Table II. Average Maleic Hydrazide Content of Treated Sandy Loam Soil after 8 Weeks

Treatment, P.P.M.	Residue, P.P.M.
0.2	0.01
0.5	0.06
1.0	0.11
2.0	0.19
5.0	0.48

run-off and precluded loss of MH by leaching. The harvested leaves were oven-dried, ground in a Wiley mill, and analyzed for MH. Soil from each pot was sampled, oven-dried, and analyzed for MH by the modified method described above.

Results and Discussion

Figure 1 is a graphical representation of the persistence of MH in the three types of soil tested. In all cases, MH content decreased with time. This decrease was very rapid for sand and muck, reaching very low values by the end of 6 weeks. The 1 p.p.m. treatments resulted in negligible residue for sand and muck in this period. Highest residues were present in clay where the decrease was marked but not as rapid as with the other soils.

The MH content of the sandy loam soil on which the tobacco plants were grown in greenhouse is shown in Table II. Approximately 10% of the original amounts added remained in the soil at the end of the 8-week growing period. Again, the decrease in the MH content of the soil was very rapid, although some of this may have been due to uptake by the plants.

The apparent breakdown of MH in the soils tested can be attributed to the action of the various soil microorganisms. The levels of MH used in this experiment were well within the nontoxic range as discussed by Fletcher (*6*). Moreover, Levi and Crafts (*10*) found indications that MH decomposed fairly rapidly in soils under moist, warm conditions. Using much higher levels of MH, these authors also concluded that MH was inactivated slowest in a clay loam. This is similar to the above finding regarding the comparatively slow decrease of MH in clay.

Analysis of the green leaves from the plants grown on treated soils showed the complete absence of any MH residue except in the case of the highest treatment, which was equivalent to at least five times the amount normally recommended for field use. In spite of this high level of treatment, the average residue was only 0.9 p.p.m.

Figure 2 shows a plant from this treatment (*B*) along with an untreated check (*A*). The stunted growth is evident as well as the typical formative effects on the lower leaves. As the plants grew and the MH content in the soil decreased, these effects were overcome and normal growth was resumed.

It can be concluded that there would have to be an enormous amount of MH in the soil before any would be found in the plant. The chemical method for the determination of MH in soils permitted the use of agriculturally effective levels of MH and avoided the danger inherent in biological indicator methods which require extreme experimental conditions.

Acknowledgment

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HERBICIDE METABOLISM

Formation of a Water-Soluble, 3-Chloroaniline-Containing Substance in Barban-Treated Plants

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BARBAN [4-chloro-2-butynyl *N*-(3-chlorophenyl)carbamate] is the active ingredient of Carbyne (Spencer Chemical Co.), a herbicide used commercially for control of wild oats (*Avena fatua*) in field crops. In the analytical procedure for the determination of barban residues in crop samples, barban is extracted from plant tissues by ethylene dichloride or a similar nonpolar solvent, and hydrolyzed to yield 3-chloroaniline which is then determined colorimetrically (*6*). It was found that ethylene dichloride–extracted plant tissue still contained a substance (or substances) which gave, upon hydrolysis, a positive test for 3-chloroaniline. This substance was not removed by continued extraction of the plant tissue with ethylene dichloride, but could be extracted with water. Apparently some of the barban, which is only 11 p.p.m. soluble in water, had been converted by the plant into a water-soluble, 3-chloroaniline-containing substance. This discovery prompted the assaying of all barban-treated crop samples for the water-soluble, 3-chloroaniline-containing substance as well as for barban, and initiated investigations on its formation and nature. For convenience, this water-soluble, 3-chloroaniline-containing substance which arises in plants treated with barban will be designated as *X*.

Analytical Procedure

The analytical procedure for *X* is based on the method used for the determination of barban (*6*). Barban is extracted from treated plants with ethylene dichloride or a similar nonpolar solvent. *X*, being water-soluble, is not extracted by these solvents and remains in the plant tissue. Essentially the same analytical procedure is used for the determination of barban and for *X*. For the former, the organic-soluble residues are analyzed, while for the latter the extracted tissues are analyzed for their 3-chloroaniline content.

