PLANT METABOLIC ACTIVATION OF THE HERBICIDE ATRAZINE TO MUTAGENIC DERIVATIVES

by

Peter A. Petrofes
Department of Biology

Submitted in Partial Fulfillment of the Requirements of the University Undergraduate Fellows Program

1980-1981

Approved by:

Ethel A. Tsutsui

Ethel Ashworth- Tsutsui, Ph.D.
Department of Biochemistry & Biophysics

April 1981

ABSTRACT

A rapid method was proposed to detect whether the herbicide atrazine could be converted into a mutagenic compound by plant metabolism. The method is based on the use of *Vicia texana* plants. Extracts were prepared from the plant tissues to be tested for mutagenic capability and metabolite identification. The mutagenic tests were to be carried out at the University of Texas System Cancer Center, Science Park Laboratory.

ACKNOWLEDGEMENT

Supported in part by the Texas A&M University Honors Fellowship Program, Texas A&M University. The author wishes to express his appreciation to Dr. Ethyl Ashworth-Tsutsui of the Texas A&M University Biochemistry Department for her continued and valuable interest and assistance. I acknowledge the technical assistance of Tom Luntz and Susan Midkiff, also of the Biochemistry Department.

CONTENTS

PLANT METABOLIC ACTIVATION OF THE HERBICIDE ATRAZINE TO MUTAGENIC DERIVATIVES

INTRODUCTION

The aim of this research was to set up a simple system that could simulate *in vitro* plant metabolism of atrazine to determine whether enzymes involved in plant metabolism could transform the initial herbicide into a mutagenic derivative. Atrazine (A Atrex 80W, Ciba- Geigy, active ingredient 2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a herbicide used widely to control broad-leaved weeds in sorghum and corn acreages.

The plant chosen, the common pinto bean (*Vicia texana* T. et G.) may be easily grown in laboratory cultures using vermiculite. For the purpose of this research, the effects of *Vicia* cell metabolism on atrazine were tested.

LITERATURE REVIEW

There is much knowledge concerning the metabolism of atrazine in corn plants. Atrazine is known to be converted in corn and maize into a mutagenic compound which causes point mutation in yeast(*Saccharomyces cerevesiae*) (Plewa and Gentile, 1976). Shimabukuro, 1967, reported that the metabolism and detoxification of atrazine in higher plants occurred via 2-hydroxylation and N-dealkylation pathways. Both pathways are found in corn (*Zea maya* L.) (Shimabukuro, 1967). In sorghum, only the N-dealkylation pathway is thought to occur. The products of these two pathways were identified as 2-chloro-4-amino-6-isopropylamino-s-triazine and 2-chloro-4-amino-6-ethylamino-s-triazine for the N-dealkylation pathway and 2- hydroxyatrazine for the hyroxylation pathway (Shimabukuro,1967; Shimabukuro, 1968). Both of these pathways are represented in figure 2.

Lamoureux et al (1970) found that atrazine could be metabolized into the same products that Shimabukuro had isolated, in excised sorghum leaf sections. This isolated the possible location of enzyme systems responsible for the conversion of atrazine(Lamoureux et al, 1970).

Benigni et al(1979) found that atrazine was metabolized to mutagenic products in tobacco (*Nicotiana alata* L.).

