organophosphates may allow degradation to a variety of toxic agents other than the insecticide as manufactured. Phosphorothionates may undergo oxidation or isomerization (*18*). Transalkylation may yield degradation products of greater mammalian toxicity (*12*). Hydrolysis may destroy the biological activity of the insecticide (*18*), or may yield ions which reattack the organophosphate to yield highly toxic materials, such as the formation of tetraethyl monothionopyrophosphate from Diazinon (*15*). These reactive organophosphoric ions might also combine with certain of the formulation constituents.

The solvent characteristics and toxicological properties of 2-methoxyethanol suggested it as a solvent for formulation of dimethoate [Rogor, *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] for use as a plant systemic. Routine checks on toxicological properties of formulated material revealed an unexpected toxic hazard. A preliminary report on the potential hazard from formulating dimethoate in 2-methoxyethanol has been published (*4*). Experimental details are considered here.

Materials and Methods

Insecticides and Solvents. Technical grade solvents were used for the formulation studies, although highly purified 2-methoxyethanol gave the same results as technical material. The insecticides, as obtained from their basic manufacturers, were of technical purity except for dimethoate where samples of varying purity were investigated. Dimethoate-P^{32} was prepared, purified, and characterized by a described procedure (*6*).

Fractionation of Dimethoate Derivatives. To the decomposed dimethoate in solvent was added 20 or more volumes of water. The aqueous solution was then neutralized and extracted twice with equal volumes of chloroform to remove the neutral phosphorus esters. The percentage hydrolysis was calculated from the proportion of the total phosphorus which appeared in the aqueous layer as phosphoric acid salts following the first extraction. After drying the chloroform with anhydrous sodium sulfate and evaporating the solvent, the neutral esters were fractionated on a silica gel column with *n*-hexane and chloroform (*6, 7*). Hydrolysis products were fractionated on an ion-exchange column (*6, 7, 19*), but remained largely uncharacterized. The insecticides other than dimethoate were similarly fractionated by

Dimethoate increases in toxicity to mammals on storage in certain hydroxylic solvents, particularly 2-alkoxyethanols. The reaction of dimethoate with 2-methoxyethanol at elevated temperatures yields at least six phosphorus-containing ionic products, seven neutral phosphate esters other than the original compound, and the disulfide of *N*-methylmercaptoacetamide. No evidence was obtained for formation of stable pyrophosphates. The product of highest mammalian toxicity was a *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate with one or both O-methyl groups replaced by O-(2-methoxyethyl) groupings. The toxicity to mammals of a few other phosphorothionate insecticides also increased on reaction with 2-methoxyethanol. Certain preparations of technical dimethoate contained an impurity, which somewhat increased the toxicity of dimethoate to several organisms, including the rat, following oral administration. Purified dimethoate reacted with lithium chloride or potassium O,O-dimethyl phosphorodithioate yielding a dimethoate potentiator, probably through O-demethylation as the initial reaction.

extraction from neutral aqueous solutions into chloroform. With Dipterex, the number of chloroform extractions was increased from two to four because of the low partition coefficient of this material into chloroform (*1*).

Toxicity Determinations. Rat oral LD_{50} determinations were made with male or female albino rats (130 to 210 grams) of the Wistar or Rolfsmeyer strains. The toxicant in a minimum volume of glycerol formal (*20*) was introduced via a stomach tube, the progress of symptoms observed, and the mortality data were recorded at greater than 48 hours. Six or more rats were used for approximating the LD_{50} values. Female albino mice (23 to 26 grams) of the Rolfsmeyer strain were used for intraperitoneal toxicity studies. Solutions of the toxicants in water, propylene glycol, or corn oil were injected at 0.10 ml. per 20 grams of mouse weight. LD_{50} values were based on 24-hour mortality data with 20 to 30 mice per compound. Housefly (*Musca domestica* L.) mortality was determined 24 hours after topical application of acetone solutions of the toxicants to 4-day-old adult females (*14*). In tests for potentiation with rats, theoretical LD_{50}'s of mixtures were calculated on the basis of the toxicities of the components, as defined by the reciprocal LD_{50}'s being additive with respect to concentration according to Finney (*9*) as reviewed by DuBois (*8*).

Other Methods. Other procedures not considered in detail were as previously reported (*6, 7*). Infrared spectra were made with a Baird infrared spectrophotometer with NaCl optics using 10% solutions of the organophosphates in carbon tetrachloride and carbon disulfide except where solubility difficulties necessitated the use of chloroform.

