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Mutagenicity of Ambient air at selected sites in the United States
Using Tradescantia as a Monitor*

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Abstract

The damaging impact of environmental mutagens and toxicants of natural or industrial origin on the health and well being of higher plants, animals and overall ecology of our biosphere has been documented and is no longer speculative. Many biological assay systems have been developed to assess the mutagenicity of aqueous and particulate compounds, but few are efficient for gaseous compounds. To fulfill the need for monitoring ambient airborne chemicals the Tradescantia stamen hair system has been adapted for use in a mobile laboratory. Controlled laboratory exposures of Tradescantia flower buds to several gaseous mutagens were positive with significant responses at < 0.1 ppm of 1,2-dibromoethane. Mutational response increases linearly with duration of exposure up to about 21 days for both physical (radiation) and chemical mutagens. Hence the stamen hair system achieves its greatest sensitivity with chronic exposures typical of those in populated areas near industrial pollution sources.

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A mobile monitoring vehicle was assembled utilizing controlled-environment growth chambers for both ambient air fumigations and concurrent clean-air control exposures. Industrial sites monitored by the mobile laboratory were selected because of known increased coincidence of human cancer and/or high levels of pollutants in the vicinity. The mobile laboratory traveled more than 12,000 miles and monitored the ambient air downwind from 14 different industrial sites throughout the United States. The ambient air at all sites near petroleum refineries gave positive mutagenic responses in the Tradescantia stamen hairs ranging from an increase over background of from 16.6 to 90.6%. Smelters and chemical industries showed more varied responses from 4.8 to 51.4% increases above background. Preliminary analyses indicate that the highest biological activity was associated with highest levels of vapor-phase organics, especially at the New Jersey site.

Introduction

The theme of the conference on "In vitro toxicity testing of environmental agents: Current and future possibilities" concerned the state of the art of bioassay systems involving cultured organisms and their application to the assessment of human health effects of environmental contaminants. The major emphasis has been placed on bacteria and mammalian cell lines but for increased perspective on the state of the art of specific in vitro assays it is important to consider the environmental impact on whole organisms by reviewing the contributions made by in vivo

assays. The more classical non-mammalian in vivo systems such as Drosophila, Zea mays and Tradescantia are characterized by well defined genetic bases, versatility in mode of treatment, relatively low cost, short term and/or ^{high} sensitivity to both physical (radiation) and chemical mutagens (Underbrink, et al, 1973(b); Vogel and Sobels, 1976; Nix and Brewen, 1978; Plewa, 1978; Schairer, et al, 1978). This paper will deal exclusively with the Tradescantia stamen hair assay, demonstrating its relevance to environmental mutagen assessment, describing the system briefly and discussing its adaptation for in situ ambient atmosphere monitoring.

As a result of very great increases in world population and expansion in industrial and agricultural development, man is being exposed to an ever increasing level of environmental pollutants. The sources of these chemical and physical environmental contaminants are varied. They include human and industrial pollution of streams and ground water (Brittin, 1972), automotive and industrial combustion products (Bond and Straub, 1973), pesticides (White-Stevens, 1971; Epstein and Legator, 1971), commercially used chemical additives, solvents or catalysts (Fishbein, et al, 1970; Flamm and Mehlman, 1978) and natural or artificial sources of radiation (Ionizing radiation, 1972; Eisenbud, 1973). In the past few years there has been an accelerating effort to detect and identify the most serious or most toxic pollutants (Hindawi, 1970; Vogel and Röhrborn, 1970;

Oser, 1971; Flamm, 1974; Neel, 1974; Waters, et al, 1978). It is to this end that we are exploiting the very sensitive Tradescantia plant somatic mutation test system to study the genetic effects of physical and known or suspected chemical mutagens.

The Tradescantia stamen hair system

The stamen hair system has been described in detail elsewhere (Underbrink, et al, 1973^(b); Sparrow et al, 1974; Schairer, et al, 1978) so only certain features will be reviewed here. The plant used exclusively in the field studies to be described here is clone 4430, a diploid interspecific hybrid (T. subacaulis x T. hirsutiflora) bred at Brookhaven (Figure 1a). This clone is a hybrid between pink and blue flowering parents with blue being dominant over pink. The visible marker used in this test system is the phenotypic change in pigmentation from blue to pink in mature flowers. The pigmentation change (hereafter called mutational or pink event) is induced in young developing floral tissue and is expressed 5 to 18 days later as isolated pink cells or groups of pink cells in the stamen hairs of mature flowers (Figure 1c,d). The pink events are essentially nonlethal so large mutant sectors indicate genetic injury early in the development of that tissue.

