Metabolism of Triazine Herbicides by Plants

MARVIN L. MONTGOMERY and VIRGIL H. FREED
Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.

The metabolism of the triazine herbicides by plants appears to be a general phenomenon. Although there is a good correlation between resistance and extent of metabolism, even the highly susceptible plants have a limited capacity for degrading these chemicals. A common pathway of degradation is indicated by the presence of the 2-hydroxy analogs in plants treated with different triazines. Paper chromatography of plant extracts indicates that basic metabolites are also produced. A degradation scheme to account for these products is proposed.

Many substituted, symmetrical triazines possess considerable herbicidal activity (*2*, *12*, *13*). Their high biological effectiveness against a wide spectrum of plants has made them quite useful as soil sterilants and as selective herbicides on crops. The selectivity of these herbicides is markedly influenced by the substituent groups attached to the triazine nucleus (*13*). Most of the triazines possessing herbicidal activity have alkyl-substituted amino groups in the four and six positions. The selectivity can be modified somewhat by varying the alkyl substituents on these amino groups. However, major changes in selectivity are brought about by varying the substituent in the two position. Chlorine, methoxy, and methylmercapto substitutions in the two position have shown the most herbicidal activity (*13*). Table I gives the structures of the different types of triazine herbicides.

Selective Toxicity of the Triazine Herbicides

The selective toxicity of the triazine herbicides varies widely with structure. Frequently, closely related plants exhibit marked differences in tolerance to a given triazine. Also, certain plants which are very resistant to the chlorotriazines are quite sensitive to other types of triazines, such as methoxy- or methylmercapto-substituted derivatives. These observations have stimulated considerable interest in the interactions of these compounds with the biochemical systems of the plant. A number of investigations (*4*, *8*, *10*, *18–20*) have been undertaken to determine the factors responsible for the tolerance of certain plants to the triazines. These investigations have involved mode of action as well as metabolism studies.

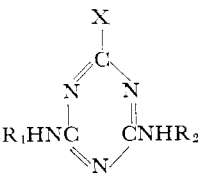
Several factors could be involved in resistance of plants to triazines: the apparently resistant plants absorb very little herbicide; the biochemical systems inhibited in the sensitive plants are not affected in the resistant plants; and the resistant plant is able to metabolize or detoxify the herbicide, thereby escaping its toxic action. This last property is probably a predominant factor in the resistance of plants to the triazine herbicides.

Studies have shown that the low absorption of the herbicide by apparently resistant plants is a factor in the selectivity of certain triazines on a limited number of plants (*13*). This is illustrated by the selective use of simazine on certain deep-rooted crop plants. Simazine has a very low water solubility and is strongly adsorbed by soils so that very little herbicide is found below the top 3 or 4 inches of soil (*3*, *13*, *16*). This property, coupled with the fact that simazine is poorly absorbed through foliage, means that deep-rooted plants will receive little exposure to the chemical. Another possibility for decreased exposure of a plant to the herbicide is that the resistant plants, for some physiological reason, take up less chemical. However, with the triazines, this behavior has not been noted. In studies where the amount of herbicide taken up by resistant and susceptible plants has been compared, little difference was found between the two types of plants (*7*, *13*). In most cases, apparently, the resistance of plants to the triazine herbicides cannot be explained by the amount of chemical absorbed.

Another possible explanation for the resistance of certain plants is that the herbicide is not active against the enzymatic processes inhibited in the susceptible species. Or, the resistant plant might have an alternate pathway whereby it could overcome the blockage of a given enzymatic process. This explanation is not readily proved or disproved since the mode of action of the triazines has not been fully elucidated. However, investigations have indicated that the triazines interfere with the photosynthetic mechanism (*8*, *21*). Carbon dioxide fixation and accumulation of starch are inhibited (*1*, *11*). Also, a number of investigators (*4*, *18*) have shown that the triazines inhibit the Hill reaction in concentrations of the same order of magnitude as the urea herbicides (*5*, *24*). These observations indicate that the action of the triazine herbicides, at least in part, involves the inhibition of the reactions involved in photosynthesis. Chloroplasts from corn, which is quite tolerant to simazine, are as susceptible to inhibition as chloroplasts from susceptible plants (*19*). Thus, the tolerance may not be due to biological inertness of the herbicide to the biochemical systems of the tolerant plants.

