SYMPOSIUM ON MODE OF ACTION OF HERBICIDES

Introduction

The first successful attempts to control undesirable plant species selectively with the herbicide 2,4-D were described in the early 1940's. Since that time, a wide variety of sophisticated herbicidal chemicals have been developed. Significant advances have been made in our understanding of the practical application and of the fate of these chemicals, but little concentrated effort has been made to understand their effect in the plant. Consequently, mechanism research has lagged behind other areas of herbicide research.

Two important principles have been elucidated in mode of action research. First, many herbicides act on biochemical processes that are probably unique to the higher plant. Photosynthesis was early recognized as a site of action. Second, herbicides act at more than one site. Consequently, a number of indirect effects have been observed with most herbicides.

This symposium was organized to consider the present status of research on herbicidal mechanisms. Outstanding authorities discussed recent advances in their respective fields. Nine papers were presented orally at the San Francisco meetings. A group of five papers is published here. Papers not published at this time include: Role of RNA Metabolism in the Action of Auxin-Herbicides by J. B. Hanson and F. Slife, Effects of Herbicides on Electron Transport and Phosphorylation Reactions in Chloroplasts and Mitochondria by D. E. Moreland and W. G. Blackman, The Relation of Metabolism to Mode of Action of Phenoxy Herbicides by D. G. Crosby and H. Tutass, and A Biochemical Basis for the Selective Herbicidal Action of 3,4-Dichlorobenzyl Methylcarbamate by R. A. Herrett. Persons desiring to obtain a complete set of papers are urged to contact the authors directly. Finally, it is hoped these papers will serve as a stimulus for new advances in mode of action research.

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Analysis of the Mode of Action of Herbicidal α-Chloroacetamides

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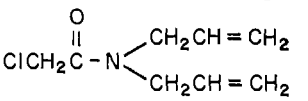
Selectivity of α-chloroacetamides is based on differential rates of metabolism or detoxification between sensitive and resistant plant species. However, physiological effects cannot be explained. Investigations have now focused attention upon protein synthesis as the general locus of interaction, although the reactivity of the α-chloroacetamides with nucleophiles suggests the possibility of multiple sites of action.

While the subject for discussion pertains to the mode of action of α-haloacetamides, it may be helpful to describe briefly the current understanding of the basis for selectivity of these herbicides.

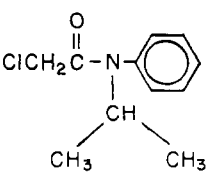
The two compounds that will be discussed in detail are shown in Figure 1. Both are pre-emergent herbicides used primarily in corn and soybeans, but they are also used in many other crops. They are especially effective against a wide spectrum of grassy weeds such as foxtail, bromegrass, cheatgrass, and crabgrass, and certain broadleaf weeds such as pigweed and lambsquarter.

UPTAKE AND METABOLISM OF CHLOROACETAMIDES BY GERMINATING SEEDS

Studies by Smith *et al.* (1966) defined the possible relationship between the degree of susceptibility of four plant species to α-chloroacetamides and differential uptake or metabolism of the compounds by the plants. The seeds used in these studies were corn, oat, soybean, and cucumber. Oat and cucumber seeds were representatives of moderately susceptible species, and corn



CDAA



PROPACHLOR

Figure 1. Structures of *N,N*-diallyl-2-chloroacetamide (CDAA) and *N*-isopropyl-2-chloroacetanilide (propachlor)

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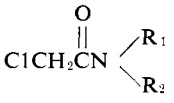
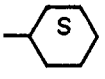
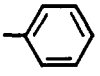
Table I. Growth Inhibition by α-Chloroacetamides[a, b]



Derivative		Corn	Oats	Soybean	Cucumber
R_1	R_2				
$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	−	++	−	+
H	$-CH_2CH=CH_2$	−	++	−	+
$-CH_2CH_2CH_3$	$-CH_2CH_2CH_3$	−	+	−	+
H	$-CH_2CH_2CH_3$	−	++	−	+
H	$-CH(CH_3)_2$	−	+	−	+
H	$-(CH_2)_5CH_3$	−	+	−	+
H		−	+	−	+
H		−	+	−	+