Conventional plant breeding tests were made with clone 4430 and appropriate parental stocks to confirm the genetic basis for the pink color locus. Classical Mendelian segregation ratios of 3:1 and 1:1 (blue to pink) were obtained indicating single gene control of flower color (Thompson and Nawrocky, 1979; Thompson and Nawrocky, unpubl.). Other endpoints documented by radiobiological studies and available for environmental chemical assessment in clone 4430 include colorless (non-pigmented) stamen hair cells, stunted hairs (cell death or growth inhibition) and aborted pollen grains as shown in figure 2.

Figure 2 b shows one and two-celled hairs; such hairs and others less than 12 cells in length are called stunted and are scored as nonsurvivors. Thus it is possible to construct survival curves as well as mutation dose-response curves for stamen hairs after mutagen treatments (Underbrink, et al, 1973; Sparrow, et al, 1974). Hence environmental stress expressed as both genetic and toxic effects can be measured with this system.

The stock plants are easily maintained by vegetative propagation and flower continuously throughout the year in controlled-environment growth chambers. The material treated consists of a group of unrooted, fresh cuttings containing young inflorescences each with 12 - 18 flower buds in a range of developmental stages (See figure 1 b,c). Following exposure to either chemical or physical mutagens, the cuttings are grown in aerated Hoagland's nutrient solution under standard conditions and the flowers are analyzed each day as they bloom for

approximately three weeks after treatment. Induced pink-event rates are expressed as the mean of the rates for several consecutive peak response days, usually days 11 to 15 for acute X rays and 7 to 12 for acute chemical exposures. Detailed descriptions of laboratory techniques for radiation and chemical exposures and calculating mutation rates are given elsewhere (Underbrink, et al, 1973; Sparrow, et al, 1974; Schairer, et al, 1978).

Radiobiology

Extensive use of Tradescantia in radiobiological studies has provided much information about somatic mutation and cell death produced by x, gamma, neutron and heavy ion radiations (Sparrow, et al, 1972; Underbrink, et al, 1973(a)). Many data exist on induced chromosome aberrations in both somatic and gametic tissues (Savage, 1975; Ma, 1978). Our studies have included investigations of increased rates of chromosomal aberrations and of somatic mutations (Marimuthu, et al, 1970). The classical linear, non-threshold, dose-response curves established for several endpoints in Tradescantia serve as models for chemical mutagen responses including predictions and extrapolation to very low dose effects (Figures 3,4). Recent work in our laboratory has demonstrated that the methods used to study the genetic or cytogenetic effects of physical mutagens can be applied directly to chemical mutagen studies (Underbrink, et al, 1973).

Chemical exposures under laboratory conditions.

The techniques developed for vapor phase chemical exposures

in the laboratory have been described in detail elsewhere (Sparrow, et al, 1974; Nauman, et al, 1979), so comments here will be limited to a few pertinent observations.

Exposures to a standard chemical mutagen, the alkylating agent 1,2-dibromoethane (DBE), in the gaseous state, showed that the number of mutational events increased linearly with the product of concentration and hours of exposure to DBE, at least over the range from 2 to 144 hours. These data may be expressed in terms of total dose by plotting induced mutation frequency against the product of concentration (ppm) and duration of exposure (hours) (Figure 5). For purposes of comparison, a standard curve for X-ray effect is shown in rads. Slope and shape of the curve for DBE induction of color change resemble those for radiation responses.

Of particular interest are the shapes of the dose response curves following DBE exposures of 6 and 144 hours (Figure 6). Data from 6-hour exposures show a significant decrease in slope in the low dose range. Although the curve for the 144-hour exposure shows the higher level of response or cumulative effect of chronic exposure, the data are consistent with the shallow, low dose slope shown for the 6-hour exposures. Differences in mechanisms responsible for mutation at high and low doses are unknown for chemicals but, as with radiation results (Figures 4,5,6), the response per rad or per ppm-hr is greater at low doses. Extrapolation from high to low levels of mutagen exposures would

lead to an underestimate of the hazard (Naumen, et al, 1977).