Since plants have a complex biochemical system which is able to metabo-

Table I. Structure of the Triazine Herbicides



	X	R_1	R_2
Chlorotriazines			
Simazine	Cl	C_2H_5	C_2H_5
Atrazine	Cl	C_3H_{7i}	C_2H_5
Propazine	Cl	C_3H_{7i}	C_3H_{7i}
Methoxy triazines			
Atratone	OCH_3	C_3H_{7i}	C_2H_5
Prometone	OCH_3	C_3H_{7i}	C_3H_{7i}
Methyl mercaptotriazines			
Ametryne	SCH_3	C_3H_{7i}	C_2H_5
Prometryne	SCH_3	C_3H_{7i}	C_3H_{7i}

lize numerous types of organic compounds, they can degrade a foreign organic molecule. However, this fact is no more important than the rate at which the herbicide is metabolized. If the rate of absorption is much greater than the rate of metabolism, a lethal concentration of herbicide may accumulate. With triazines, different plants possess varying capacities for detoxification. A number of investigations (*6, 7, 9, 13, 20, 23*) have shown a good correlation between the extent of metabolism and the degree of resistance to the triazines. Thus, apparently the metabolism of the triazine herbicide plays an important role in a plant's resistance to these chemicals.

Metabolism of the Chlorotriazines

Chlorazine [2-chloro-4,6-bis (diethylamino)-*s*-triazine] was the first triazine herbicide, followed by simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] and atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine). Simazine and atrazine metabolism have received the most attention. The marked tolerance of corn to the chlorotriazines indicated that this plant would be well suited for metabolism studies. Roth was the first to observe that simazine was being extensively degraded by corn (*20*). By incubating simazine with expressed corn sap, he found that after 100 hours only a small percentage of the added simazine could be recovered from the mixture. In contrast, over 90% of the simazine added to wheat juice could be recovered in a similar experiment. This observation was quite significant inasmuch as wheat is sensitive to simazine. In a similar experiment with C^{14}-atrazine and expressed corn juice, Montgomery and Freed (*15*) found that atrazine undergoes extensive conversion to a new compound. On the basis of the chromatographic behavior of this compound, it was suggested that the product was the hydroxy analog of atrazine, the chlorine atom having been replaced with a hydroxyl group. This conversion can be readily made in the laboratory under strongly acidic conditions. These observations were confirmed by Castelfranco *et al.* (*4*), who characterized the constituent responsible for the conversion. The properties of the active constituent strongly indicated that it was not enzymatic or proteinaceous.

This constituent was subsequently isolated and identified by Roth and Knülsi (*22*) and, independently, by Hamilton and Moreland (*14*). It was found to be the cyclic hydroxamate, 2,4-dihydroxy-3-keto-7 methoxy-1,4-benzoxazine. In vitro conversion of simazine to the hydroxy analog was demonstrated by using a crystalline sample of the cyclic hydroxamate or its glucoside. The hydroxy analog was also found in extracts of corn plants exposed to simazine, demonstrating in vivo conversion (*14*). This reaction is probably a common one for all of the chlorotriazines, since propazine undergoes the same conversion when incubated with corn juice. This rapid conversion of the chlorotriazines to the hydroxy analogs appears to be the primary factor in the high tolerance of corn to these herbicides. The hydroxy analog appears to be biologically inactive on plants, even on ones which are quite susceptible to the chlorotriazines. (*9*).

Metabolism studies have also been carried out in which whole plants were exposed to simazine and atrazine in nutrient media. Even the susceptible plants have a limited capacity for degrading these herbicides. Davis *et al.* (*7*) studied the uptake and translocation of simazine in corn, cotton, and cucumber plants. Using C^{14}-labeled herbicide, they showed that the amount of simazine taken up by these plants was of the same order of magnitude. However, they found quite a difference in the solubility of the C^{14}-labeled material in the treated plants. Using a chloroform extraction, which removes unaltered simazine as well as certain metabolites, they found that only 5% of the radioactivity in treated corn was chloroform-soluble. The per cent chloroform-soluble for cotton and cucumber plants was 25 and 50%, respectively. These results conclusively show that all three plants are capable of degrading simazine to varying extent. Of equal significance is the fact that the extent of degradation is in good agreement with the relative susceptibilities of these plants. Corn is quite tolerant to simazine, cotton is moderately sensitive, and cucumber is quite sensitive.