Results

Toxicity of Pure Dimethoate Compared with Technical Material. Technical dimethoate (Montecatini, Milan, Italy) varied in rat acute oral LD_{50} from about 215 to 350 mg. per kg. while the highly purified material gave values of about 550 for male and 650 mg. per kg. for female rats. The percutaneous toxicity of propylene glycol or 2-methoxyethanol solutions to male rats with 22 hours covered contact was about 700 to 1100 mg. per kg. with either the technical or highly purified material. The toxicity difference between the technical and pure material appeared in the acute oral LD_{50}'s (mg. per kg.) with the rabbit (500 pure, 300 to 450 technical), less so with the guinea pig (550 pure, 350 to 600 technical) and hen (50 pure, 25 to 40 technical), but not with the mouse (60 for either) or pheasant (12 to 20 pure, 15 to 20 technical). These figures for rabbit, hen, and pheasant are very tentative because of the small number of animals involved. No toxicity difference was observed between the technical and pure dimethoate following injection into locusts, or topical application to locusts, aphids, or flies.

The rat, rabbit, and guinea pig, but not the mouse, hen, or pheasant quickly developed a deep narcosis after administration of pure dimethoate. No such narcosis occurred with the technical material in any of the species. Narcosis preceded typical anticholinesterase signs by at least an hour, and had usually abated by the time that the typical symptoms of dimethoate poisoning began. As this narcosis was an effect caused by pure material but not by impure material at the same doses, it is not possible at present to provide an explanation for the mechanism. Similar narcotic effects have been reported by Vandekar (*22*), but only with unpurified organophosphorus compounds of which larger doses could be given intraperitoneally.

Evidence for a Potentiating Agent in Technical Dimethoate. Further study on the oral toxicity of technical dimethoate to rats demonstrated that a component of the technical material served to increase or potentiate the toxicity of pure dimethoate. A small amount of technical dimethoate administered with pure dimethoate increased its toxicity far more than anticipated for an additive effect (Table I).

Table I. Potentiating Action of Impurities in Technical Dimethoate on the Toxicity of Pure Dimethoate to Rats

Contaminant in Mixture, % (W./W.)	Oral LD_{50}, Mg./Kg. Actual	Calcd.[a]
TECHNICAL DIMETHOATE		
0	648 (529–795)[b]	(648)
3.3	436 (367–519)	629
10	400 (328–489)	593
30	400 (328–489)	507
100	336 (263–429)	(336)
PES + PEI FRACTIONS FROM TECHNICAL DIMETHOATE		
0	648 (529–795)	(684)
1	476 (400–566)	643
3	336 (283–400)	633
9	308 (259–367)	606
27	336 (283–400)	537
100	367 (308–436)	(367)

[a] Calculated according to Finney (*9*).
[b] 95% Confidence limits.

Technical grade dimethoate was separated into four fractions to determine the nature of the potentiator. These fractions were an organic-insoluble (EI) which failed to dissolve initially in diethyl ether, the dimethoate which was crystallized out from the ether at −78° C., and two organic-soluble impurity fractions obtained from the ether-soluble material after crystallizing out the dimethoate, evaporating the solvent, and washing the residue with cooled petroleum ether to yield a soluble (PES) and insoluble (PEI) fraction. A typical sample of technical dimethoate yielded 3.5% EI, 2% PES, and 17% PEI. Adequate solvent volumes were used at each stage to yield maximum differentiation of the fractions and to approximate a complete crystallization of the dimethoate. The EI fraction was a mixture of hydrolysis products, and the PES material was predominantly *O,O,S*-trimethyl phosphorodithioate. The PEI fraction contained at least four phosphorus-containing materials based on paper chromatography, was mostly ionic, and had an atomic

Table II. Potentiating Action of the PEI Fraction from Technical Dimethoate When Added at 10% (W./W.) to Several Organophosphorus Compounds

Compound	Rat Sex	Rat Oral LD_{50}, Mg./Kg. Actual	Calcd.[a]	Potentiation Ratio[b]
Dimefox	M	1.7[c]	2.2	1.3
Dimethoate	F	285 (240–339)[d]	586	2.1
O,O-Dimethyl *S*-carbamoylmethyl phosphorodithioate	M	925 (655–1308)	2000	2.2
Malathion	M	550 (369–821)	1486	2.7
Methyl demeton	F	>55	82	<1.5
Parathion	F	2.8[c]	3.3	1.2
Schradan	M	>3.3	5.5	<1.7

[a] Calculated according to Finney (*9*). [b] Calculated LD_{50}. [c] Approximation. [d] 95% Confidence limits.