Originally, *X* was determined by re-

A water-soluble, 3-chloroaniline-containing substance is formed in plants after treatment with barban. It has been found in the 13 plant species studied and may account for 60% of the applied barban 3 days after treatment. The pattern of its formation and decline has been studied in detail. Other carbamates were found to form similar water-soluble products.

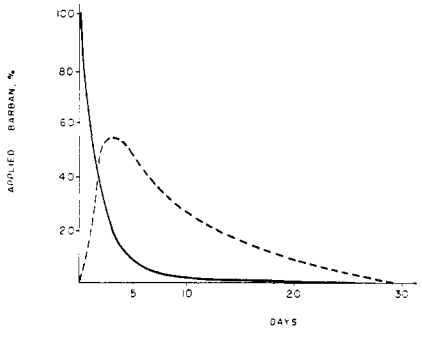


Figure 1. *X* formation curve

———Barban
– – – – –*X*

extracting the ethylene dichloride–extracted plant tissue with water; however, this aqueous extraction was soon found to be unnecessary since identical results could be obtained by determining *X* directly in the ethylene dichloride–extracted tissue.

After air drying to remove any remaining solvent, the ethylene dichloride–extracted tissue is hydrolyzed with 10% aqueous sodium hydroxide to free any 3-chloroaniline bound in the tissue in the form of *X*. Occasionally, a dried plant tissue, especially dried grain samples, foams badly. This difficulty may be overcome by using smaller samples of tissues (50 grams of tissue per 500 ml. of base), large refluxing flasks, and ample addition of Dow-Corning Antifoam A. The 3-chloroaniline is then steam distilled and determined as in the procedure for barban.

Experimental

Plant Species. Following the discovery of *X*, all plant species used in the barban decline studies were assayed for this fraction as well as for barban. The plants included greenhouse and field-grown barley, flax, peas, wheat, sugar-beets, oats, safflower, sunflower, rapeseed, mustard, clover, alfalfa, and wild oats. These crops were treated with an emulsifiable concentrate of barban at the rate of 1 pound of barban per acre in 10 gallons of water. *X* was present in all 13 of the barban-treated plant species. As much as 60% of the 3-chloroaniline applied (as barban) was transformed into *X*. The content rose rapidly in all plants after the application of barban, peaked on the second or third day, and then slowly declined, approaching a zero value well in advance of crop maturity. The general pattern of *X* formation and decline is shown in Figure 1.

Temperature and Plant Age. The effect of temperature and plant age on the decline of barban and the appearance of *X* was studied in wheat and wild oat plants grown in constant temperature rooms. One group had 40° F. night and 60° F. day temperatures, and the other had 70° F. night and 80° F. day temperatures. They were sprayed with barban at a 0.5 pound per acre rate either at the 1-, $1^1/_2$-, 2-, or 3-leaf stage of growth. Plants of each species, age, and temperature group were analyzed at intervals over a 3-week period for residual barban and for *X*. Plant age had no effect on barban decline or *X* formation curve. Wheat and wild oat plants 1 week old had the same capacity for converting this quantity of barban to *X* as had plants 5 weeks old. This is considered to be important, since barban is a herbicide against young plants, rarely affecting older growth.

No gross differences were seen in the *X* formation pattern of wild oats, a barban-sensitive crop, and of wheat, a resistant one. By the fourth day, the wild oats had ceased growing due to the action of barban; however, over 80% of the barban had disappeared during this period so that only minor differences in decline between wheat and wild oats could be noticed.

Barban declined slightly faster at the higher temperatures. The increase in decline rate (Q_{10} of 1.1) was identical for both wheat and wild oats. This faster decline made less barban available for conversion, therefore correspondingly less *X* was formed at the higher temperatures. Two weeks after treatment, roughly the same amount of barban and *X* was present at each temperature.