The extensive degradation of simazine and atrazine by corn plants was also demonstrated by Montgomery and Freed (*17*) in residue and metabolism studies. In residue studies, plants were grown in soil treated with C^{14}-labeled herbicide. Plants were harvested periodically and analyzed for total radioactivity as well as for chloroform-soluble radioactivity, which would include any unaltered triazine. The radioactivity in the chloroform extract was not necessarily the parent triazine, since any fragment of degradation which was chloroform-soluble would appear in the extract. Therefore, the chloroform extracts were fractionated by paper chromatography and ion exchange resins to ascertain how much of the chloroform-soluble radioactivity could still be the parent triazine.

The amount of chloroform-soluble radioactivity as well as total radioactivity was appreciable at each harvest, which ranged from 2 weeks to 3 months. The per cent chloroform-soluble declined at each time of harvest indicating that the triazines were metabolized and the fragments incorporated into plant constituents. This was confirmed by analysis of the chloroform extracts. Paper chromatography of the chloroform extract showed that very little, if any, radioactivity was still in the form of the parent triazine. Similar results were obtained with ion exchange fractionation. Thus, apparently, corn readily degrades these triazines.

More direct evidence that these triazines were completely degraded by corn plants came from metabolism studies using C^{14}-labeled herbicide. Plants were placed in an aqueous solution containing the triazine, and the carbon dioxide given off by the plants was collected over a 3-day period and analyzed for radioactivity. Preliminary experiments with labeled herbicides in water alone indicated that none of the herbicide would volatilize and thus interfere with the determination.

Significant amounts of radioactive carbon dioxide were evolved from plants treated with either simazine or atrazine. In one experiment (*17*), plants were kept in the dark during the experiment, and, as would be expected, a greater percentage of the triazine taken up was metabolized to carbon dioxide, since the $C^{14}O_2$ would not be fixed by photosynthesis. These findings clearly demonstrate the ability of corn to degrade the triazine nucleus and metabolize the fragments, inasmuch as the C^{14} label was located in the triazine ring.

Similar results were obtained by Funderburk and Davis (*10*). These investigators compared the metabolism of ring and side-chain labeled simazine by corn plants. Paper chromatography of the extract of treated plants indicated that hydroxy simazine and an unidentified C^{14} product are formed with either type of labeled herbicide. Also, appreciable amounts of labeled carbon dioxide are given off by plants treated with either compound. These findings show that all portions of the triazine ring are subject to complete oxidation by corn, cotton, and soybean plants.

Other plants beside corn metabolize the chlorotriazines. As indicated earlier, cotton and cucumber possess this ability, as shown by the incorporation of the C^{14} of the simazine into chloroform-insoluble products. Funderburk and Davis (*10*) showed that cotton and soybeans exposed to labeled herbicide give off appreciable amounts of radioactive carbon dioxide, showing these plants can completely metabolize a portion of the absorbed triazine. Studies in this laboratory have shown that a number of plants are capable of metabolizing atrazine (*9*). Plants were exposed to C^{14}-labeled herbicides through root uptake, and the $C^{14}O_2$ evolved by the treated plants was precipitated and analyzed for radioactivity. This procedure measures only

completely oxidized triazine, so there was undoubtedly a more extensive degradation of triazine than was indicated by $C^{14}O_2$ evolution. Table II gives the relative extent of breakdown of atrazine by a number of plants, with corn being given an arbitrary rating of 100.

None of the more sensitive plants comes close to being as active as corn in the degradation of atrazine. However, oats, which are susceptible to atrazine, can break down the hydroxy analog as rapidly as corn breaks down the parent chlorotriazine. This demonstrates, in the case of corn, the importance of the ability to rapidly convert the chlorotriazine to the hydroxy analog and gives strong supporting evidence for the direct relationship between resistance and metabolism of the triazines by plants.