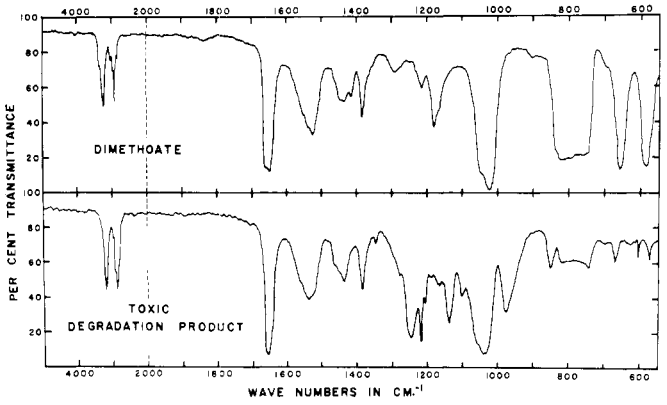

Figure 1. Comparison of infrared spectra (10% solutions in carbon tetrachloride) of dimethoate and a toxic degradation product from reaction with 2-methoxyethanol (the last phosphorothiolate fraction eluted from silica gel columns as discussed in the text)

Table III. Stability of Dimethoate at 40% (W./V.) in Various Solvents at 70° C.

Conditions (Solvent Concn. and Temp.[a])	% P^{32} in $CHCl_3$ Phase After Days:[b] 2	8	Mouse LD_{50} (ip., Mg./Kg.) After Days:[c] 2	8
None, 100%, 70° C.	83	18	70	>160
Water, 3.2%				
28° C.	95	92	145	125
70° C.	7	0.7	>160	>160
Hydroxylic organic solvents, 40%, 70° C.				
2-Ethoxyethanol	91	63	20	3
Glycerol formal	84	25	50	45
Methanol	68	9	>100	>160
2-Methoxyethanol	85	43	20	2.5
Isopropanol	87	46	40	28
Propylene glycol	60	9	83	>160
Nonhydroxylic organic solvents, 40%, 70° C.				
Cyclohexane	93	81	75	70
Diethyl carbitol	95	90	75	50
Dioxane	94	85	75	83
Methyl isobutyl ketone	95	...	75	65

[a] Once recrystallized technical dimethoate containing microgram amounts of dimethoate-P^{32} dissolved in commercial grade solvents. The temperature for methanol was 65° C.

[b] At zero days, the percentage of P^{32} partitioning into chloroform from an equal volume of water was 95 to 97% with no differences between formulating solvents.

[c] Mouse LD_{50} values based on dimethoate equivalents. The administration solvent was propylene glycol with all materials except dimethoate originally dissolved in water. The zero day LD_{50} was 105 mg. per kg. with no difference between formulating solvents. Similar toxicity relationships were found in a separate study with rats following oral administration of the formulations.

ratio for phosphorus:sulfur:nitrogen of 1:2.38:0.53.

Addition of 10% (w./w.) of fraction EI or PES to pure dimethoate failed to yield significant potentiation. The potentiating agent appeared in the PEI fraction, as shown by factors for potentiation of 1.95 and 1.77 from two separate preparations. An equal mixture of the impurity fractions (EI, PES, and PEI) was tested, but no potentiation was found between the impurity fractions. The PEI fraction was further fractionated by washing with xylene, the potentiating agent appearing in the xylene-insoluble fraction. Distillation of the PEI fraction at 0.7 mm. Hg with a slow leak of nitrogen through the liquid yielded distillate fractions at a vapor temperature of 51° and 60° C. which were active in potentiation, but the distillation yielded no concentration of the potentiating agent, and some decomposition occurred during distillation. The potentiating material was difficult to remove from dimethoate on purification by crystallization since the potentiator carried along with the crystals through one or two crystallizations from ether unless the crystals were thoroughly washed with cold ether. The difficulty in purifying out the potentiating agent was further shown by the finding that dimethoate must be recrystallized several times from diethyl or diisopropyl ether and preferably once or twice subsequently from xylene before no further toxicity reduction results from recrystallization. The activity of trace amounts of the potentiating material in increasing the oral toxicity of dimethoate to rats is illustrated in Table I. The activity in potentiating the toxicity of other organophosphate insecticides is shown in Table II.

