phate. This suggests how it is possible for phytoplankton blooms to persist in water containing only a few hours' supply of dissolved phosphate. The observations presented here suggest that a rapid flux of phosphate is typical of highly productive systems, such as blooms, and that the flux rate is more important than the concentration of dissolved phosphate in maintaining high rates of organic production.

It would be of interest to learn what factors tend to stabilize the flux of phosphate over a wide range of phosphate concentrations and what factors induce a more rapid flux in certain circumstances (*6*).

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Biological Effects of a Chemical Mutagen, Diepoxybutane, on Tomato

Abstract. Tomato seeds were treated with three concentrations of 1:2, 3:4-diepoxybutane. Seedling mutations were induced in high amounts with all doses, but other biological effects in the treated generation including delayed germination, chlorophyll-deficiency sectoring in the first true leaves, and failure of plants to bear fruit, were found only with the highest concentration. Neither a lethal nor a partially lethal dose was applied.

Diepoxybutane has been demonstrated to be a highly effective mutagenic agent in barley by Ehrenberg and Gustafsson (*1*), in *Neurospora* by Kolmark and Westergaard (*2*), and in *Drosophila* by Bird (*3*) and others. Other biological effects, occurring simultaneously with the mutational effect of diepoxybutane in these biological materials, included sterility and lethality, which increased with increased chemical dose. However, in this study lethality was absent in all treatments, and sterility was restricted to only the highest concentration, while the mutational effect was observed with all doses of diepoxybutane applied.

Tomato seeds, 300 per treatment, of the highly inbred O18 strain of the Red Cherry variety of *Lycopersicon esculentum*, were treated with three concentrations of 1:2, 3:4-diepoxybutane—0.2, 0.5, and 0.8 percent—in aqueous solution and with distilled water. Preliminary investigations indicated the 50 percent lethal dose to be somewhere between 0.1 and 1.0 percent diepoxybutane when the seeds, presoaked for 24 hours in distilled water, were treated in the chemical for 1 hour under vacuum followed by 5 hours at normal pressure. The same conditions of treatment were followed throughout.

Table 1. Number of mutations recovered in the second generation following treatment of tomato seed with various concentrations of diepoxybutane, or (control group) with distilled water.

Treatment	No. of transplanted seedlings	No. of mutants recovered	Percent of plants producing mutants
Control (distilled H_2O)	69	0	0.0
0.2 percent diepoxybutane	110	5	4.5
0.5 percent diepoxybutane	104	4	3.8
0.8 percent diepoxybutane	40	7	17.5

Following treatment, the seeds were thoroughly washed and planted in soil. After 2 weeks the seedlings were transplanted to pots on a raised bench in a greenhouse maintained at 65°F. night temperature. The number of transplanted seedlings in each treatment were as shown in Table 1. The plants, trained to one main axis, were grown for 7 months and seeds from the first four fruit-bearing inflorescences were recovered separately.

For the determination of induced mutations the seeds from the first and last inflorescence of each plant were grown to the seedling stage of the second generation and screened for seedling abnormalities. Lines segregating for color, rate of growth, or morphological seedling abnormalities were grown to the third generation to determine the inheritance of the abnormalities recovered. Abnormalities segregating in a 3:1 ratio in the seedling stage of the third generation were classified as mutations.

Mutations were recovered from all three diepoxybutane treatments but none from the control lots. The number and percentage of mutations recovered from each diepoxybutane treatment are listed in Table 1. Only a single mutant type was recovered from any one treated plant, but the mutation could be found in either or both of the tested inflorescences of this plant. The majority of the mutants were chlorophyll-deficient types; in the remainder, rate of growth and morphological characteristics were affected.

Apparent pleiotropic effects on rate of growth and morphological development were characteristic of many of the chlorophyll mutants. Lethality in the seedling stage or sterility of most of the mutants prevented the recovery of the mutations in a homozygous condition. This fact might be an indication of chromosomal deletions or rearrangements in the mutants rather than true gene changes.