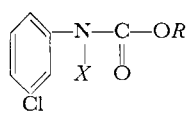
In Vitro Studies. All attempts to form *X* in vitro failed. Wheat plants were homogenized in cold *M*/40 phosphate buffer at pH 6.8 with a sufficient amount of barban added to half-saturate the brei. In some trials, sugars, Krebs cycle, or amino acids were introduced into the system. These homogenates were then held for varying times at different temperatures and pH's, exposed to light and dark. Pure systems were also tried in which similar compounds were added to barban solutions. No *X* was found in any of these homogenates or pure systems.

Movement. The formation of *X* is possibly a means by which barban can be moved in the plant as a water-soluble substance. To study the movement of barban and *X*, wheat plants in the 2-leaf stage were treated with a single spot application of formulated barban at the basal, mid, or terminal section of either leaf. At intervals, samples of the plants were harvested, the leaves sectioned, and each section analyzed for barban and for *X*. Sufficient plants were used for each sample so as to permit detection of 3% of the applied barban. Neither material was found anywhere in the plant other than the initial site of barban application, indicating little, if any, translocation. This is in accord with Foy, who, using radioautographic techniques, found that carbon-14–labeled barban was not readily translocated (*3*). Within the area of barban application, patterns of barban decline and of *X* formation were normal.

Structural Requirements. With the formation pattern of *X* well established and shown to be consistent, the next investigations concerned its nature. Was this formation in the plant unique to barban, or could other carbamates be similarly involved? To check this, minor structural modifications were made on the barban molecule. These included replacing the hydrogen on the nitrogen with a hydroxyl group, substituting another ester group, or modifying the chlorobutynyl ester group by changing the triple bond or the terminal chlorine. These analogs are listed in Table I.

The chemicals were formulated and

Table I. Structural Analogs of Barban



X	R
OH	—CH_2—C≡C—CH_2Cl
H	—CH_2—C≡C—CH_2OH
H	—CH_2—C≡C—CH_2NH_2
H	—CH_2—C≡C—COOH
H	—CH_2—CH=CH—CH_2Cl
H	—CH_2—$(CH_2)_2CH_2Cl$
H	—CH=CH—CH_3
H	—CH_2—CH_2—CH_3
H	—$CH(CH_3)_2$
H	—$C(CH_3)_3$
H	—$CH_2CH(CH_3)_2$

applied at a rate of 1 pound per acre to pots of wheat and wild oats in the greenhouse. The wild oats were in the 1 1/2-leaf stage and therefore susceptible to barban treatment. None of the compounds gave visible injury to either crop. The biologically active compounds were detected by their stunting effect on wild oats. Barban and its *N*-hydroxy analog were the only carbamates of those listed in Table I showing any biological activity against wild oats; the latter compound being less active than barban.

Wheat plants were used to study the decline of the carbamates and the formation of *X*. Although there were differences in the rapidity of decline between the compounds in the wheat plant, all were capable of forming a water-soluble, 3-chloroaniline-containing substance. Thus, no specific structural requirements can be assigned for the formation of *X* other than the fact that it has a 3-chloroaniline moiety.

Purification. Although the isolation of *X* from barban-treated plants has not been achieved, the compound has been purified. The purification has been followed by hydrolysis of samples and analysis for their 3-chloroaniline content. Isolation techniques have included liquid-liquid extractions and the use of Magnesol, Florisil, and silicic acid in column chromatography. These methods have shown that a fractionation of the 3-chloroaniline bound as *X* occurs. *X* is probably a mixture of closely related substances. Extraction procedures with 1% sodium hydroxide and 10% sodium bicarbonate suggest the presence of a weakly acidic group in *X*.

The purest fractions contain about 1% of bound 3-chloroaniline. They are light yellow, dialyzable, and show no ultraviolet fluorescence. Alumina, charcoal, and most ion-exchange resins hold these substances tightly, as they do barban, and release them slowly only under drastic conditions. Paper chromatography or electrophoresis gave no further separation of any fractions. No proteinaceous material was found in *X;* however, several fractions yielded sugars upon hydrolysis. The ultraviolet and infrared spectra of these fractions are not definitive. Studies are continuing on the isolation and identification of *X*.