Although a large percentage of the effort of this group has been spent on the development of the mobile monitoring vehicle, a number of chemicals have been tested in the laboratory to validate the system as a monitor for gaseous mutagens. Chemicals such as the gasoline additives 1,2-dibromoethane (DBE) and trimethyl phosphate (TMP) were found to be potent mutagens while SO_2 , NO_2 , vinyl chloride, and freon-22 were weak mutagens according to this test system. These and other chemicals or air pollutants tested are listed in Table 1. The concentration listed is the lowest value tested that showed a significant mutagenic response.

Benzo (a) pyrene and other mutagens (numbers 24-34 in Table 1) were applied topically in solution in DMSO. The aqueous exposure results should be considered preliminary but are generally consistent with predicted mutagenic responses.

Ambient air exposures

Laboratory studies with both radiation and chemicals indicated that the stamen hair system was highly sensitive to radiation and gaseous chemicals (Sparrow, et al, 1974; Nauman, et al, 1976) and should respond to in situ exposures to industrial pollution at ambient levels. The criteria for monitoring air pollution for mutagenicity include: (1) a roadworthy vehicle to house the test organism during exposures; (2) exposure of the test organism under suitable culture conditions; (3) a constant flow of untempered ambient air; and (4) a semichronic exposure

capability simulating natural exposures of plants and animals. A detailed description of the design and operation of the mobile monitoring vehicle (MMV) has been given elsewhere (Schairer, et al., 1978). The trailer shown in Figure 7 was insulated and air conditioned to permit year-round operation of the laboratory. Three Model M-13 growth chambers (Environmental Growth Chambers, Chagrin Falls, Ohio) were installed. One of the chambers serves as a clean air control, the second is used for ambient air exposures. The chambers are designed to maintain any desired standard laboratory condition or to simulate fluctuations in the temperature and relative humidity of the ambient air outside. Ambient air is drawn into the fumigation chamber through a four-inch glass duct at continuous flow rates up to about 18 cubic feet per minute, a maximum of one air change every two minutes. An air filter train is used to scrub the air continually in the chamber serving as the concurrent control.

Field exposures were accomplished in the following manner: fresh cuttings of *Tradescantia* clone 4430 were made from stock plants grown in controlled environment chambers at Brookhaven National Laboratory; they were hand carried to the test site by car or airplane; cuttings were placed in the chambers in glass containers filled with Hoagland's nutrient solution (Figure 1b); and exposures were made for a ten-day period. At the end of the exposure the cuttings were taken back to Brookhaven National Laboratory for posttreatment analysis of the flowers as they

bloomed each day. Exposures of a few hours to several weeks could be made but ten days was chosen for the Tradescantia plants because it was long enough to maximize the sensitivity of the system and to simulate semichronic natural exposures yet short enough to permit sufficient flower analysis to be done back at Brookhaven. The peak mutation response period following a ten-day exposure is 11 to 17 days after the start of the exposure. The mean of the mutation rates for a 5- to 7- day scoring period resulted in an observed rate for a given test site based on an average stamen hair population between 300,000 and 400,000. A population of 300 cuttings in each ambient air and control chamber will yield enough data to resolve as small as a 10% increase in pink events over the background frequency.

The first field trials for the MMV were conducted in the summer of 1976 in Elizabeth, N.J. This site was characterized by high levels of automotive exhaust and petroleum refining effluents. Over the period of the next 2 1/2 years, twelve other sites were monitored (Figure 8 and Table 2). These sites were selected because of known high levels of human cancer incidence or the presence of high levels of suspect compounds in the atmosphere. One exception was the site at Grand Canyon, Az. It was deemed essential to conduct a "clean ambient air" exposure to verify the efficiency of the filter on the concurrent control chamber and to eliminate the possibility of an artifact generated in the fumigation chamber. Two exposures at Grand Canyon produced similar results with no significant difference in

background mutation frequency between the control and ambient air samples. Since the Grand Canyon test represented a good baseline exposure to clean air in situ and included all of the stress of shipping plant material, field handling, etc., this mutation frequency was established as the standard for comparison for all other field sites monitored. The results of mutagenicity monitoring at the remaining sites are shown in Figure 8 as the net induced mutation frequency following subtraction of the Grand Canyon rate. Clearly significant increases ^{in mutation frequency} at the flower color locus were observed at most industrial sites. The most consistent mutagenic response was that associated with processing of petroleum products (Table 2, lower). The induced mutation frequency ranged from 16.6% at Elizabeth in January to 90.6% in September. Some of this variation in response was undoubtedly due to seasonal change and varying wind direction; prevailing winds in the summer and fall are SW while winter winds are NW. The fixed location of the Elizabeth site placed petroleum refining operations directly upwind during the summer.