The action of the potentiating agent in rats was briefly examined. By injecting (ip.) the potentiating fraction (PEI) at 40 mg. per kg. into female rats and then administering pure dimethoate orally at 400 mg. per kg. after 4, 24, and 96 hours, the potentiating effect was not transient but persisted for at least 24 hours but not up to 96 hours. In this test, the narcosis caused by the pure dimethoate was not reduced by the potentiator. The potentiating agent was ineffective in potentiating the dermal toxicity of pure dimethoate as determined by concurrent intraperitoneal administration of the PEI fraction and dermal administration of pure dimethoate with the amount of potentiator administered comparable to 10% of the weight of the dimethoate administered. The lack of action of the potentiator with percutaneous application was also indicated by the lack of dermal toxicity difference between the pure and technical dimethoate. Failure of the potentiator to act for dermally applied, pure dimethoate was therefore not due to inability of the potentiator to penetrate the skin since injected potentiator was also ineffective. It is possible that in the process of penetration through the skin the potentiator is formed while the liver is less significant in formation of the potentiator. Attempts to demonstrate an increased oral toxicity of pure dimethoate after incubation with shredded rat skin in Ringer solution were inconclusive, due to difficulties in homogenizing the products for administration.

Several suspected or known decomposition products of dimethoate or related compounds were tested as possible potentiators by adding them at 10% (w./w.) to pure dimethoate and administering orally to female rats. The materials which failed to potentiate pure dimethoate were as follows: $(CH_3O)_2P(S)SH$, pure and impure; $(CH_3O)_2P(S)SCH_3$; $(CH_3O)_2P(S)SSP(S)(OCH_3)_2$; $(CH_3O)_2P(S)SCH_2C(O)OH$; $(CH_3O)(HO)P(S)SCH_2C(O)OH$, impure; $HSCH_2C(O)NH_2$; $HSCH_2C(O)NHCH_3$; and $(SCH_2C(O)NHCH_3)_2$.

A potentiator was formed when pure dimethoate was treated with dealkylating

Table IV. Decomposition of Dimethoate in 2-Methoxyethanol (40% W./V.)

Property	Technical Dimethoate, 7 Months at 25° C.	Pure Dimethoate, Hours at 100° C. 15	30	52	91	138	303
Total neutral phosphate esters, %[a]	59	80	57	36	22	15	6.5
Percent of total P^{32} as:[b]							
Neutral phosphate esters eluting in the position of:[c]							
Phosphorodithioate A	4.2	1.45	3.09	0.95	0.26	0.00	0.00
Dimethoate	47.8	63.32	34.58	10.71	6.98	0.23	0.18
Phosphorodithioate C	0.8	8.05	10.17	8.64	4.96	1.49	0.44
Phosphorothiolates	6.0	7.17	9.16	15.69	9.80	13.28	5.87
Acidic phosphate esters eluting in the position of:[d]							
$(CH_3O)_2P(O)OH$	28.0	9.51	20.27	31.63	45.88	58.58	86.06
Unknown A	6.9	1.53	6.57	9.63	6.31	2.77	1.33
$(CH_3O)_2P(S)SCH_2C(O)OH$	2.7	6.49	12.54	16.79	19.91	15.91	1.51
$(CH_3O)_2P(S)OH$	2.5	1.67	2.64	4.13	4.41	6.41	4.61
Unknown B	0.7	0.10	0.34	1.06	1.39	1.20	0.00
$(CH_3O)(HO)P(S)SCH_2C(O)NHCH_3$	0.4	0.69	0.64	0.76	0.12	0.10	0.00
Mouse LD_{50}, ip., mg./kg.[e]	9	16	5	4	16	65	>250

[a] Proportion of total phosphorus partitioning into an equal volume of chloroform from neutral aqueous solution.

[b] The phosphorus determinations were made colorimetrically for the technical dimethoate solution and by radioassay in the other cases where dimethoate-P^{32} was used in the reaction with 2-methoxyethanol.

[c] Separation on a silica gel column by the procedure of Dauterman *et al.* (*6*). Phosphorodithioate A may have contained a small amount of a second phosphorodithioate, particularly in the case of the technical dimethoate. The phosphorothiolate fraction consisted of at least five materials as indicated in the text.

[d] The acidic phosphate esters eluted in the same position indicated in Figure 2 of the paper by Dauterman *et al.* (*6*). Fraction designations indicate elution position and not structures of the hydrolysis product since it is probable that many of these materials contain the 2-methoxyethyl grouping replacing either the *O*-methyl or *N*-methylamide grouping.