Discussion

Barban, as well as certain related analogous carbamates, is converted by plants into water-soluble, 3-chloroaniline-containing substances (*X*). From 10 to 60% of the aniline moiety of the carbamates can be converted into *X*. This formation appears to be a general reaction as shown in the effect on barban by the 13 plant species and the action of wheat upon analogous carbamates. The formation pattern of the *X* compounds of all the carbamates studied to date was consistent, with the maximum amount appearing in the plant on the second to fourth day after treatment. In some cases, the carbamates disappeared too rapidly in the plant to yield much *X*. Here, the peak of formation was reached on the second day and *X* declined thereafter. In a few cases, factors such as a slower penetration into the plant gave a maximum *X* buildup in 4 days. *X* then declines in the plant and approaches a zero value in 4 to 6 weeks.

No movement of barban or of *X* in plants has been demonstrated. Although in these experiments the analytical determinations were pushed to the limit of their sensitivity, possibly undetectable amounts of either substance may be moving through the plant and these may exert a herbicidal action. However, neither substance accumulates in any portion of the plant other than *X* at the site of barban application. It thus seems that the herbicidal action of barban is not dependent upon barban translocation via solubilization through *X* formation.

The formation of *X* could be achieved in the plant by the metabolism of barban to a water-soluble substance or by the combination of barban, a metabolite, or its 3-chloroaniline moiety with plant components to form a water-soluble product.

For the purpose of studying barban metabolism, the molecule was considered as having four sites where degradation could occur—the aromatic ring, the carbamate linkage, the triple bond, and the chloromethyl group. The metabolism of the ring was investigated and 2-chloro-4-aminophenol (bound in plant tissues) was detected in barban-treated plants. This metabolite will not steam distill and cannot be confused with 3-chloroaniline which forms an azo dye exhibiting very specific chromatographic properties. It is certain that *X* contains bound 3-chloroaniline; therefore, the solubilization could not have occurred through changes in the aromatic ring.

The next site examined was the carbamate linkage. Baskakov and Zemskaya (*1*) have suggested that herbicidal carbamates are metabolized to their *N*-hydroxy derivatives and are thereby activated. Melinkov (*5*), in a patent, claimed a greater water solubility for these derivatives than for the parent carbamates. The *N*-hydroxy derivative of barban was prepared; however, its water solubility (and partition coefficient between water and ethylene dichloride) was only slightly increased over that of barban, ruling it out as *X*. Thus, if *X* is a metabolite of barban, its water solubility apparently would have to be imparted through alterations of the triple bond or of the chloromethyl group. Studies with 4-chloro-2-butynyl-1 - C^{14} *N* - (3 - chlorophenyl)carbamate showed that the alkynyl group is degraded, as C^{14}-labeled C_2–C_4 organic acids were isolated from wheat plants 2 days after their treatment with labeled barban. Barban analogs were synthesized also, in which the triple bond was hydrated or oxidized to give 2,3-dihydroxy-4-chlorobutyl, 2 (or 3)-keto-4-chlorobutyl, 2,3-diketo-4-chlorobutyl, carboxymethyl, 4-hydroxy-2-butynyl, or 3-carboxy-2-propynyl groups as the ester portion. These compounds all have approximately the same solubility and partition coefficient as barban. All lacked the required partition coefficient to be considered as *X* per se. From this evidence, *X* does not appear to be a direct metabolite of barban.

This then leaves the possibility that *X* is a combination product of barban or a barban metabolite with a naturally occurring plant component. This could happen since alien substances frequently are bound by plants. *X* could be formed by the attachment of barban or its alkynyl degradation product to a solubilizing group, by transesterification with multiple plant components, by association with plant constituents, or by the inclusion or attachment of 3-chloroaniline with soluble plant fractions. Any of these reactions could result in a water-soluble 3-chloroaniline-containing fraction arising from barban. No free 3-chloroaniline has ever been found in barban-treated plants. All 3-chloroaniline, applied as barban, exists in the plant in a bound form and must be liberated by hydrolysis prior to its determination. It is not known if the 3-chloroaniline in *X* is still present as barban. The conditions necessary to free 3-chloroaniline from *X* also hydrolyze 3-chloroaniline from barban. Experiments designed to learn if the C^{14}-labeled alkynyl group of barban was incorporated into *X* were clouded by the rapid metabolism of that group into contaminating water-soluble compounds.

