Chemistry and Toxicological Properties of *O*, *O*-Diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) Phosphorothioate (Diazinon)

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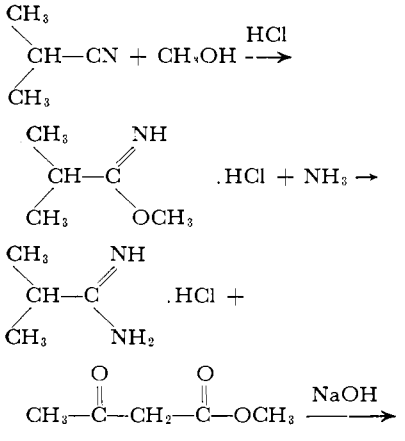
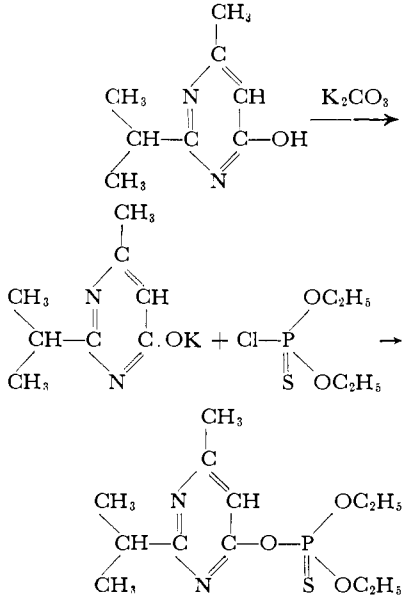
The insecticide O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (Diazinon) is prepared by condensation of 2-isopropyl-4-methyl-6-hydroxypyrimidine with O,O-diethyl chlorothiophosphate. The presence in the chloro intermediate of small amounts of mono and triethyl thiophosphates gives technical Diazinon which contains as impurities triethylthiophosphate and dipyrimidinyl ethyl phosphorothioate. Diazinon is obtained in the pure state by distillation or through formation of a mineral acid salt. Pure Diazinon is not a potent cholinesterase inhibitor, but on distillation or long standing increased cholinesterase inhibitory activity is found. A number of possible isomerization and decomposition products have been prepared, but the compound responsible for the anticholinesterase activity has not yet been found.

As the result of an extensive study of organic phosphorus compounds by Schrader (*11*) in Germany, a new group of biologically active compounds was found. Of these organophosphates, the condensation product of 4-nitrophenol with *O*,*O*-diethyl chlorothiophosphate (parathion) is still widely used against a variety of insects in agriculture. In 1950, a thorough study was started at the Geigy Research Laboratories to find insecticides of activity comparable to that of parathion, but showing lower toxicities to warm-blooded animals (*5*). From these studies came the organic phosphate insecticide Diazinon, *O*,*O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate, which was recently introduced to the agricultural and sanitary fields (*4*, *7*).

The synthesis of technical Diazinon, its purification, the by-products formed, and their respective properties are described below.

Synthesis of Technical Diazinon



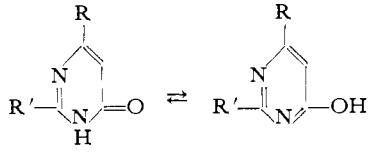


2-Isopropyl-4-methyl-6-hydroxypyrimidine. The synthesis of 2-isopropyl-4-methyl-6-hydroxypyrimidine is based on the procedure of Pinner (*10*), who, at the end of the last century, described the synthesis of 6-hydroxypyrimidines. The instability of the imido esters and amidines, intermediates in the synthesis of hydroxypyrimidines, made these compounds unavailable commercially. It could be shown that the reaction of aliphatic nitriles with hydrochloric acid in methanol under strictly anhydrous conditions leads to the respective imido ester in good yields. Methyl isobutyrimidine hydrochloride was prepared from isobutyronitrile in yields as high as 97%. Reaction of the methyl isobutyrimidine hydrochloride with aqueous ammonia gave the isobutyramidine hydrochloride in 8% yield. By condensing an aqueous solution of isobutyramidine hydrochloride with methyl acetoacetate, it is possible to get pure 2-isopropyl-4-methyl-6-hydroxypyrimidine in 93% yield (see flow diagram).

