metaphase II, anaphase II and the quartet stage were analyzed in each sample as well as from untreated sibling controls to detect any abnormalities which might be caused by DPX 3778.

At each stage, almost all of the cells were normal. Although a very low frequency of abnormal cells was detected at certain stages in these samples, a similar frequency of these abnormal cell types was also observed in untreated control plants.

Plants of the inbred A632 were also treated in a similar way at a rate of 4 lb/A, and microsporocyte samples were taken at 30 and 105 hours after treatment. Meiosis in these samples also appeared no different from meiosis in control, untreated sibling plants.

Thus, meiosis in DPX 3778-treated plants does not appear to differ from meiosis in untreated control sibling plants. (I would like to express my thanks to Dr. Charles Laible for treating the plants utilized in this study. I would also like to thank Funk's Seeds International for providing nursery space in which these and other plants were grown).

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A maize-microbe bioassay for the detection of proximal mutagenicity of agricultural chemicals — We have devised a bioassay to detect the presence of proximal mutagens of agricultural chemicals. A proximal mutagen is a mutagen that arises from the host metabolism of a non-mutagenic substance or "promutagen" (Brusick, D. J., and V. W. Mayer, Environ. Health Perspec. Experimental Issue No. 6:83-96, 1973). Although we believe the wide use of pesticides is necessary, we suggest that the present monitoring systems used to detect genetic damage are inadequate. Since the possibility exists that mutagenic agents may be passed along the food chain, we contend that additional genetic monitoring systems should be developed and tested for their accuracy and economic feasibility. The recent citation of aldrin and dieldrin as carcinogenic compounds (Carter, L. J., Science 186:239-242, 1974) amplifies our concern, especially since many scientists hypothesize that carcinogens cause cancer by somatic mutations (Ames, B. N., Genetics 78:91-95, 1974). Thus a rapid method to detect mutagens arising from the use of agricultural chemicals is urgently needed.

This bioassay is based on the host-mediated scheme. In this experiment we used a maize-yeast system. The chemical studied was atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine].

One hundred kernels of inbred H51 were planted in each of eight trays filled with equal amounts of vermiculite. Into each tray a final volume of 1.5 l of distilled water plus a known concentration of atrazine was added. The concentrations of atrazine used were 0 ppm (control), 1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm. This concentration gradient encompasses the concentrations of atrazine used in commercial applications (0.5-5 ppm). The trays were put into an environmental chamber set to days with a 14 h photoperiod. The day temperature was 30^{o} C and 20^{o} C at night. The plants were allowed to grow for one week. Once during this period 500 ml of distilled water was added to each tray.

The seedlings were harvested after one week, and root tips were collected for chromosome analysis. The roots, stems and leaves were collected and placed on ice. Approximately 50 g of tissue from each tray was gathered. Tissues from the plants of each of the trays were homogenized separately with a Virtis blender in 200 ml of sterile water. The material was filtered through "Miracloth" and the filtrate was passed through No. 4 filter paper. The filtrate was centrifuged at 1000xg for 20 min. The supernatant fluid (1S fraction) was saved and lyophilized. Each sample, in powder form, was placed into a vial, whiffed with nitrogen and sealed. The vials were coded to introduce a blind, and the reversion tests were conducted using Saccharomyces cerevisiae.

The presence of a proximal mutagen in the 1S fraction was tested by reversion analysis using three S. cerevisiae strains. H201.14.4 is a haploid ade^{-} base pair substitution auxotroph; D4 is a diploid ade^{-} base pair substitution heteroallele; and DW844 is a haploid frameshift nuclear petite. Each strain was plated on complete media at a population density of 10^{7} cells/plate. The plates were incubated at 30^{o} C for 6 h. After incubation there was a confluent growth of cells covering each plate.

Each lyophilized 1S fraction was sterilized and diluted in sterile distilled water to a concentration of 12 mg/ml. 0.1 ml of the sterile 1S fraction was placed onto the middle of each plate and incubated for 18 h at 30^{o} C. Each plate was then replica-plated onto minimal media, incubated at 30^{o} C for 72 h and scored. A 0.1 ml, 1000 ppm solution of atrazine was also tested on H201.14.4 as described above.