To be characterized, *X* must be further purified and its solubilizing components identified. That more than one component is involved is shown by the different solubilities of fractions of crude *X*. At present, it is dialyzable, nonproteinaceous, contains acidic groups neutralized by sodium hydroxide, but not sodium bicarbonate, may contain sugars, and is associated in solubility with the flavanoids.

The toxic effects of barban on plants—growth retardation, greenish-blue coloration, plant brittleness, and abnormalites of shoot apex—show up at the time of maximum *X* formation, i.e., 2 to 3 days after treatment (*2, 4*). Nevertheless, the herbicidal activity of barban as a postemergence spray does not appear to

be dependent upon the formation of *X* since all carbamates studied, active or inactive, give a similar compound. The formation of *X* may be taken as evidence that these compounds penetrated the plant, at least to the site of their conversion to *X*. Thus, the lack of herbicidal activity of carbamates analogous to barban cannot be blamed on penetration failure.

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SOIL EFFECTS ON HERBICIDES

Influence of Soil Properties on the Phytotoxicities of the *s*-Triazine Herbicides

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The initial and residual toxicities of nine *s*-triazines were compared in five soils, and the relationship of simazine phytotoxicity to organic matter, clay content, cation exchange capacity, and pH of soils was investigated. The chloro derivatives with a single methyl, ethyl, or isopropyl substituent on each amino group were more toxic than those with a single substituent on an amino group and two substituents on the other. The chloro derivative with two substituents on each amino group was least phytotoxic. In simple correlation analyses, the simazine ED_{50} value was positively correlated with soil organic matter and cation exchange capacity, and negatively correlated with pH. Multiple regression analyses suggested that the negative correlation between the simazine ED_{50} and pH was a consequence of the correlation between organic matter and pH. The relative values of the four soil properties for dosage predictions are discussed. Organic matter was best for predictions; and the use of other properties with organic matter did not improve predictability greatly. Under experimental conditions which prevented leaching, simetone was the most persistent of the nine herbicides. Atrazine, propazine, and norazine were only slightly less persistent than simazine in most soils.

SEVERAL *s*-TRIAZINES are currently used as selective pre-emergence herbicides in crops. The rates required for weed control vary with the chemical and weed species, but 2 to 6 pounds per acre are adequate for most weeds.

At rates used for selective weed control, the *s*-triazines may remain active in the soil for several months after application. At rates of 20 to 30 pounds per acre, simazine and several analogs are effective semipermanent soil sterilants. All of the *s*-triazines included in these experiments are rather persistent; however, in Europe and the U. S., simazine has been the most satisfactory *s*-triazine for soil sterilization.

A major problem in the efficient use of these chemicals as selective herbicides on crop plants is their persistence in soils. Knowledge of the relationship of phytotoxicity to soil properties should aid in establishing safe, effective rates of these herbicides.

Limited data are available on the effect of soil properties on the phytotoxicity and persistence of simazine. Investigators reported that to produce the same degree of injury, less simazine was required in sand than in clay soils and soils high in organic matter (*1, 2, 4, 12*). Soil temperature appeared to influence the toxicity of simazine to corn (*2*); but whether the differences were caused by variations in availability of the chemical in the soil solution or absorption of the chemical by plant roots was not determined. In experiments by Aelbers and Homburg (*1*), simazine was inactivated more rapidly in clay and peat than in sand soil. Dewey's (*4*) results showed that simazine was inactivated under conditions favoring growth of microorganisms, but little or no loss occurred under conditions unfavorable to microbial growth. Guillemat (*5*) reported that several species of fungi would inactivate simazine, particularly in the presence of other carbon and nitrogen sources. Therefore, simazine activity in the soil appears to follow patterns similar to those of other relatively persistent, organic herbicides with respect to soil properties and soil environment.

The objectives of the experiments reported here were to compare the initial and residual toxicities of nine *s*-triazines in five soils and to relate the phytotoxicity of simazine to organic matter, clay content, cation exchange capacity, and pH of soils.

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