Biological effects in the treated generation, excluding the mutational effect, resulting from diepoxybutane treatment were found only with the highest concentration, 0.8 percent diepoxybutane. The seed treated with 0.2 and 0.5 percent diepoxybutane developed in all respects the same as the control. The mutational effect in seed treated with these two concentrations was the only observable difference from the seed treated with distilled water.

The three characteristic effects of the 0.8 percent dieoxybutane treatment in the treated generation included delayed germination, chlorophyll-deficiency sectoring in the first true leaves, and the production of fruitless plants.

While the control, 0.2 percent, and 0.5 percent diepoxybutane treatments yielded 90 percent germination of the treated seed within 10 days of planting, during the same period only 15 seeds, or 5 percent, in the 0.8 percent diepoxybutane treatment germinated. However, 3 weeks later germination had increased to 90 percent, thus demonstrating the failure to administer even a partially lethal dose.

All of the seedlings that germinated in the 0.8 percent diepoxybutane treatment exhibited chlorophyll-deficiency sectoring in the first true leaves. All other leaves developed normally.

In this experiment, sterility was determined by the number of plants failing to produce any fruit; a treated plant was considered fertile if it produced at least one fruit on any of the tested inflorescences. In the 0.8 percent diepoxybutane treatment, eight plants, or 20 percent, failed to produce fruit. In the other two chemical treatments together, only one plant, of the 0.2 percent diepoxybutane treatment, was fruitless. However, all of the plants which failed to produce fruit in the greenhouse on the main axis, when they were pruned back to the first leaves and set in the field, produced fruit on their axillary branches in all respects the same as normal plants.

The tomato appears in this experiment to respond differently from other biological materials to diepoxybutane treatment. While the mutational effect increased with increased chemical concentration, at least between the two higher concentrations, neither sterility nor lethality patterns were the same. Sterility was manifested only at the highest concentration of diepoxybutane, while even a partially lethal dose was not demonstrated with any of the concentrations employed. At the same time, two previously unreported effects, chlorophyll-deficiency sectoring and delayed germination, were produced by diepoxybutane treatment.

All of these effects, excluding the mutational effect, were restricted in tomato to the 0.8 percent diepoxybutane treatment. In addition, chlorophyll-deficiency sectoring in first true leaves and delayed germination were relatively constant features of this treatment.

The constancy of delayed germination and chlorophyll sectoring in the 0.8 percent diepoxybutane treatment indicates possibly that there is a relatively invariable response of the individual tomato seeds in a treatment to a given concentration of chemical. And, too, if one assumes these effects to be the result of gross chromosomal aberrations, the ratio of normal to damaged meristematic cells in the seed treated with 0.8 percent diepoxybutane has reached the threshold point necessary for the manifestation of these effects. On the other hand, if lethality is dependent on all meristematic cells of a seed being irreparably damaged, then the dosage necessary for this condition had not yet been administered in this experiment.

The results of this study may thus indicate the tomato to be a valuable material on which to study the quantitative effects of chemical mutagens in higher plants. First, diepoxybutane has demonstrated mutagenic effects over a wide range of chemical concentrations (0.2 to 0.8 percent) without being limited by lethality or sterility. Second, the seed itself demonstrated few properties that would cause variations in the effects of a given concentration of chemical with an individual treatment (*4*).

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Effect of Synthetic Smog on Spontaneous Activity of Mice

Abstract. Mice allowed to run in a revolving-drum activity cage are sensitive indicators of air pollution. They respond to the presence of synthetic smog by diminishing their 24-hour activity in the revolving wheels. The reduction in wheel activity is comparatively greater for larger amounts of smog.

Since Stewart's pioneering experiments on the measurement of the activity of rats and mice with revolving wheels and kymographs, numerous investigators have studied the factors which influence the behavior of rats in this type of apparatus (*1*). Attempts have been made to calibrate or improve the wheels, or invent new means of measurement, such as tambour-mounted or tilting cages, photoelectric or magnetic devices, and a variety of mazes, most of which measure different quantities (*2*). Furthermore, many environmental and biological factors affect activity, yet relatively little is known about the motivation involved, despite several investigations of this phase of the problem (*3*).