The data concerning the reversion and induced recombination analysis are presented in Table 1. The data indicate that a mutagen is present in the 1S fraction of plants exposed to various concentrations of atrazine. That the

Table 1. Summary of yeast reversion or recombination data and maize chromosome observations.

1S fraction	*S. cerevisiae* strains			Chromosome morphology of maize root tips
	H201.14.1	D4	DW844	
CONTROL (0 ppm)	0	37	0[a]	CONTROL[b]
1 ppm	++	1000	0	normal
5 ppm	++	700	0	normal
10 ppm	+	900	0	normal
15 ppm	+++	2000	0	normal
20 ppm	++++	55	0	normal
25 ppm	+++	20	0	normal
30 ppm	+++	30	0	normal
Atrazine (1000 ppm) applied to plate	+	NA[c]	NA	NA

[a]Zero indicates that the reversion rate was not above the spontaneous reversion rate.

[b]The control was considered the "normal" chromosome morphology and was compared with the morphology of the chromosomes from plants exposed to the various concentrations of atrazine.

[c]Not applicable.

mutagen is not a natural component of maize is shown by the control. The very high concentration of atrazine applied directly to the yeast cells did induce a low reversion rate; however, the general increase in the amount of reversion of H201.14.4 is directly related to the amount of atrazine to which the maize plants were exposed. D4 also shows a high recombination rate up to the 15 ppm 1S fraction. The recombinants were confined to an area within the drop of the 1S fraction. The lack of recombinants above the spontaneous rate in 1S fractions above 15 ppm may be due to lethality.

In both base pair substitution mutant stocks, H201.14.4 and D4, there is an increase in the reversion or recombination rate induced by the 1S fraction of plants exposed to atrazine. However, DW844, a frameshift mutant reverted by ICR-170, does not revert by the proximal mutagen present in the 1S fractions. From these data we suggest that the proximal mutagen derived from atrazine primarily induces base pair substitution mutations.

We were unable to detect any chromosome damage induced by atrazine in exposed seedlings. Breaks induced by atrazine in meiotic chromosomes of barley have been reported; however, the plants were exposed to 1000 ppm (Wuu, K. D., and W. F. Grant, Cytologia 32:31-41, 1967).

We propose to extend this bioassay to include the effects of long term growth after an initial exposure to a pesticide. Maize pollen grains can be used to detect chromosome damage and forward and reverse mutation rates. The amount of pollen abortion can be used as an index of chromosome aberrations; if a proximal mutagen causes breaks in meiotic chromosomes, we could monitor these effects over an exposure concentration gradient by the increase in pollen abortion. Forward and reverse mutation rates could be measured on populations based on 10^5 to 10^6 pollen grains by studying mutation rates at the waxy locus. We have experiments in progress that should determine whether or not the above suggestions are feasible.

We contend that this bioassay could be used routinely by industry to test the agricultural chemicals they produce prior to their introduction into the environment. We believe that such information is essential in making rational decisions concerning agriculture and its ecological impact. (Partially funded by a D. F. Jones Fellowship, Research Corporation, New York).

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Topographical structure of the R region in R-ch complexes — Recombination studies involving a number of R-ch isolates revealed that the R region in these stocks consists of a number of closely linked discrete genetic units, each controlling anthocyanin pigmentation in a plant part or tissue. Recombinational analyses in the testcross progenies from G R-ch/g r-g heterozygotes of five cherry isolates show the following linkage relationships among the anthocyanin traits: pigmentation in aleurone (S), anther (P), silk (Si) and pericarp (Ch) (Table 1). Probable gene sequences in these isolates can be constructed as:

Ecuador R-ch: G - S - P - Ch - Si
New Mexican R-ch: G - Si - S - Ch - P, or g - P - Ch - Si - S
Standard R-ch: G - Si - Ch - S - P
Pueblo R-ch: G - S - Ch - Si - P
P.C. 150 R-ch: G - S - Ch - Si

In addition to these four anthocyanin traits, pigmentation in glume (Glm), auricle (Au), leaf margin (Lm), nodal ring (Nr) and coleoptile (Co) appear to be controlled by independent discrete units. Leaf color factor (Lc) expression was poor and