[a] No inhibition −; slight inhibition +; marked inhibition ++.
[b] Inhibitor concentrations 10 p.p.m. except for diallyl derivative where concentration was 100 p.p.m.

and soybeans represented highly resistant plant species. The compounds studied are shown in Table I, along with the relative sensitivities of the plant species to the chloroacetamides. The seeds used in these studies were surface sterilized with hypochlorite and placed in Scientific Product seed packs containing the chloroacetamide solutions. All of the chloroacetamides were labeled at the carbonyl carbon with ^{14}C. The concentration of the test solutions was 10 p.p.m. with the exception of *N,N*-diallyl-2-chloroacetamide (CDAA), which was used at 100 p.p.m. due to its low specific radioactivity. At these concentrations seed germination was not affected. Following various times of germination, the seeds were removed from the pouches, rinsed, and dried. The tissues were homogenized in 80% aqueous acetone, and extracts were assayed for radioactivity.

Two types of uptake curves were observed. Corn, soybeans, and oats yielded the parabolic uptake curve shown in Figure 2. While the uptake of only the diallyl derivative by oats is shown, the curve was typical for all derivatives. The uptake curve for cucumbers shown in Figure 3 was sigmoidal in nature and was the same for all derivatives. A statistical analysis of the data from all sampling times indicated that all chloroacetamide derivatives were taken up to the same extent by a given seed species. Corn, one of the more resistant species, invariably took up less chemical than other seeds. Soybeans, the other resistant species, took up more than any other seed, about three times the amount for corn. Since oats and cucumbers are more susceptible than corn and soybeans, it appears that susceptibilities are not determined by the amount of chemical absorbed.

The extent of metabolism of these chloroacetamides was studied by determining the amount taken up and metabolized in 6 and 48 hours. The radioactivity of

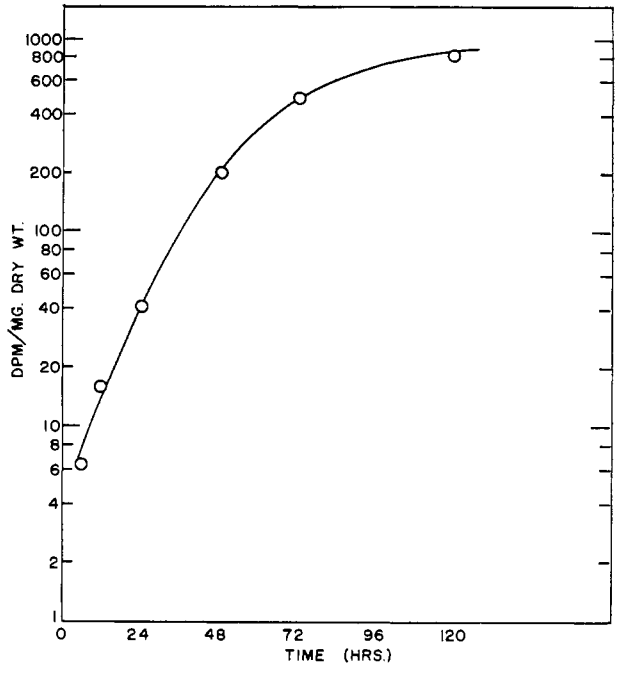


Figure 2. Uptake of ^{14}C-labeled *N,N*-diallyl-2-chloroacetamide by germinating oat seeds

the plants was extracted and partitioned between chloroform and water, since the chloroacetamides were found to have very high chloroform-water partition coefficients ranging from 5 to 25. Suspected breakdown products, such as chloroacetic and glycolic acids (Jaworski, 1964), had chloroform-water partition coefficients of less than 0.01. It was assumed that the radioactivity found in the chloroform fraction was due to nonmetabolized chemicals; the radioactivity remaining in the aqueous phase was attributed to metabolites.