[e] Initial mouse LD_{50} for the purity of dimethoate used was 145 mg. per kg.

agents under mild conditions. The most effective agent for yielding the potentiator was potassium *O,O*-dimethyl phosphorodithioate, while lithium chloride also showed some activity. Potassium *O,O*-dimethyl phosphorodithioate (5.9 grams) was refluxed with pure dimethoate (10.7 grams) for 2 hours in 50 ml. of anhydrous acetone, evaporated, and the residue washed with several portions of petroleum ether to remove *O,O,S*-trimethyl phosphorodithioate (*2*) and then with several portions of chloroform to remove unreacted dimethoate. The residue after evaporating the solvent was 8.6 grams of material which when added at 10% (w./w.) to pure dimethoate yielded potentiation by a factor of about 2.2 in the oral toxicity to female rats. This active potentiating material was further separated into three fractions of about equal weight by precipitating from an acetone solution at −78° C. by slowly adding diethyl ether. All three fractions gave potentiation of pure dimethoate by factors of 1.8 to 2.6. Pure dimethoate refluxed with potassium *O,O*-dimethyl phosphorodithioate in anhydrous methanol for 8 hours and then fractionated in a similar manner also gave an organic-insoluble fraction which potentiated pure dimethoate. Refluxing of *O,O*-dimethyl phosphorothioic acid with equimolar pure dimethoate-P^{32} in acetone for 36 hours resulted in about 40% breakdown of the dimethoate to ionic products based on chloroform to water partitioning properties of the P^{32}, but failed to yield material with significant activity in potentiating pure dimethoate. However, when an aqueous solution of the impure potassium desmethyl dimethoate, $(CH_3O)(KS)P(O)SCH_2C(O)NHCH_3$, was treated with excess hydrochloric acid and extracted with chloroform, the chloroform-soluble materials yielded a potentiation factor of 1.6. Oxidation of desmethyl dimethoate by the general procedure of Foss (*11*) yielded a more toxic product (LD_{50} of approximately 100 mg. per kg.) with little activity in potentiating pure dimethoate. A potentiator (potentiation factor 2) for pure dimethoate was formed by refluxing equimolar lithium chloride and dimethoate in acetone for 4 hours, evaporating the solvent, and washing thoroughly with chloroform to leave a hygroscopic salt which when dry yielded a two-factor potentiation of pure dimethoate. Elemental analyses on this hygroscopic sample yielded 12.29% phosphorus, 25.51% sulfur, and 5.73% nitrogen for an atomic ratio of 1.00:2.01:1.03 and a calculated molecular weight of 252 instead of the theoretical 221.

Stability of Dimethoate in Different Solvents. Dimethoate is relatively stable in nonhydroxylic organic solvents but undergoes both hydrolysis and toxicity changes in hydroxylic organic solvents (Table III). Water for this hydrolysis reaction may be present as an impurity or absorbed from the air. The toxicity to mice of dimethoate was reduced in water, propylene glycol, and methanol, but increased in glycerol formal, isopropanol, and 2-ethoxy- and 2-methoxyethanol during an 8-day storage at 70° C. A similar relationship was found in a separate experiment where the change in rat oral toxicity was used as the criterion of stability. Further studies were made with 2-methoxyethanol and isopropanol to determine the mechanism of reaction forming toxic materials or combinations of materials from dimethoate.

Degradation of Dimethoate in 2-Methoxyethanol. The rate of dimethoate decomposition in 2-methoxyethanol at 100° C. is indicated by Table IV. There was a marked loss in thiono sulfur both in the neutral and acidic esters. No correlation was evident between the amount of any one fraction and the toxicity of the total reaction mixture to mice. Several of the fractions contained mixed components. This was particularly true for the neutral phosphorothiolate ester fraction. It was impossible to state a structure for the ionic degradation products based on their elution position from the ion-exchange column because of the great number of possible acidic esters that could be formed when the 2-methoxyethanol can replace either or both of the *O*-methyl and *N*-methylamide groupings before hydrolysis. It appears likely, however, that considerable hydrolysis occurred at the carboxyamide grouping. The ratio of products in a solution prepared by dissolving technical dimethoate in 2-methoxyethanol and storage for 9 months at room temperature was quite different than that from the purified dimethoate in 2-methoxyethanol held at 100° C. until the same degree of breakdown had occurred. The difference may represent the effect of impurities in the technical dimethoate and/or a change in the balance of products with reaction temperature. The last component of the neutral phosphorothiolates eluted from the silica gel columns with *n*-hexane: chloroform (20:80) was almost identical with respect to infrared spectrum for both technical and purified dimethoate held at elevated temperature in 2-methoxyethanol.