The hydroxypyrimidine appears, even as a technical product, in high purity, having an active ingredient content between 97 and 99.9%. Upon recrystallization of the technical product, no other heterocyclic compound than the mentioned hydroxypyrimidine was ever isolated. The properties of this compound are: colorless needles, melting point 173–4° C.; median acute oral lethal dosage to mice (abbreviated as LD_{50} mouse per os): 2700 mg. per kg.; median pseudocholinesterase inhibition (abbreviated as ChE i_{50}) > 20 mg. % [enzyme to human blood plasma 1 to 10, substrate: acetyl choline 6.6 × 10^{-3} mole in Ringer–sodium bicarbonate 37° C. (*1*)].

In order to decrease the production costs of 2-isopropyl-4-methyl-6-hydroxypyrimidine, a continuous procedure was developed in the production department at Geigy (*12*).

The hydroxypyrimidines exist in two isomeric forms, as ketone and as enol.

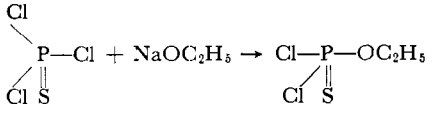


In alkaline solution the hydroxypyrimidines easily form salts which can be isolated after the azeotropic evaporation of the water with an appropriate solvent. It is important to use a sufficient amount

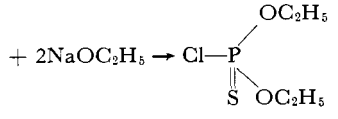
of solvent such as benzene in order to get rid of traces of water. The most convenient salts for the further condensation reactions are the potassium and sodium salts, respectively. Instead of isolating the alkali salt of hydroxypyrimidine, it is possible to heat a mixture of hydroxypyrimidine with potassium carbonate in benzene until no more water is distilled off. During this procedure the alkali salt of the hydroxypyrimidine is formed as a fine dispersion which is very suitable for the next condensation step.

***O,O*-Diethyl Chlorothiophosphate.** The synthesis of diethyl chlorothiophosphate is described in the literature and in several patent applications.

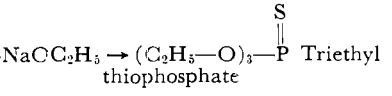
Trichlorothiophosphate, which can be made from phosphoryl chloride plus sulfur, reacts with sodium alcoholate to form:



O-Ethyl dichlorothiophosphate (dichloro ester)



O,O-Diethyl chlorothiophosphate (monochloroester)

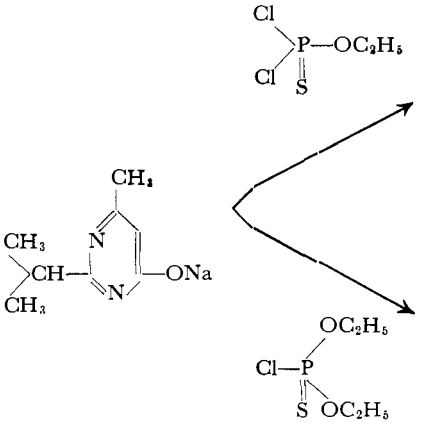


Triethyl thiophosphate

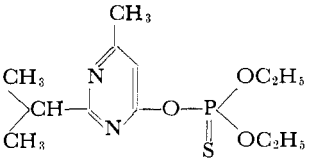
Although two of the three chlorine atoms are considerably more active, it is not possible to get a chemically pure diethyl monochlorothiophosphate using 1 mole of trichlorothiophosphate and 2 moles of sodium ethylate under technical conditions. Small amounts of by-products—namely, *O*-ethyl dichlorothiophosphate and triethylthiophosphate—are always formed. It is advantageous to limit the percentage of the dichloroester to a minimum and to use a small excess over 2 moles of sodium ethylate. Under technical conditions, a successful separation of the reaction products by distillation of the mixture is not possible. The ratio of the three reaction products is as follows:

O-Ethyl dichlorothiophosphate	2 to 4%
O,O-Diethyl chlorothiophosphate	90 to 92%
Triethyl thiophosphate	5 to 6%

Technical Diazinon. During the condensation with hydroxypyrimidines both chlorine containing compounds of the reaction mixture as mentioned above lead to the respective pyrimidinyl esters, while triethyl thiophosphate remains unchanged:



O-Ethyl *O,O*-bis(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (dipyrimidyl ester)



Diazinon

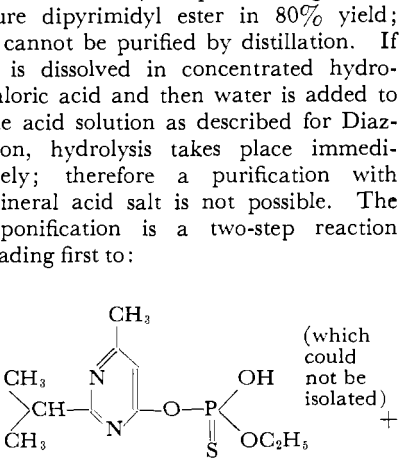
After purification of the crude reaction product, the ratio of dipyrimidyl ester and Diazinon is not the same as the ratio of the chloroesters (*O,O*-diethyl chlorothiophosphate/*O*-ethyl dichlorothiophosphate) in the original reaction mixture, because the dipyrimidyl ester is considerably less stable to hydrolysis. The purification of the technical condensation product is effected in the following way. The crude benzene suspension is cooled to room temperature and then water is added in an amount sufficient to dissolve the inorganic salts. After separation of the aqueous layer, the benzene solution is treated with dilute sodium hydroxide solution, then with water, and finally with dilute mineral acid. Depending on the temperature, pH, and time required for the separation of the layers, the amount of the dipyrimidyl ester saponified will vary and thus the amount of dipyrimidyl ester present in technical Diazinon will be lower than that calculated, on the original dichloroester content. As the difference in stability to hydrolysis between Diazinon and the dipyrimidyl ester is not very pronounced, a complete hydrolysis of the dipyrimidyl ester would always cause a considerable loss of Diazinon. Therefore, the technical Diazinon contains always a small percentage of dipyrimidyl ester which varies from 1 to 3%. The properties of this compound are: yellow oil, nondistillable; n_D^{20} 1.5224; LD_{50} to mice per os: 325 mg. per kg.; ChE i_{50} 0.4 mg. %; insecticidal activity approximately 0.1 of that of Diazinon on flies.

By-products

Triethyl Thiophosphate. Triethyl thiophosphate, which is formed during the synthesis of the *O,O*-diethyl chlorothiophosphate, remains unchanged during the condensation of the hydroxypyrimidine salt with the chlorothiophosphate mixture as well as during the purification of technical Diazinon.

The pure chemical has a boiling point of 90–1° at 12 mm. of mercury. It is relatively stable to hydrolysis, has an LD_{50} to mice per os: 750 mg. per kg. and does not show any noticeable inhibition of cholinesterase. It has no insecticidal properties.

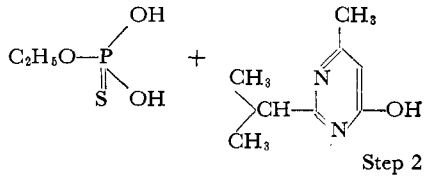
***O* - Ethyl - *O,O* - bis(2 - isopropyl - 4 - methyl-6-pyrimidinyl) Phosphorothioate (Dipyrimidyl Ester).** Upon heating 2 moles of the potassium salt of 2-isopropyl-4-methyl-6-hydroxypyrimidine and 1 mole of ethyl dichlorothiophosphate in benzene for 18 hours at 50° to 60° C., the dipyrimidyl ester is formed. If the reaction solution is treated several times with 1*N* sodium hydroxide solution and 1*N* hydrochloric acid and then washed several times with water until the aqueous phase is neutral, it is possible to get 99% pure dipyrimidyl ester in 80% yield; it cannot be purified by distillation. If it is dissolved in concentrated hydrochloric acid and then water is added to the acid solution as described for Diazinon, hydrolysis takes place immediately; therefore a purification with mineral acid salt is not possible. The saponification is a two-step reaction leading first to:



(which could not be isolated) +

Step 1

and then to



Step 2

Figure 1 shows the degree of hydrolysis of each step at varying pH.