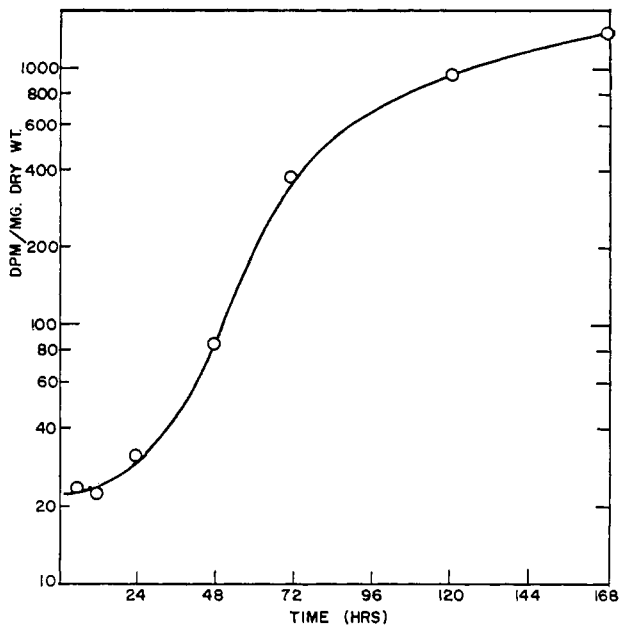


Figure 3. Uptake of ^{14}C-labeled *N,N*-diallyl-2-chloroacetamide by germinating cucumber seeds

As shown in Figure 4, both corn and oats metabolized a large fraction of the chemical absorbed in 48 hours. Thus, both resistant and susceptible species apparently have the ability to metabolize chloroacetamides. At 6 hours there was a definite difference between the amount of metabolism by corn and oats. Corn metabolized significant amounts in a short time, but oats, the more susceptible species, metabolized essentially none.

As shown in Figure 5, a similar relationship was found between soybeans and cucumbers. Both metabolized large fractions in 48 hours, but only the resistant soybeans metabolized a significant amount in 6 hours.

The degree of susceptibility of various seeds to chloroacetamides could be directly related to the time required to initiate metabolism of these chemicals. Those species which were able to metabolize the chemical as soon as it entered, or within a short time thereafter, had only small amounts of the chemical present internally at any time. On the other hand, those species with delayed or slow metabolic capabilities accumulated relatively higher and therefore potentially lethal concentrations. Thus, the basis for selectivity in the case of chloroacetamide herbicides may be the ability of resistant plants to metabolize them at a rate sufficient to keep their levels below that required for growth inhibition. Based on these studies, it is reasonable to conclude that the locus of action of chloroacetamides does not necessarily require a unique enzyme system in susceptible *vs.* resistant species.

MODE OF ACTION

Studies on the mode of action of chloroacetamide herbicides have been very limited, considering the fact that CDAA and propachlor are among the most interesting herbicides known, from the standpoint of unit activity and selectivity.

Studies were conducted some time ago to evaluate the effects of CDAA upon respiration of germinating seeds (Jaworski, 1956). These are summarized in Table II. While these data suggested that CDAA could inhibit certain sulfhydryl-containing enzymes involved in respiration, the basic lethal effect must involve some mechanism more intimately connected with growth.

Chloroacetamides are frequently more toxic to small seeded plants, especially those known to have high carbohydrate stores. Upon germination of such seeds in the presence of the herbicides, they appear to shrivel and die as though energy was limiting growth. Furthermore, these compounds have a negligible effect upon plants that are actively photosynthesizing. Based on these observations, an examination was conducted on the ability of chloroacetamides to inhibit crystalline