Nature of the Neutral Esters Formed on Reaction of Dimethoate with 2-Methoxyethanol. Chromatography of the neutral esters from the reaction of dimethoate with 2-methoxyethanol (40% w./v. dimethoate in 2-methoxyethanol, 8 days, 70° C.) resolved eight phosphorus-containing components. These components in the order of their elution made up the following percentages of the total phosphorus: 11.2, 52.1, 4.4, 2.4, 1.3, 0.5, 6.8, and 21.3%. Each

fraction was contaminated with nonphosphorus-containing degradation products of *N*-methylmercaptoacetamide and 2-methoxyethanol. Elemental analyses on the samples were meaningless because only small amounts were available and the samples were used first for spectral examination which left traces of carbon tetrachloride in the samples even after careful evaporation at reduced pressures. The nature of the fractions was interpreted from the following absorption bands in their infrared spectra: when present the $-C(O)NHCH_3$ grouping gave absorption bands at 1525 to 1535, 1645 to 1655, and a doublet at 3200 to 3400 cm.$^{-1}$; the $-C(O)OCH_2CH_2OCH_3$ grouping at 970 to 985, and 1130 to 1135 cm.$^{-1}$ in addition to the 1020 to 1030 cm.$^{-1}$; the P=O group at 1250 cm.$^{-1}$ and the P=S at 580 to 590, and 653 to 658 cm.$^{-1}$; and the $POCH_3$ at 1180 cm.$^{-1}$. The nature of the fractions was as follows:

Phosphorodithioate A, a phosphorodithioate eluting with hexane-chloroform (80:20) which probably contained a carboxylic ester rather than an amide grouping with a possible structure of $(CH_3O)_2P(S)SCH_2C(O)OCH_2CH_2OCH_3$.

Dimethoate, eluting with hexane-chloroform (80:20).

Phosphorodithioate C, a phosphorodithioate eluting with hexane-chloroform (80:20) with a carboxylic amide peak evident in the infrared spectrum. One possible structure would be $(CH_3O)(CH_3OCH_2CH_2O)P(S)SCH_2C(O)NHCH_3$.

Three phosphorothiolates eluting with hexane-chloroform (50:50). The first of these may have been $(CH_3O)(CH_3OCH_2CH_2O)P(O)SCH_2C(O)OCH_2CH_2OCH_3$.

A phosphorothiolate eluting with hexane-chloroform (20:80), the known elution position on this column of $(CH_3O)(CH_3S)P(O)SCH_2C(O)NHCH_3$, with an infrared spectrum very similar to this known compound.

A phosphorothiolate eluting with hexane-chloroform (20:80) which contained an impurity which crystallized out from carbon tetrachloride. This impurity was identified as the disulfide of *N*-methylmercaptoacetamide as indicated later. The infrared spectrum on the remaining soluble fraction is compared with that of dimethoate in Figure 1. This spectrum clearly indicates that the degradation product is a phosphorothiolate with the carboxylic amide group intact. The absorption peaks at 975 and 1135 cm.$^{-1}$ are associated with the formation of a 2 methoxyethyl phosphate group. This conclusion is based on examination of the spectra of several known 2-alkoxyethyl phosphate esters. Elemental analyses indicated one sulfur per molecule, though the samples still contained carbon tetrachloride and the disulfide of *N*-methylmercaptoacetamide. The most probable structure appeared to be $(CH_3O)(CH_3OCH_2CH_2O)P(O)SCH_2C(O)NHCH_3$. Neither the elemental analyses nor the infrared spectrum give sufficient evidence to rule out the structure, $(CH_3OCH_2CH_2O)_2P(O)SCH_2C(O)NHCH_3$, although it appears less likely than the structure with only one ester group exchanged. Attempts to prepare $(CH_3O)(CH_3OCH_2CH_2O)P(O)SCH_2C(O)NHCH_3$ through a variety of routes were not successful.

The first seven fractions were not lethal to mice when injected at 40 mg. per kg. The eighth and last fraction had a mouse intraperitoneal LD_{50} of 0.5 mg. per kg. and a rat oral LD_{50} of 1.0 to 1.5 mg. per kg. The fly topical LD_{50} as μg. per gram was 0.4 for dimethoate, 0.9 for the dimethoate after reaction with 2-methoxyethanol, and 1.2 for the separated phosphorothiolate fraction eluted last from the silica gel column.