Pure Diazinon

By Distillation. Technical Diazinon can be refined by distillation under reduced pressure. The small amount of solvents present can be easily separated off when a 10-cm. column is used. The triethyl thiophosphate can be eliminated only by repeated distillation or by a chemical refinement as described below. Pure Diazinon is a colorless liquid, having a boiling point of 82–4° C. at 0.0002 mm. of mercury. Above 100° C. Diazinon is sensitive to oxidation. Traces of oxygen being present during distillation lead to a partial decomposition. Temperatures higher than 120° C. lead to a considerable deterioration of Diazinon even in the absence of oxygen; the breakdown is, however, higher in the presence of oxygen. This leads to the assumption that Diazinon is oxidized first to the corresponding phosphate ester which, being less stable than the sulfur analog, is decomposed during the heat treatment. If the distillation is carried out carefully at a temperature of 100° C. or below in an oil bath, the amount of decomposed Diazinon is negligible, being lower than 0.5% even in the presence of oxygen. A degradation product occurring during the distillation procedure is 2-isopropyl-4-methyl-6-hydroxypyrimidine. It is not clear how this hydroxypyrimidine is formed, although there is no water present. Obviously the hydrogen-donor must be in the phosphorus containing part of the molecule.

By Extraction. Technical Diazinon can be purified by dissolving it in 20% hydrochloric acid and extracting the neutral by-products (triethyl thiophosphate) with an organic solvent (ethyl ether). The hydrochloric acid solution is then diluted with water, whereby two layers are formed. The acid, aqueous layer is discarded and the Diazinon layer dissolved in a low boiling organic solvent. After thorough drying with sodium sulfate, the solvent is evaporated in vacuum and Diazinon is obtained in very high purity (99.5 to 100%).

Syntheses with Highly Purified Intermediates. Another way to obtain pure Diazinon consists in using highly purified intermediates for syntheses. A chemically pure 2-isopropyl-4-methyl-6-hydroxypyrimidine, which is transformed with pure potassium hydroxide into its potassium salt, is condensed with pure *O,O*-diethyl monochlorothiophosphate at 50° to 60° C. The resulting product is, after purification, as described above, practically 100% pure Diazinon.

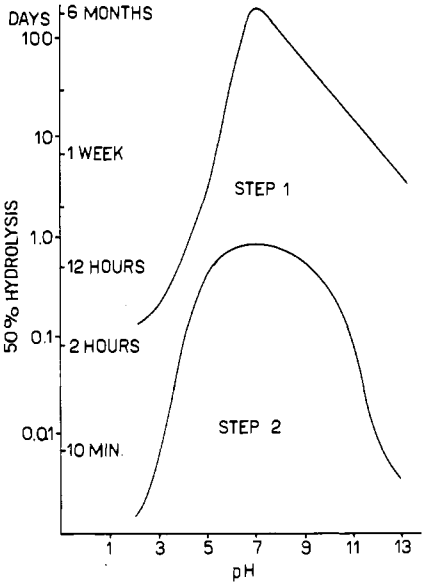


Figure 1. Effect of pH on degree of hydrolysis of O-ethyl-O,O-bis(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

Analytical Problems

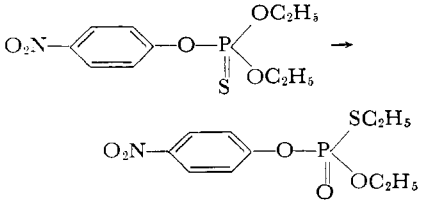
The anticholinesterase activity of such a nondistilled Diazinon is lower than the activity of the compound obtained from the technical product by distillation. This fact leads to the assumption that a very potent cholinesterase inhibitor is formed during the distillation. As the amount of such a decomposition product (or products) must be very small, extremely sensitive analytical methods were necessary for its detection.