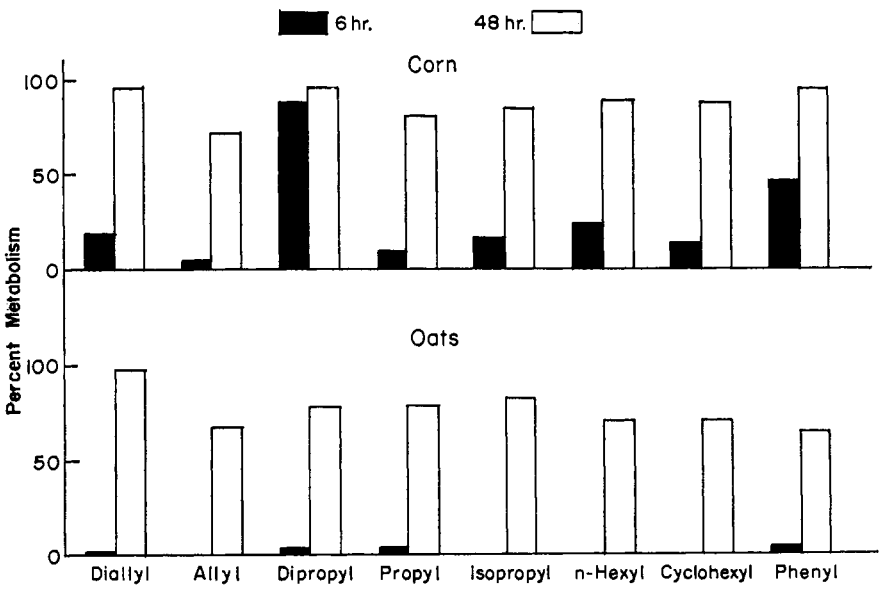


Figure 4. Metabolism of 2-chloroacetamides by corn and oats

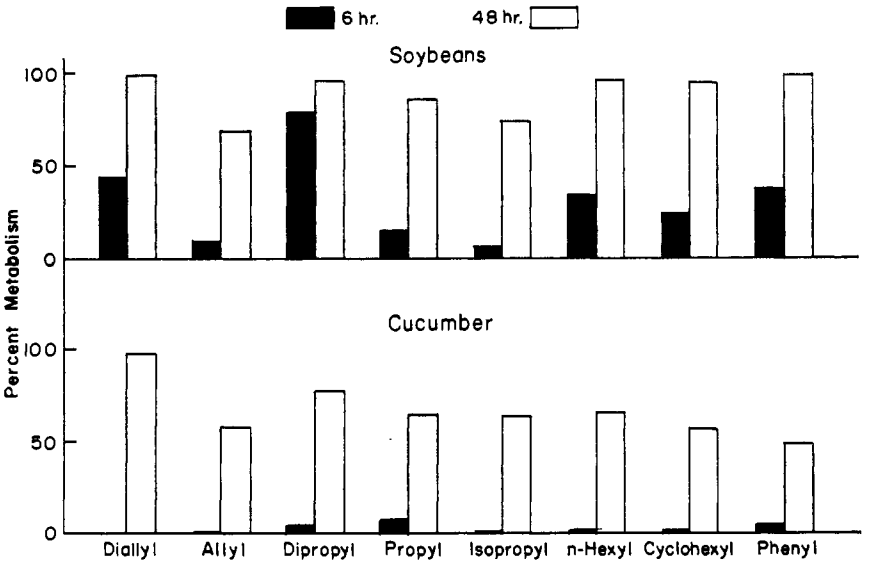


Figure 5. Metabolism of 2-chloroacetamides by soybeans and cucumber

Table II. Effect of CDAA on Ryegrass Respiration

Treatment, P.P.M.	O_2 Uptake, μl./Hr./100 Mgs.	R.Q.	Cotyledon Growth (Mm.)
Control	16	1.0	21
CDAA (10)	3	0.6	0
GSH (15)	17	1.0	21
CDAA + GSH	19	0.9	0

