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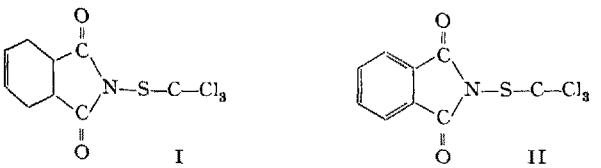
Reactions of Captan and Folpet with Thiols[1]

Captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) (I) and Folpet (N-trichloromethyl-thiophthalimide) (II) both have extensive application; the former being utilized primarily as a general fungicide for the treatment of a wide variety of foliar, soil and seed-borne diseases[2–5]. In addition to being a pre-harvest pesticide, Captan also has a wide use as a post-harvest chemical[6]. Folpet is also a general fungicide used in the treatment of foliage and soil infections on fruit, vegetables and ornamentals.



Our studies with Captan and Folpet involve a variety of metabolic and feeding investigations. In recovery studies of Captan and Folpet from animal tissues and whole blood, it was found that the amounts of the fungicides recovered from mice injected s.c. and then sacrificed at time intervals were very small (ca. 5% for a 1 mg/animal dose) (the whole mouse carcass was extracted with solvent and the Captan or Folpet residue analyzed colorimetrically by the resorcinol method of KITTLESON[7]).

Since earlier work with spiked mouse extract of both Captan and Folpet indicated a recovery of greater than 90%, the low recovery from injected animals was suggestive of hydrolysis and/or binding of both Captan and Folpet. LUKENS and SISLER[8] have shown that Captan or trichloromethylsulfenyl chloride ($ClSCCl_3$) reacts with cysteine to form UV-absorbing products, and in the case of Captan the isolated product was proven to be 2-thiazolidine-thione-4-carboxylic acid. Recently RICHMOND and SOMERS[9] reported on the reaction of Captan with cell thiols of *Neurospora crassa conidia*.

In order to elaborate the rate of thiol interaction, the reactions of Captan and Folpet with blood was studied with the aid of both the sulfhydryl reagent 5, 5′-dithiobis (2-nitro-benzoic acid) (DTNB) and *p*-nitrothiophenol.

When Captan or Folpet are added to a system in which DTNB has been allowed to react with the free sulfhydryl groups in buffered blood solutions, decolorization of the yellow DTNB-complex is observed. The rate and extent of decolorization is shown in Figure 1. It can be seen that the initial rate of reaction for both Captan and Folpet in this milieu is extremely rapid, with a maximum of reactivity being reached in less than 1 min for Captan and ca. 3 min for Folpet (both at concentrations of 15 $\mu g/\mu l$ blood).

When DTNB is added to a system in which buffered blood has been incubated with Captan or Folpet, formation of the DTNB-complex is inhibited to some extent. A summary of the data is given in the Table.

The reaction of Captan and Folpet with *p*-nitrophenol (NTP) was next studied. The disappearance of the absorbance maximum of NTP at 410 nm was indicative of reaction, and was followed over a wide pH range. The data are summarized in Figure 2. Since the extent of loss of yellow color denotes the extent of reaction, note that the lower the % when compared to the control, the

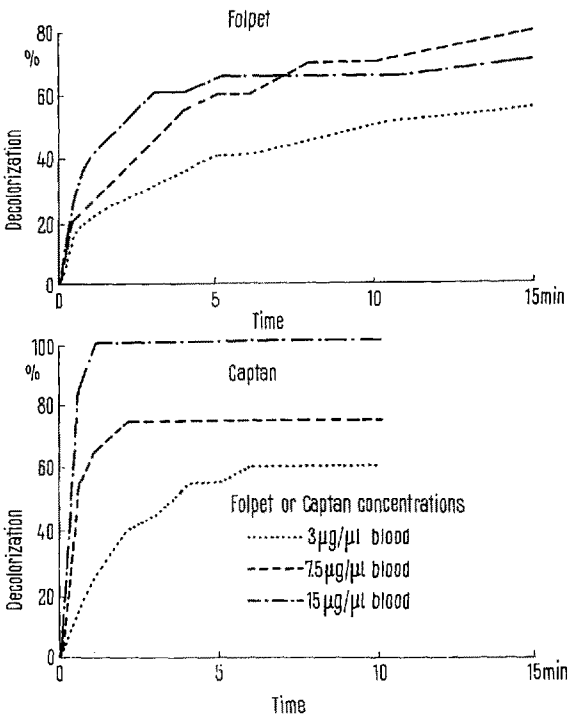


Fig. 1. Decolorization of DTNB-blood sulfhydryl complex by Folpet and Captan.

[1] This study was supported by Research Contract No. PH 43-64-57, National Cancer Institute, National Institutes of Health, Public Health Service, and represents Paper No. 33 of this Contract.