Results from other sites are shown in Table 2, upper). The increases in mutation rate observed in Houston, El Paso, Lake Charles and Upland are considered negative or of marginal significance. It is important to note that atmospheric monitoring at fixed sites is very dependent upon wind direction and speed. Although trailer locations were selected downwind from the desired source of pollution, a false negative may result

from unpredicted wind changes, extended periods of rain or other environmental factors. Adverse weather conditions were experienced at some of the "negative" sites.

Preliminary results of exposing potted plants and cuttings to diesel exhaust for 1 and 2 hours respectively suggest mutagenic activity. A two-fold increase in pots over cuttings does not imply a greater sensitivity in potted plants but presumably was due to differences in exposure techniques. In both exposures significant responses were obtained suggesting the suitability of this system for use in diesel exhaust studies, e.g. Fig.8 NYC bus terminal.

Summary and Conclusions

Final assessment of human health effects resulting either directly or indirectly from exposure to harmful environmental agents may rest with mammalian test system results. In vitro systems including cell and tissue cultures offer the most potential for extrapolation to humans. However the present consensus of opinion is that no single assay system is adequate; discrepancies have been observed between false negatives, false positives and metabolic activation of promutagens in established genetic systems such as Salmonella, Saccharomyces, Drosophila and Zea Mays. The more expensive long-term tests must be augmented by multiple assays designed for mere redundancy or to uniquely fill gaps in present state of the art of environmental monitoring. The Tradescantia stamen hair test system is one such assay which can offer redundancy as well as fill the gap of monitoring ambient air for mutagenic agents.

The flower color locus in heterozygous clones of Tradescantia has been shown to mutate when exposed to various categories of agents from fumigants (ethylene dibromide and trimethylphosphate), solvents (hexamethylphosphoramide and trichloroethylene), chemical additives or catalysts (vinyl chloride) and to compounds requiring activation such as benzo (a) pyrene. This laboratory validation of the stamen hair system supports its use as a short-term bioassay for chemical mutagens.

The most unique application of this plant system is its ability to respond to low levels of airborne compounds. Without the constraints of sterile culture and other demanding experimental requirements the Tradescantia plant is very adaptable to in situ monitoring at suspect industrial sites. Collection of data on the mutagenicity of ambient air pollution is an area of testing not amenable to mammalian or in vitro systems and hence an area in which Tradescantia can make a unique contribution.

Controlled laboratory exposures to x rays and the chemical mutagen 1,2-dibromoethane have given straightline dose response curves with no indication of a threshold at doses as low as 0.25 rad and 0.1 ppm respectively. The similarity of the shapes of these curves supports the use of the large amount of radiobiological data available for Tradescantia for extrapolation or predictive hypotheses for chemical mutagen responses. Both radiation and chemical dose response curves showed a reduction of

slope at low doses; a fact which indicates that extrapolation from high dose to low dose effects would result in an underestimate of the effect or risk assessment.

The high sensitivity and versitility of the Tradescantia stamen hair system were put to good use by employing the plant as an in situ monitor for mutagens in ambient air in polluted industrial sites. Twelve out of 18 exposures showed a highly significant increase in mutation rate ^{with one} approaching a background doubling. The environment most consistantly mutagenic was that downwind from petroleum refineries. No specific compounds or groups of compounds have as yet been correlated with the positive sites; studies involving the fractionation and analysis of complex mixtures are continuing.