Titration. A method for the exact determination of the Diazinon content in products has been developed by Suter, Delley, and Meyer (*13*), using the titration with perchloric acid in acetic acid and 1-naphthol–benzene as an indicator. This method is appropriate, providing that other perchloric acid–consuming compounds are absent. In technical Diazinon this is not the case, as small amounts of 2-isopropyl-4-methyl-6-hydroxy-pyrimidine as well as dipyrimidyl ester are always present. It was necessary to eliminate these by-products interfering with the perchloric acid titration method. For this purpose a more accurate analysis was developed (*14*). When 3*N* sulfuric acid is used for the treatment of technical Diazinon, it is possible to eliminate all perchloric acid–consuming by-products without a loss of Diazinon and the subsequent titration affords exact Diazinon data. The technique is as follows.

A 1% solution of Diazinon in petroleum ether is shaken for 30 seconds with 3*N* sulfuric acid. The aqueous layer is separated off and extracted twice with additional quantities of petroleum ether. The combined petroleum ether extracts, which are washed with water, then with 0.05*N* sodium hydroxide solution and finally with water again, are carefully dried. The solvent is evaporated, the residue dissolved in anhydrous acetic acid, and the Diazinon content determined by potentiometric titration with perchloric acid. The yield during this purification process can never be 100%; it was possible to get 99% yield of Diazinon which limits the amount of by-products to less than 1%.

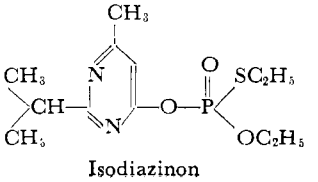
Paper Chromatograms. Paper chromatograms were prepared from different samples of the technical and of the pure Diazinon synthesized from chemically pure intermediates or purified over a mineral acid salt. The technique (*2*) was as follows:

A droplet containing 2 mg. of Diazinon was applied to Whatman paper No. 3 treated for reverse phase chromatography. For the development of the chromatogram the paper strip was immersed in water for 30 minutes and then dried. The dry strip was exposed to bromine vapor and then sprayed with fluorescein solution. In this way it was possible to make clearly visible by-products present in amounts as low as 1%. In chromatograms of technical batches, spots indicating small amounts of 2-isopropyl-4-methyl-6-hydroxypyrimidine were visible near the front of the mobile solvent and one or several spots with R_F values of 0.7 to 0.8 could be detected. Diazinon as well as the dipyrimidyl ester remain at the starting point, so that by-products must be responsible for the above mentioned spots, which were formed during either synthesis or purification of the technical Diazinon. During the synthesis of parathion (*9*) as well as of Systox (*6*), a partial isomerization took place:

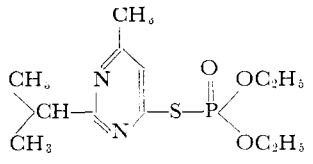


A similar phenomenon could also occur during the formation of Diazinon. On the other hand, such a Diazinon isomer should not migrate on the paper in an entirely different way than Diazinon.

An attempt was made to synthesize this compound, called hereafter Isodiazinon, as well as another potential isomer—namely, Thioldiazinon:

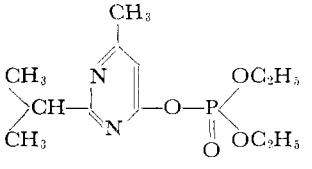


Isodiazinon



Thioldiazinon

Using 2-isopropyl-4-methyl-6-mercaptopyrimidine for the condensation with *O,O*-diethyl chlorophosphate, Thioldiazinon should result. A wide variety of reaction conditions was chosen, without getting even small amounts of a pure Thioldiazinon. Under mildest condensation conditions at temperatures as low as 30° C. the reaction between 2-isopropyl-4-methyl-6-mercaptopyrimidine and *O,O*-diethyl chlorophosphate did not take place in the expected way, and under more drastic conditions nothing but the intermediates could be isolated in a pure state. So it seems very unlikely that Thioldiazinon can be formed during the synthesis of technical Diazinon effected at temperatures of 80° C. or more. The stability of Thioldiazinon or the tendency for its formation is evidently poor even at considerably lower temperatures. Similar attempts to make Isodiazinon, using 2-isopropyl-4-methyl-6-hydroxypyrimidine as a starting material and *S*-ethyl *O*-ethyl chlorophosphate, failed completely. This second theoretical possibility of an isomerization can be also excluded. The oxidation product of Diazinon, *O*-Diazinon, was taken into consideration, as a further potential candidate with a high anticholesterase activity.



O-Diazinon

Using 2-isopropyl-4-methyl-6-hydroxypyrimidine and *O,O*-diethyl chlorophosphate as starting materials under similar condensation conditions as for Diazinon, the oxygen analog (*O*-Diazinon) was obtained. The purified and distilled product boils at 123–5° C. at 0.03 mm. of mercury. This *O*-Diazinon always contains, after distillation, small amounts of 2-isopropyl-4-methyl-6-hydroxypyrimidine and probably small amounts of TEPP which may be formed due to the influence of heat. *O*-Diazinon is considerably less stable to hydrolysis than Diazinon, at a pH of 7 the rate being about 10 times as high. The anticholinesterase activity proved to be by far higher than the anticholinesterase activity of Diazinon. *O*-Diazinon could explain the higher cholinesterase inhibition of partly decomposed Diazinon.

Cook (*3*) demonstrated that spots of pure Diazinon on paper chromatograms exhibited very high cholinesterase inhibition after bromine treatment, the inhibition being about 5000 times greater than that of the untreated, pure Diazinon. Cook assumed that in the case of parathion, the oxygen analog, paraoxon, product of bromine treatment, was responsible for the increased ChE inhibition. Cook's inhibition values found with his paper chromatogram technique are in good agreement with the figures obtained by measuring Diazinon and synthetic *O*-Diazinon with a method mentioned earlier, similar to the one used by Cook. The amount causing 50% inhibition of cholinesterase is 1 mg. %, it is higher for purified nondistilled Diazinon and about 0.0002 mg. % for *O*-Diazinon.

A fact excluding *O*-Diazinon as the substance causing the mentioned spots in paper chromatography with an R_F value of 0.7 to 0.8 is, however, the observation that washing of Diazinon with diluted acid or alkali hydroxide solution did not eliminate the occurrence of the spots.

Further investigations based on paper chromatography of technical, distilled, and purified Diazinon, known and potential by-products, did not lead to the determination of the compounds or breakdown products responsible for the increased anticholinesterase activity of distilled Diazinon, even occurring when oxygen is carefully excluded.

The fact that none of the above cited decomposition or by-products was able to explain the increased mammalian toxicity as well as the higher cholinesterase inhibition found in technical Diazinon indicated that a special study was needed to clarify the phenomena which can occur when technical Diazinon or its formulations are stored. The results of this special study have been reported (*8*).

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ACARICIDE FORMULATION

Stabilization of Aramite by Glycols

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STABILIZATION OF TOXIC INGREDIENTS in dusts and wettable powders is a major problem of the pesticide formulator. Technical Aramite is an example of a toxic compound which undergoes decomposition in formulated products. The active ingredient of Aramite (a trade-mark of the U. S. Rubber Co.) is 2-(*p-tert*-butylphenoxy)-1-methylethyl-2-chloroethyl sulfite, which is used as a miticide on agricultural crops. The technical material contains propylene oxide as a heat and light stabilizer, which protects the active ingredient in the unformulated state, under ordinary temperature conditions (*2*).

Technical Aramite, formulated in wettable powders and dusts, decomposes in most commercial diluents when stored in paper bags and cartons at temperatures normally encountered in warehouses. Experience has shown that when propylene oxide is added to these formulations in excess of that which is present in the technical product, the Aramite is not protected against decomposition. This lack of stabilization is probably due to the volatility of propylene oxide.

Various factors have been studied