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greater the reaction. The pH range in which the reactions could be studied is limited to between 5 and 8 pH units because at lower than 5 pH units, the thiophenol itself was only a pale yellow color, and at higher than 8 pH units the color was intense and became more so with addition of Captan or Folpet. This appeared to indicate reactions different from those occurring in the 5–8 pH range, but the nature of the reactions was not investigated further. The reactions of Captan and Folpet with NTP were followed at a concentration of 3.3 μg of compound/ml buffer solution. Concentrations as low as 0.33 μg/ml were found to be reactive.

The reaction of Captan and Folpet with the biologically important thiols cysteine and glutathione over a wide pH range was studied with results shown in Figures 3 and 4 respectively. Thiol groups are very reactive at physiological pH. The reaction of Captan, trichloromethylsulfenyl chloride and Folpet respectively with cysteine essentially does not occur below pH 4 and its rate rises rapidly above pH 6.0 as illustrated in Figure 3.

As shown in Figure 4, the reaction of Captan, trichloromethylsulfenyl chloride and Folpet respectively with glutathione does not occur below pH 5.0 and its rate also rises rapidly above pH 6.0.

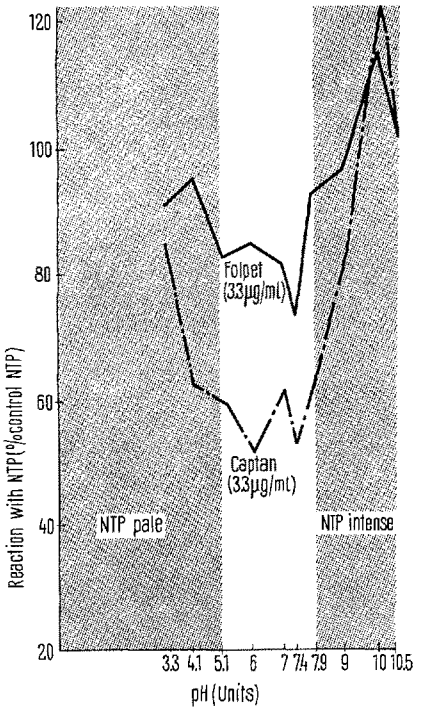

Fig. 2. Reactions of Folpet and Captan with *p*-nitrothiophenol.

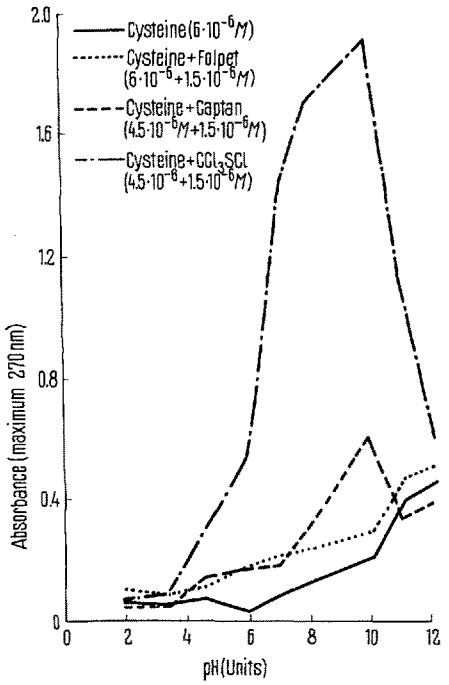

Fig. 3. UV-reaction products of Folpet, Captan and trichloromethylsulfenyl chloride with cysteine.

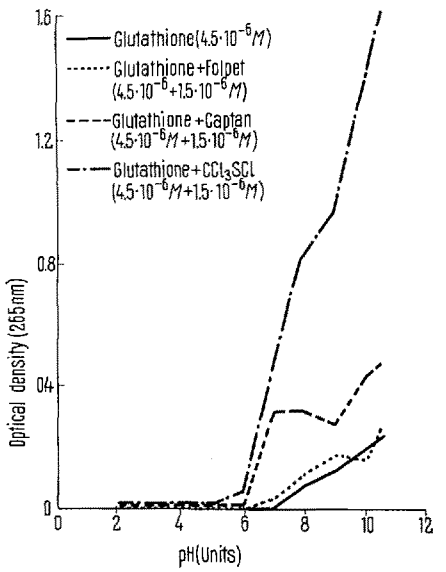

Fig. 4. UV-reaction products of Folpet, Captan and trichloromethylsulfenyl chloride with glutathione.

Compound	Concentration, μg/μl blood/ml total volume	% estimated prevention of complex formation
Captan	20/6/3	65
	50/6/3	65
	100/6/3	65
Folpet	20/6/3	30
	50/6/3	45
	100/6/3	70

Zusammenfassung. Es wird gezeigt, dass die Pflanzenschutzmittel Captan und Folpet mit Verbindungen, die Thiolgruppen enthalten, unter physiologischen Bedingungen (gepuffertes Blut) reagieren.

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