Methylmercapto Triazine (Prometryne)

The metabolism of triazines other than the chloro-substituted members has received only a limited amount of investigation. Corn is moderately sensitive to the methoxy and methylmercapto triazines. Incubation of corn juice with C^{14}-labeled prometryne (2-methylmercapto-4,-6 - isopropylamino - *s* - triazine) revealed that no significant amount of hydroxy propazine was formed. Apparently the system responsible for conversion of the chlorotriazine to the hydroxy triazine is not active on the methylmercapto derivative. Further, corn plants exposed to labeled prometryne do not give off significant amounts of $C^{14}O_2$. However, some of the absorbed herbicide is metabolized. A study was carried out with corn to compare the metabolism of propazine and prometryne (*9*). Plants were exposed to labeled triazine in nutrient media. Three and 8 days following exposure to the herbicide, plants were exhaustively extracted with chloroform to remove unaltered triazine as well as chloroform-soluble degradation products. The radioactivity in the extracted residue and in the chloroform extract was determined. The chloroform extract was then subjected to paper chromatography to determine the amount of radioactivity still in the form of the parent triazine. The results of this experiment are given in Table III.

These data show that corn metabolizes propazine more rapidly than it does prometryne. With prometryne, there is a significant increase in the amount of chloroform-insoluble radioactivity between the 3- and 8-day harvest, indicating metabolism is occurring. However, paper chromatography of the chloroform extracts reveals that there is little change in the percentage of radioactivity which represents unaltered prometryne. In the case of propazine, the chloroform-soluble radioactivity which chromatographs as propazine declines markedly between the third and eighth day following exposure. This finding illustrates the remarkable ability of corn to degrade the chlorotriazines. It also demonstrates its limited ability to metabolize prometryne. Thus, corn should be more sensitive to prometryne than propazine, since it cannot readily detoxify this methylmercapto triazine.

Wheat is another plant which has a limited ability to metabolize prometryne. The extent of metabolism was measured in a similar experiment as carried out with propazine and prometryne with corn (*9*). Plants were exposed to labeled herbicide in nutrient media and extracted with chloroform 3 and 8 days following exposure. The radioactivity in the extracted residues and chloroform extracts was measured, and the percentage of radioactivity in the chloroform extract which could still be prometryne was determined by paper chromatography. The results are given in Table IV.

Apparently wheat can degrade a significant amount of prometryne. However, the rather slow decline in the percentage of radioactivity which represents prometryne indicates that the tolerance of wheat to this chemical should not be large. This agrees fairly well with greenhouse trials using prometryne on wheat (*9*).

Since carrots are moderately resistant to prometryne, the ability of this plant to degrade prometryne was measured. No significant amounts of $C^{14}O_2$ were evolved from carrot plants exposed to C^{14}-labeled herbicide through root uptake. However, in this 3-day study, up to 25% of the absorbed herbicide was altered. This was indicated by behavior of the radioactivity in the chloroform extract on ion exchange resins and incorporation of the C^{14} label into chloroform-insoluble constituents. These data are supported by residue studies on plants grown in prometryne-treated soil. Plants were harvested periodically and extracted with chloroform. The percentage of chloroform-soluble radioactivity declined at each harvest, indicating that prometryne was being metabolized.

Further evidence that prometryne is degraded by carrots came from studies in which labeled prometryne was incubated with carrot tissue. The chemical was placed in the tissue by vacuum infiltration, and after an incubation period of from 4 to 8 days, the tissue was extracted with chloroform.

Table II. Metabolism of Atrazine by Various Plants as Measured by $C^{14}O_2$ Evolution

Plant	Chemical	Rating
Corn	Atrazine	100
Oats	Atrazine	14
Alfalfa	Atrazine	12
Cucumber	Atrazine	12
Oats	OH Atrazine	100

Table III. Metabolism of Propazine and Prometryne by Corn Plants

Days Following Treatment	% of C^{14} Chloroform Extractable	% of C^{14} That Could Be Parent Triazine
	PROPAZINE	
3	96.0	89.4
8	92.3	48.2
	PROMETRYNE	
3	90.1	83.4
8	78	84.3

Table IV. Metabolism of Prometryne by Wheat Plants

Days After Exposure	% of C^{14} Chloroform Extractable	% of C^{14} That Could Be Parent Triazine
3	78.5	73.2
8	76.8	62.5