α-amylase hydrolysis of soluble starch using the starch-iodine assay. This enzyme would be produced in germinating seeds to hydrolyze stored starch for energy. Neither CDAA nor propachlor would inhibit α-amylase at $10^{-3}M$. The chloroacetamides might interfere with the gibberellic acid induced amylase production, as demonstrated in barley endosperm (Varner, 1964). Significant inhibition of GA_3-induced α-amylase production was achieved at $10^{-4}M$ (Figure 6). This was found with CDAA and propachlor. The inhibitory response was assumed to represent an effect on protein synthesis or some molecular level preceding protein synthesis since the effect of CDAA was not reversed at high levels of GA_3. Similar experiments using intact barley seeds are shown in Figure 7. At $10^{-4}M$, CDAA gave a 27% inhibition of amylase production, and at $10^{-2}M$ complete inhibition of amylase production was achieved. These levels of CDAA inhibited growth of barley shoots by 78% when measured over a 73-hour growing period. The results are shown in Figure 8. The growth of barley shoots was stimulated approximately 13% by GA_3 at $10^{-5}M$. However, CDAA caused approximately the same degree of inhibition of growth in this instance as with non-GA_3 treated barley seed.

This correlation between growth inhibition and inhibition of α-amylase induction suggested that protein synthesis may be affected by the chloroacetamides.

Mann *et al.* (1965) surveyed a large variety of herbicides and their effects upon protein synthesis. In these

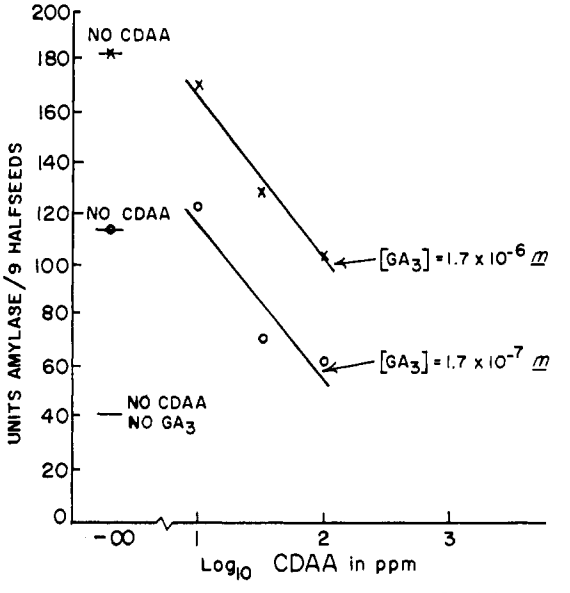


Figure 6. Effect of gibberellic acid and CDAA on α-amylase activity in barley endosperm and aleurone

studies, barley coleoptiles and Sesbania hypocotyls were pre-incubated with CDAA for 1 hour. Leucine-1-^{14}C was then added and incubated for an additional 2 hours. Extraction of the tissue with hot ethanol and determination of residual radioactivity indicated a substantial inhibition of leucine incorporation into protein, as shown in Table III. Mann concluded that the inhibition of protein synthesis by CDAA is probably due to a more fundamental action, since even the uptake of amino acids (α-aminobutyric acid) was somewhat inhibited by CDAA. This latter effect could be attributed to the inhibition of respiration and subsequent effects on active uptake of amino acids by the plant tissue.

Probably the most elegant studies that have been conducted on the mode of action of chloroacetamides are those by Duke (1967) at the University of Illinois