Presence of a Disulfide and Absence of Thionopyrophosphates in Dimethoate Decomposed in 2-Methoxyethanol. In the decomposition of dimethoate in 2-methoxyethanol, the disulfide of *N*-methylmercaptoacetamide is formed. A decomposed formulation was dissolved in water, neutralized, and extracted with chloroform. The chloroform was dried with sodium sulfate, evaporated, and the residue dissolved in diethyl ether. On cooling to $-78°$ C., a portion of the material precipitated. The cold supernatant was decanted off and the procedure repeated to wash the ether-insoluble material three times with fresh ether. Some of the ether-insoluble materials, when dissolved in a chloroform–carbon tetrachloride mixture, crystallized on cooling. Recrystallization from chloroform–carbon tetrachloride yielded a material identical to the known disulfide of *N*-methylmercaptoacetamide in melting point and mixed melting point (124° C., uncorrected), infrared absorption spectrum, and elemental analysis (calculated; C—34.59, H—5.81, N—13.45, and S—30.80%; actual; C—34.50, H—6.05, N—13.60 and S—30.67%). This same material was also recovered as indicated previously as an impurity in the toxic degradation product after chromatography on silica gel.

It appeared possible that the high toxicity of decomposed dimethoate might be associated with thionopyrophosphates based on the high toxicity to mammals of these materials (*17*) and the isolation of tetraethyl monothionopyrophosphate as a degradation product of Diazinon on storage where trace amounts of water are involved in initiating the decomposition (*15*). On analogy with the decomposition of Diazinon and the known toxicity of tetramethyl monothionopyrophosphate, this material was prepared by the procedure of McIvor *et al.* (*17*), and the product yielded the infrared spectrum and mouse intraperitoneal toxicity reported by these workers (*16*, *17*). Chromatography of tetramethyl monothionopyrophosphate showed that it eluted from the silica gel column coincident with the elution position of dimethoate. This toxic pyrophosphate was not a constituent of dimethoate decomposed in 2-methoxyethanol since: the chromatographic characteristics were different than that of the toxic agent derived from dimethoate, the dimethoate fraction recovered from chromatography was not greatly more toxic than known dimethoate and this fraction would contain tetramethyl monothionopyrophosphate if it were present, and the infrared spectrum of the toxic agent when isolated differed greatly from that of the pyrophosphate.

Degradation of Dimethoate in Isopropanol. A 40% solution (w./v.) of dimethoate-P^{32} in isopropanol held 8 days at 70° C. decreased in mouse LD_{50} to about 28 mg. per kg. (Table III). Fractionation of this material in a similar manner to that described for the products from the dimethoate-2-methoxyethanol reaction yielded 17% dimethoate, three other less polar phosphorodithioates (1, 3, and 12% of the total phosphorus), three phosphorothiolates (6, 4, and 3% of the total phosphorus) and 54% of the phosphorus-containing materials as hydrolysis products. The mouse LD_{50} for each of these components other than the phosphorothiolates was greater than 80 mg. per kg. The major phosphorodithioate (12%) other than dimethoate appeared from the infrared spectrum to be $(CH_3O)_2P(S)SCH_2C(O)OCH(CH_3)_2$. The most toxic material was the phosphorothiolate comprising 6% of the total and eluting with hexane-chloroform (50:50) as a distinct peak just after the dimethoate. This phosphorothiolate had a mouse LD_{50} of 10 to 15 mg. per kg. All three phosphorothiolates had the $=P(O)SCH_2C(O)NHCH_3$ portion of the molecule intact. The first two (6 and 4%) had at least one isopropyl group introduced based on a shift in the P—O—(C) infrared absorption band to 999 cm.$^{-1}$ from the 1020 to 1030 cm.$^{-1}$ position in $(CH_3O)_2P(O)SCH_2C(O)NHCH_3$ and $(CH_3O)(CH_3S)P(O)SCH_2C(O)NHCH_3$, and the appearance of the appropriate bands associated with the isopropyl group, particularly at 1383, 1369, 1170, and 1143 cm.$^{-1}$. The infrared spectrum for the most toxic material (6% of the total phosphorus) had P—O—(C) bands at both 999 and 1043 cm.$^{-1}$ and was consistent with a structure of $(CH_3O)((CH_3)_2CHO)P(O)SCH_2C(O)NHCH_3$ or $((CH_3)_2CHO)_2P(O)SCH_2C(O)NHCH_3$. The 3% component eluted last from the column appeared to be $(CH_3O)(CH_3S)P(O)SCH_2C(O)NHCH_3$.