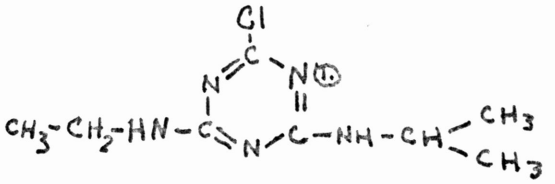

Fig. 1 Atrazine: 2-chloro-4- ethylamino-6-isopropylamino-s-triazine

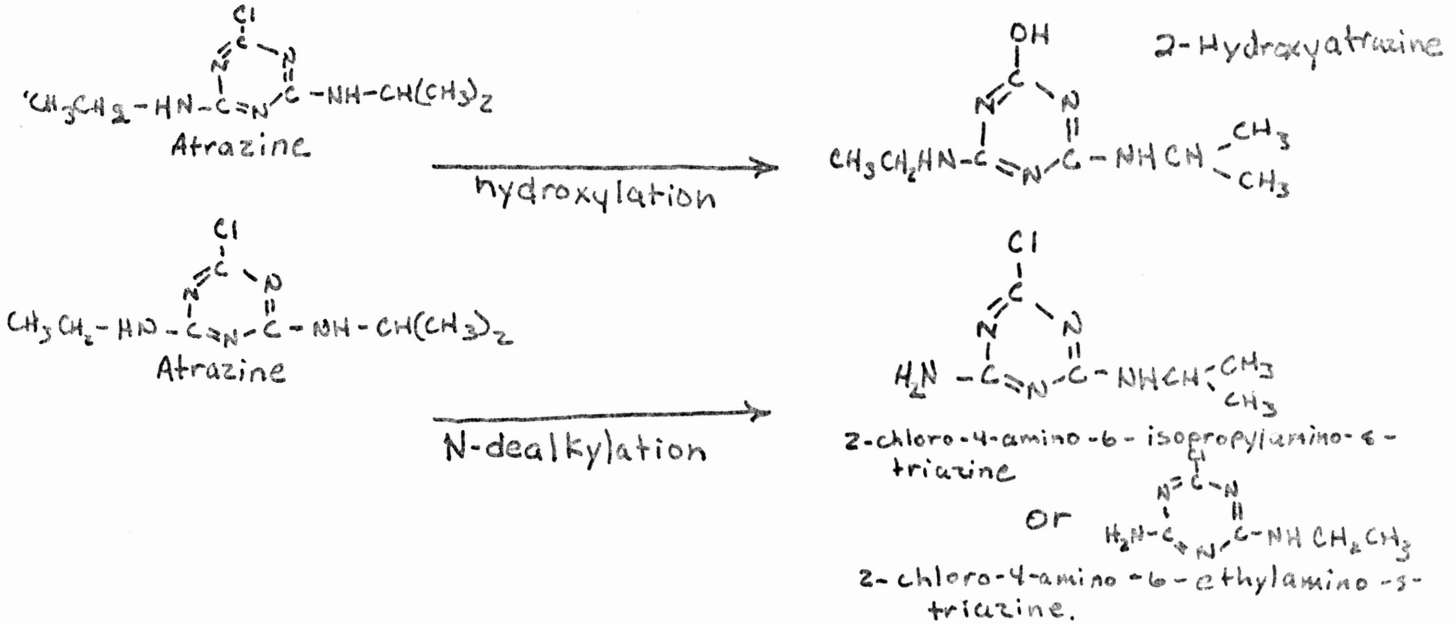

Fig. 2. Possible metabolic pathways of atrazine and the products of these pathways.
(Shimabukuro, 1968)

It is not yet known whether Nicotiana metabolism resembles that of corn or that of Sorghum (Benigni et al, 1979).

Since plant metabolic activation of herbicides is a fiald still in its infancy, virtually nothing is known at this time about the activating enzymes in plants.

OBJECTIVES OF RESEARCH

The objectives of this study were threefold:

(1) to set experimental conditions for the activation of atrazine by Vicia enzumes to compounds which could cause genetic mutations in fungi.

(2) to determine, if possible, which probable metabolites of atrazine are responsible for mutagenic activity in the test system.

(3) to determine some of the biochemical characteristics of activating enzymes in plants.

Subcellular fractions of plants were prepared by standard fractionation techniques and were to be assayed for mutagenic activity in collaboration with Dr. Barry Scott, microbial geneticist at the Science Park laboratory for basic research of the University of Texas System Cancer Center at Smithville, Texas. All mutagenic tests were to be carried out at Science Park.

MATERIALS AND METHODS

Beans of V. texana were placed in plastic containers filled with vermiculite. Equal volumes of water or water plus atrazine were added to each container at given time spans in terms of days. The plants were then grown in the lab with an average photoperiod of ten hours at room temperature. Different concentrations of atrazine were used in two of the boxes: 80 ppm and 100ppm respectively. The third container was simple watered as a control. Plants were grown under these conditions to the three leaf stage at which time the were havested. The three groups of plants were handled separately to prevent contamination. Initially, the roots,stems and leaves were all homogenized together in five volumes of water (weight/volume). The homogenate was filtered with number four filter paper and the filtrate was centrifuged at 5000g for ten minutes. The supernatant was collected and refrigerated until the assays could be run.

This initial preparation of extracts contained too much volume to be efficiently assayed for mutagenicity, but thin layer chomatography using cellulose plates was still possible. The chromatography system used was n-butanol-acetic acid-water (12:3:5)(Lamourex et al, 1970).