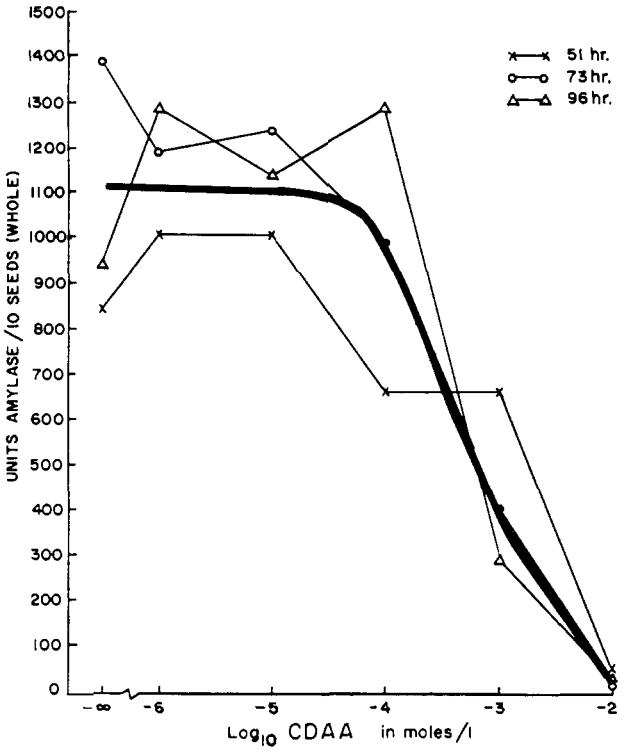


Figure 7. Effect of CDAA on α-amylase activity in intact barley seeds

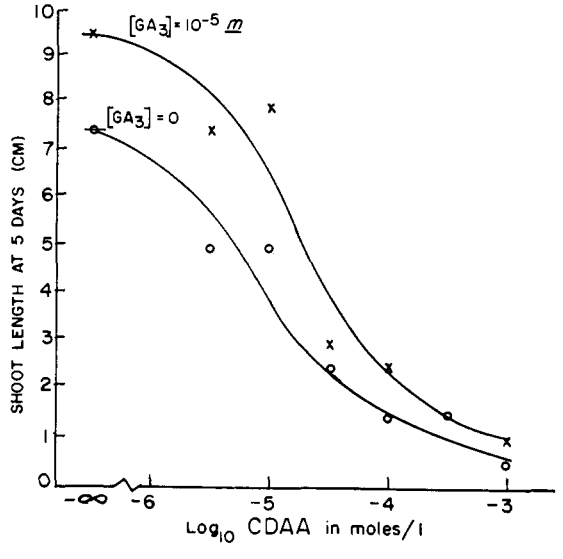


Figure 8. Effect of gibberellic acid and CDAA on the growth of barley seeds

Table III. Inhibition by CDAA of Leucine-1-^{14}C Incorporation into Protein (%)

Plant	2 P.P.M.	4 P.P.M.
Barley	51	70 (36[a])
Sesbania	58	87 (50[a])

[a] Per cent inhibition of DL-α-amino-*n*-butyric-1-^{14}C.

with propachlor. Duke showed that cell elongation and protein synthesis by normal and auxin-induced cucumber hypocotyl tissue was inhibited by propachlor at 5 p.p.m. The same concentration of herbicide that inhibited auxin induced growth also inhibited protein synthesis. Similarly, inhibition of the growth of cucumber roots was closely correlated with the inhibition of protein synthesis in root tips, and protein synthesis was significantly reduced before reduction in root growth. Duke further demonstrated that nucleic acid synthesis was inhibited; but he was able to show in time-course studies that the inhibition of protein synthesis preceded the inhibition of growth and nucleic acid synthesis. Apparently, neither oxidative phosphorylation nor the formation of messenger RNA ribosome complex was inhibited. Thus, Duke indicated that propachlor could be active by preventing the activation of amino acids and aminoacyl-tRNA formation or by interference with the transfer of aminoacyl-tRNA to the polypeptide. In this respect, he thought the herbicide appeared to act somewhat like cycloheximide, chloramphenicol, or puromycin. Based on other logic, he concluded that the mode of action of propachlor was probably like that of cycloheximide—that is, preventing the transfer of aminoacyl-tRNA to the forming polypeptide rather than affecting amino acid activation or aminoacyl-tRNA formation. The binding of chloramphenicol to ribosomes can also prevent the transfer of aminoacyl-tRNA to forming polypeptides, and propachlor could behave in a similar fashion.

Clark and Marcker (1968) have reviewed the basis for the initiation of protein biosynthesis in bacteria and have indicated the importance of methionyl-tRNA in the initiation of protein biosynthesis. In bacteria, there appears to be a specific methionine transfer RNA that is formylatable by 10-formyltetrahydrofolic acid. Formylmethionyl-tRNA then functions as the protein chain initiator. Their schematic diagram illustrates the binding of messenger RNA to the ribosome and indicates how the transfer RNA base pairs with the messenger RNA. Their current idea indicates that formylmethionyl-tRNA base pairs at a site which they have designated as the peptide site. A site adjacent to the peptide site, designated as the amino acid site, provides the position for the next tRNA-containing amino acid to be linked with the formylmethionyl-tRNA. Once the first peptide linkage is formed between formylmethionine and the adjacent aminoacyl-tRNA, the formylated-tRNA leaves the ribosome and another aminoacyl-tRNA moves into position to link with the dipeptide. In this way, the polypeptide chain is developed.