Stability of Several Phosphate Insecticides in 2-Methoxyethanol. Eleven phosphate insecticides dissolved at 40% (w./v.) in 2-methoxyethanol were held 8 days at 10° or 70° C. A great increase in toxicity to mice resulted on heating the two amide-containing phosphorothionates, dimethoate and *O*-

Table V. Toxicity to Mice of Several Insecticides Formulated in 2-Methoxyethanol (40% W./V.) and Held 8 Days at 10° C. or 70° C.

Insecticide	Mouse LD_{50}, ip., Mg./Kg.[a] 10° C.	70° C.	LD_{50} Ratio, 10° C./ 70° C.
Chlorthion	65	77	0.85
Co-Ral	50	50	1
DDVP	22	500	0.044
Delnav	230	140	1.6
Dimethoate	145	2.7	54
Dipterex	425	325	1.3
Malathion	1000	270	3.7
Methyl parathion	8	8	1
O-Methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate	50	5	10
Parathion	3	3	1
Ronnel	1100	190	5.8

[a] Propylene glycol used as solvent for toxicity determinations.

methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate (Table V). Significant increases also resulted with malathion and Ronnel, while DDVP was greatly reduced in toxicity. The heated formulations were dissolved in water, neutralized with sodium bicarbonate, and extracted with chloroform a sufficient number of times to theoretically recover 98% of the original organophosphate. Based on a single determination, the recovered chloroform-soluble materials comprised greater than 90% of the theoretical weight for no hydrolysis with Co-Ral, Dipterex, methyl parathion, and *O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate, 55 to 70% of theory with Chlorthion and parathion, and 32 to 45% of theory with DDVP, Delnav, dimethoate, malathion, and Ronnel.

Discussion

An impurity in certain batches of technical dimethoate was found to potentiate the mammalian toxicity of the main constituent. A potentiation in rat oral toxicity was also evident when this impurity was mixed with certain other carboxylic ester and carboxylic amide-containing phosphorothionates. Such potentiation probably results from one compound, the potentiator, reducing the efficiency of detoxification of the toxicant (*8*). A wide variety of organophosphates have been found to serve as potentiators for the toxicity of malathion and certain other organophosphates (*3, 8, 21*). The presence of potentiators, or indeed of any more toxic impurities or decomposition products, in technical pesticides can be detected by the routine toxicity checks normally carried out during the development of a manufacturing method.

Formulations of certain phosphorothionate insecticides in 2-methoxyethanol were found to increase in mammalian toxicity on storage at elevated temperatures. Many 2-alkoxyethyl phosphates and phosphorothionates are known to have high mammalian toxicity. Introduction of a 2-alkoxyethyl group into the molecule of a phosphate insecticide with low mammalian toxicity, particularly in place of an *O*-methyl phosphate group, would be expected to reduce or destroy the desired selective toxicity characteristics of the molecule. Hydroxylic organic solvents may catalyze the hydrolysis or directly replace ester or amide groupings within organophosphate insecticides. Where formulations contain such solvents, the storage stability of the formulation should be carefully examined for possible changes in chemical composition and selectivity of biological action. These toxicity changes can be detected during the experimental work carried out on accelerated storage of proposed new formulations.

Extensive studies on the biological fate of dimethoate have established the susceptibility of all ester and amide sites in the molecule to hydrolysis (*5–7, 13*). High humidity results in decomposition of dimethoate to methylamine, mercaptoacetic acid, and unidentified phosphorus compounds (*10*). The reaction with 2-methoxyethanol is complex, yielding at least six phosphorus-containing ionic products and seven neutral phosphorus esters other than the original compound. In addition to degradation to ionic products, the reactions involve considerable loss of thiono sulfur and incorporation of 2-methoxyethyl groups into the molecule in place of the *O*-methyl and *N*-methyl groups. The product of high mammalian toxicity is probably formed by attack of the *O*-methyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate ion on the solvent. This ion might have originated either from direct hydrolysis of dimethoate under the acid conditions of the formulation caused by degradation to ionic products, or by reaction with some *O,O*-dimethyl phosphorodithioic acid present as an impurity or degradation product. It is not clear whether one or both *O*-methyl groups are displaced by the solvent.

This investigation followed the discovery by routine toxicity checks that an unusual gain in mammalian toxicity had occurred in an early formulation of technical dimethoate. Change of solvent gave a commercial formulation of entirely satisfactory toxicological stability.

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