Some of the factors which tend to complicate the use of these techniques are age, sex, diurnal cycle, oestrus cycle, visible light (*5*), heredity (*6*), and hunger and dietary deficiencies (*7*). Drugs with both stimulating and depressing effects on activity are known (*8*). Whole-body radiation also exerts some influence, though activity is not especially responsive to this kind of insult (*9*). Tobacco smoke is also claimed to have some effects (*10*). Although other air pollutants have not been systematically studied, one of Stewart's original observations is of interest in this connection. During the course of his experiments on the effects of barometric pressure and alcohol on the activity of rats, he observed a decrease on several occasions which he attributed to the escape of gas in his laboratory. While he does not indicate whether the poisoning seriously affected his animals in other ways, he was apparently the first to observe the effect of an air pollutant on voluntary activity (*1*).

The experiments described in this report were performed in the expectation that biological methods which measure the voluntary behavior of the experimental animal would provide sensitive indicators of environmental factors such as air pollution and infectious agents, since relatively small sensory impulses may be amplified by the neuromuscular system of the animal into large changes in behavior (*4*).

For the purpose of studying these effects, we have employed two modified 100 ft^3 refrigerators. The chambers are similar and are provided with activated charcoal filtered air pulled by an exhaust blower on the roof. The temperature is approximately the same in both chambers. A mixture of ozone and gasoline vapor in air is forced into the exposure chamber. (The technique is similar to that described by Kotin and Falk, *11*.) This smog is analyzed daily for total oxidant with phenolphthalein (*12*) and for ozone by absorption in neutral potassium iodide (*13*).

The mice used for the present study were young adult C57BL/6 males; they were caged individually, and they had

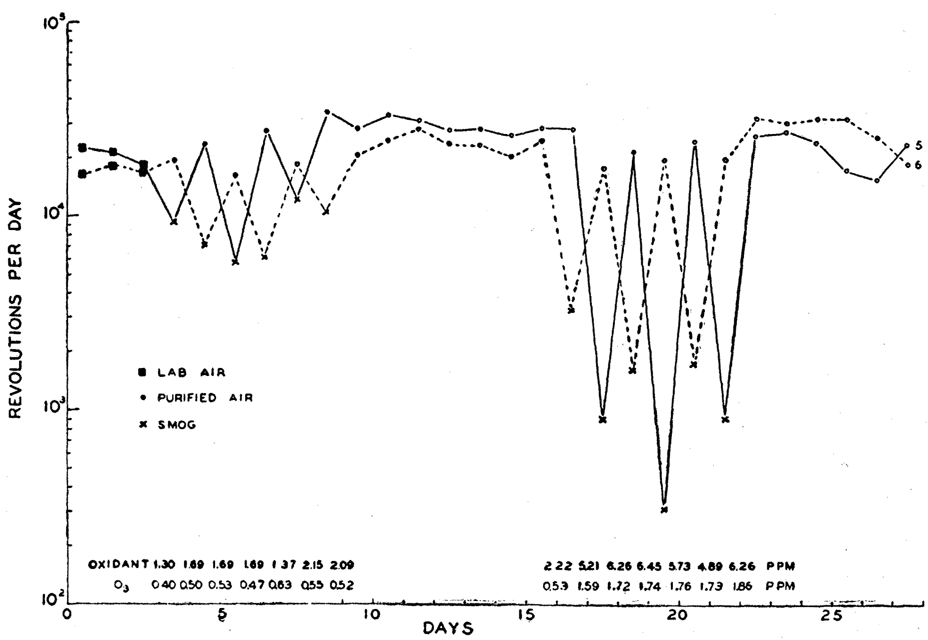

Fig. 1. Spontaneous wheel turning activity of two C57 Black male mice in different environments. The total oxidant and ozone determinations are shown at the bottom of the graph for each day of exposure to synthetic air pollutant mixture.