The above background makes it tempting to suggest that because of the reactive nature of the α-halogen in the α-chloroacetamides, a nucleophilic displacement may occur between the amino group of methionyl-tRNA and the herbicide, as seen in Figure 9. Conceivably other aminoacyl-tRNA's can be alkylated in a similar fashion. However, based on structure-activity studies with herbicide analogs, it is felt that the reaction would be quite specific for at least a limited number of aminoacyl-tRNA's. If such a reaction were to occur with methionyl-tRNA, in which the transfer RNA was spe-

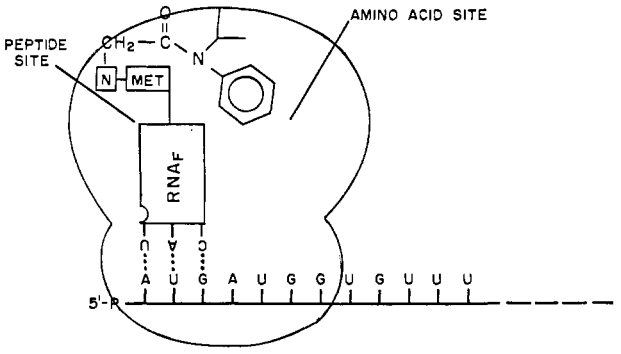


Figure 9. Schematic diagram illustrating the possible inhibition of peptide synthesis by alkylation of the amino group of the initiating aminoacyl-tRNA by propachlor

cific for the initiation step—namely, the formylatable version of methionyl-tRNA—then an interference with the mechanism of protein initiation could occur. This model would be consistent with Duke's theory, which clearly indicates an inhibition of nascent protein synthesis even though there is currently no evidence for a specific protein initiating aminoacyl-tRNA in plants. It further suggests that the effect may be more specific than merely reaction with any aminoacyl-tRNA molecules.

It is always tempting to have a single explanation for the mode of action of a given chemical. However, it is possible that the α-haloacetamides may be acting by a variety of mechanisms whose sum total results in growth inhibition and death of susceptible plant species. For example, at least 30 enzymes have been reported to be inhibited by iodoacetamide (Webb, 1966). The reversal studies on the inhibition of respiration suggest that the α-haloacetamides can generally react with sulfhydryl reagents both in vitro and in vivo. Numerous enzyme systems contain sulfhydryl groups vital to the activity of the enzyme, and it is conceivable that a large variety of enzymes might be inhibited at the concentrations which have been studied. Since these levels are comparable to those involved in the inhibition of protein synthesis, a multivalent rather than a specific inhibition may be involved.

LITERATURE CITED

Clark, Brian F. C., Marcker, Kjeld A., *Scientific Am.* **218,** 36 (1968).
Duke, William Bryant, Ph.D. Thesis, Univ. of Illinois, Urbana, Ill., 1967.
Jaworski, Ernest G., *Science* **123,** 847 (1956).
Jaworski, Ernest G., J. AGR. FOOD CHEM. **12,** 33 (1964).
Mann, Jay D., Jordan, L. S., Day, B. E., *Plant Physiol.* **40,** 840 (1965).
Smith, Gerald R., Porter, C. A., Jaworski, E. G., Abstract, 152nd National Meeting, ACS, New York, 1966.
Varner, J. E., *Plant Physiol.* **39,** 413 (1964).
Webb, J. Leyden, "Enzyme and Metabolic Inhibitors," Vol. **III,** p. 1, Academic Press, New York, 1966.

Received for review August 30, 1968. Accepted December 9, 1968. Division of Agricultural and Food Chemistry, 155th Meeting, ACS, San Francisco, Calif., April 1968.