In an effort to concentrate the final extract, a new extraction method was employed (Lamourex et al,1970). The plants were grown for twelve days in the same types of sol-

utions, except the 80 ppm atrazine solution was lowered to 50ppm. The extraction was performed on the roots and stems as one group. The leaves were then treated separately. The groups were homogenized in 80% cold methanol in a Waring blender for three minutes. The homogenate was then filtered and concentrated to near dryness. The filtrate was then partitioned with chloroform. Aliquots of both the chloroform and aqueous phases were then chromatographed and stored for mutagenic study.

An attempt at silica gel thin layer chromatography used silica gel G and a ethanol-methanol system (1:1). This method of chromatography is used to identify certypes of triazine compounds of which atrazine is one. (Ebing and Henkel, 1964).

RESULTS AND DISCUSSION

The data in Table 1 indicates the amount of atrazine added to the plants, the length that the plants were grown in the atrazine and the amount of extract prepared. The first extracts prepared contained too much volume, therefore an alternate system was developed using methanol extraction instead of water. The thin layer chromatography using cellulose plates and butanol system showed possible atrazine compound derivatives that gave Rf values similiar to those reported in other literature (Shima, 1968). The cellulose thin layer chromatography was run on the extracts which contained too much volume and could not be efficiently tested at Science Park Laboratory for mutagenic capabilities.

The next set of plants were grown in atrazine as were the first. The extraction method used methanol and was vacuum dessicated to near dryness. Thin layer chromatography was performed on silica gel plates (Ebing and Henkel), but no results were obtained from any of the plates prepared. Reasons that no results did appear from these chromatography plates include: incorrect plate spreading, which could have resulted in either the front moving completely to the top of

TABLE 1

TRIAL	ATRAZINE CONC.	DAYS GROWN	EXTRACT SYSTEM	EXT. AMT.
1	0ppm	24	water	350ml
	80ppm	24	water	250ml
	100ppm	24	water	210ml
2	0ppm	18	water	320ml
	80ppm	18	water	235ml
	100ppm	18	water	200ml
3	0ppm	16	methanol	45ml
	50ppm	16	methanol	23ml
	100ppm	16	methanol	19ml
4	0ppm	12	methanol	41ml
	50ppm	12	methanol	22ml
	100ppm	12	methanol	16ml

of the plate or the front not being able to move at all. Since time became an important factor in this project, a regular chromatography was employed using cellulose plates. The extracts were then concentrated and prepared for delivery to Dr. Scott at the Science Park Laboratory for mutagenic studies. At the time of this writing no results were available.

Atrazine was apparently metabolized by V. texana, but no actual mutagenic capabilities have yet been reported concerning this project. Other literature has found that atrazine can be metabolized by other plants into carcinogens (mutagens) which cause point mutation in microbial systems (Lameroux et al, 1970; Benigni et al, 1979; Plewa and Gentile, 1976). It is not known if the metabolites of atrazine found in Vicia are the same as these found elsewhere, or if the enzymes which activate atrazine are the same. The atrazine was found to be deleterious to the plants after repeated concentration and these deleterious affects could be seen in the plants appearance as well as the amount of extract which was finally obtained from those plants which were exposed to the atrazine.

CONCLUSION

V. texana simulated quite well an *in vitro* method for the testing of atrazine metabolism. The time necessary to treat the plants was short (2 weeks at the most) and extracts could be prepared easily. The possible metabolites could be compared with those in the literature and could be tested at the Science Park Laboratory for mutagenic capabilities. Since this did require collaboration with the Science Park Laboratory more time was required to obtain results then was available.

REFERENCES

Benigni,R.,Bignami,M.,Camoni,I.,Carere,A.,Conti,G. Iachetta, R.,Morpurgo,G.,Oratli,V. *Jour. of Tox. and Envir. Health*, 5:809-819,1979.

Ebing,M. and Henkel,R. *Jour. of Chromat.* 14:283-285, 1964.

Lamourex,G.,Shimabukuro,H.,Swanson,H.,Frear,D. *Jour of Agric. Food Chem.*, 18:81-86,1970.

Plewa,M. and Gentile,J. *Mut. Res.*, 38:287,1976.

Shimabukuro,R. *Plant Physiol.*, 42, 1269,1967.

Shimabukuro,R. *Plant Physiol.*, 43, 1925,1968.