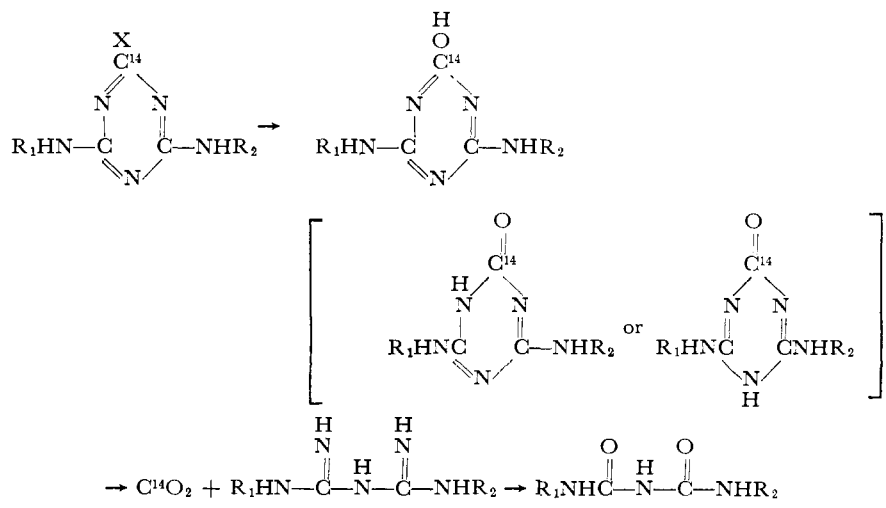


Figure 1. Proposed sequence of reactions in the metabolism of triazine herbicides

Paper chromatography of the chloroform extract indicated that two radioactive compounds were present. One of these compounds was prometryne, while the other component had an R_F value that corresponded to hydroxy prometryne, which is identical to hydroxy propazine. Thus, the carrot appears to degrade prometryne in a manner analogous to that in which corn degrades propazine.

Proposed Degradation Sequence

There has been considerable interest and speculation in the sequence of reactions leading to complete destruction of the triazine nucleus. The intervening reactions between formation of the 2-hydroxy compounds and complete oxidation to carbon dioxide have not been determined. Figure 1 shows the authors' proposed sequence of reactions in the route of degradation of the triazine herbicides, with herbicide labeled with C^{14} in the two position.

The hydroxy compound in the brackets is shown in the keto form, since the infrared spectrum of this compound indicates a carbonyl group is present.

Evidence for the formation of the suggested degradation products was obtained by paper chromatography of the extracts of corn plants treated with propazine (experiment reported in Table III). The developing solvent was isoamyl alcohol saturated with 3*M* hydrochloric acid. With this developer, the parent triazine has an R_F value of about 0.88, while that of hydroxy propazine is 0.55 to 0.60. Chromatography of the extract of propazine-treated corn 3 days following exposure showed three radioactive components to be present. Most of the radioactivity was present as propazine, with some hydroxy propazine and a small amount of a third component being present. The extract of propazine-treated corn 8 days following exposure, contained four radioactive components, three of which were found in the earlier extract.

This later extract contained appreciable amounts of the two unidentified compounds. The R_F values of these two compounds were much smaller than propazine or hydroxy propazine, which suggests they are basic compounds. Similarity of the R_F values of a substituted biguanide tested in this laboratory, analogous to the one postulated in the degradation scheme, and of one of the degradation products found in corn indicates that one of the radioactive components may very well be the postulated biguanide. Also, the difference in the R_F values for unsubstituted biuret and the second unidentified component is only 0.08, indicating that the second compound may be the substituted biuret. If the substituted biguanide is formed, it probably undergoes hydrolysis to a substituted biuret or a substituted guanidine and a substituted urea.

Acknowledgment

A grant-in-aid from the Geigy Agricultural Chemical Corp., which made this work possible, is gratefully acknowledged.

Literature Cited

(1) Ashton, F. M., Zweig, G., Mason, G. W., *Weeds* **8,** 448 (1960).
(2) Bartley, C., *Farm Chem.* **122,** 113 (1957).
(3) Burschel, P., *Weed Res.* **1,** 131 (1961).
(4) Castelfranco, P., Foy, C. L., Deutch, D. B., *Weeds* **9,** 580 (1961).
(5) Cooke, A. R., *Ibid.*, **4,** 397 (1956).
(6) Davis, D. E., Funderburk, H. H., Sansing, N. G., *Proc. Southern Weed Conf.* **12,** 172 (1959).
(7) Davis, D. E., Funderburk, H. H., Sansing, N. G., *Weeds* **7,** 300 (1959).
(8) Exer, B., *Experientia* **14,** 135 (1958).
(9) Freed, V. H., Oregon State University, Corvallis, unpublished data.
(10) Funderburk, H. H., Davis, D. E., *Weeds* **11,** 101 (1963).
(11) Gast, A., *Experientia* **14,** 134 (1958).
(12) Gentner, W. A., Shaw, W. C., 1957 Field results, USDA Progress Report, Plant Industry Station, Beltsville, Md., 1958.
(13) Gysin, H., Knülsi, E., *Advan. Pest Control Res.* **3,** 289 (1960).
(14) Hamilton, R. H., Moreland, D. E., *Science* **135,** 373 (1962).
(15) Montgomery, M., Freed, V. H., *Res. Prog. Rep. Western Weed Control Conf.* **71,** 1960.
(16) Montgomery, M., Freed, V. H., *Weeds* **9,** 231 (1961).
(17) Montgomery, M., Freed, V. H., Fang, S. C., *Res. Prog. Rep. Western Weed Control Conf. Ibid.*, **92,** 1958.
(18) Moreland, D. E., Gentner, W. A., Hilton, J. L., Hill, K. L., *Plant Physiol.* **34,** 432 (1959).
(19) Moreland, D. E., Hill, K. L., *Weeds* **10,** 229 (1962).
(20) Roth, W., *Compt. Rend.* **245,** 942 (1957).
(21) Roth, W., *Experientia* **14,** 137 (1958).
(22) Roth, W., Knülsi, E., *Ibid.*, **17,** 312 (1961).
(23) Sheets, T. J., *Proc. Weed Soc. Amer.* **44,** 1960.
(24) Wessels, J. S. C., Van der Veen, R., *Biochem. Biophys. Acta* **19,** 548 (1956).

Received for review August 19, 1963. Accepted December 9, 1963. Division of Agricultural and Food Chemistry, 144th Meeting, ACS, Los Angeles, Calif., April, 1963. Published as Oregon Agricultural Experiment Station Technical Paper No. 1712.

METABOLISM OF HERBICIDES

Tolerance of Several Grass Species to 2-Chloro-s-triazine Herbicides in Relation to Degradation and Content of Benzoxazinone Derivatives

ROBERT H. HAMILTON

Department of Botany, Pennsylvania State University, University Park, Pa.

THE 2-chloro-4,6-dialkylamino-*s*-triazine herbicides are extensively used as pre-emergence sprays for weed control in corn. Corn is not injured by applications up to 10 times the amount required for control of annual weeds. These herbicides are also used for control of weeds in sorghum, sugar cane, and a few other crops. In general, other cereal grains are susceptible to these herbicides. Photosynthesis and specifically the Hill reaction has been implicated as a sensitive site of action (*4, 13, 14*) of these herbicides. However, chloroplasts from corn are as sensitive to inhibition of the Hill reaction as those from susceptible species (*15*). It has been suggested (*5*) that metabolism of these herbicides by corn and other resistant species may be the mechanism for their tolerance. The two most widely used derivatives are 2-chloro-4,6-bis-(ethylamino)-*s*-triazine (simazine) and 2-chloro - 4 - ethylamino - 6 - isopropylamino-*s*-triazine (atrazine). The latter derivative is more water-soluble and thought to be somewhat less selective in the field than simazine.

Extracts of corn degrade simazine in a nonenzymatic manner (*3, 5, 16*). The compound responsible for the apparent catylitic degradation is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (I) or its 2 glucoside (*8, 17*), and the structure has recently been established (*6, 7, 11, 18, 19*). The major product of the degradation reaction (*8*) is 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxy simazine), which is nonphytotoxic (*5*)