Future Possibilities

As an established short-term bioassay, the stamen hair system can be used as one of a battery or tier of tests for chemical mutagen assessment. The most unique contribution will be in the area of atmospheric monitoring. A collaborative study with Research Triangle Institute, Inc., under contract with the USEPA, has shown good correlation between mutagenicity of airborne particulates (Ames/Salmonella) and vapors (Tradescantia) at the same industrial sites (Hughes et al, in press). Fractionation, quantitative analysis and bioassessment of environmental mixtures using the above and other organisms may identify specific hazardous compounds which can be tested by the

more rigorous mammalian systems. Fractination techniques have the obvious advantage of simplifying atmosphere clean-up and regulation procedures for specific hazardous compounds.

Greater use of basic radiobiological data should be encouraged. there is a very large background of radiation data describing dose response curve patterns (Nauman,et al, 1978), dose rate effects (Underbrink,et al, 1974; Nauman,et al, 1975), oxygen enhancement ratios in tissue (Underbrink, et al, 1975) and nuclear factors influencing sensitivity (Sparrow, et al, 1968). Dose response curve patterns have been shown to be similar in Tradescantia for both physical and chemical agents. Other phenomena established by radiation studies should be explored for chemicals. Predictive potential for effects of environmental chemical agents are of equal importance to earlier studies related to nuclear fallout and its impact on ecology in general and food chain in particular.

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References

1. Brittin, W. E. (Ed). 1972. "Air and Water Pollution." Colorado Associated University Press, Boulder, Colorado.
2. Bond, R. G. and C. P. Straub (Eds). 1973. " Handbook of Environmental Control." Vol. 1, "Air Pollution." CRC Press, Cleveland, Ohio.
3. Eisenbud, M. 1973. "Environmental Radioactivity." Academic Press, New York.
4. Epstein, S. S. and M. S. Legator (Eds). 1971. The Mutagenicity of Pesticides, Concepts and Evaluation." MIT Press, Cambridge, Mass.
5. Fishbein, L., W.G. Flamm and H.L. Falk. 1970. "Chemical Mutagens, Environmental Effects on Biological Systems." Academic Press, New York.
6. Flamm, W. G. 1974. A tier system approach to mutagen testing. Mutation Res. 26:329-333.
7. Flamm, W. G. and M A. Mehlman (Eds). 1978. "Advances in Modern Toxicology", Vol. 5, "Mutagenesis" Hemisphere Publishing Corporation, London.
8. Hindawi, I. J. 1970. "Air Pollution Injury to Vegetation." National Air Pollution Control Administration Publ. No. AP-71, U. S. Department Health, Education and Welfare, Washington, D. C.
9. Hughes, T. J., E. Pellizzari, L. Little, C. Sparacino and A. Kolber. 1980. Ambient air pollution: Collection, chemical characterization and mutagenicity testing. Reviews in Toxicology - Section of Mutation Res. (in press).
10. "Ionizing Radiation: Levels and Effects. " 1972. Vols.1 and 2. United Nations, New York.

11. Ma, T-H., A. H. Sparrow, L. A. Schairer and A. F. Nauman. 1978.
Effect of 1,2-dibromoethane (DBE) on meiotic chromosomes of
Tradescantia. Mutat. Res. 58:251-258.
12. Marimuthu, K. M., A. H. Sparrow and L. A. Schairer. 1970.
The cytological effects of spaceflight factors, vibration,
clinostat and radiation on root tip cells of Tradescantia.
Radiat. Res. 42:105-119.
13. Nauman, C. H., P. J. Klotz and L. A. Schairer. 1979. Uptake of
tritiated 1,2-dibromoethane by Tradescantia floral tissues:
Relation to induced mutation frequency in stamen hair cells.
Environmental and Experimental Botany 19:201-215.
14. Nauman, C. H., and A. H. Sparrow. 1978. Problems of extrapolation
from high dose to low dose in Tradescantia mutation studies.
Environ. Health Perspect. 22:161-162.
15. Nauman, C. H., A. H. Sparrow and L. A. Schairer. 1976. Comparative
effects of ionizing radiation and two gaseous chemical mutagens
on somatic mutation induction in one mutable and two non-mutable
clones of Tradescantia. Mutat. Res. 38:53-70.
16. Nauman, C. H., A. H. Sparrow, A. G. Underbrink and L. A. Schairer. 1977.
Low-dose mutation response relationships in Tradescantia;
principles and comparison to mutagenesis following low-dose
gaseous chemical mutagen exposure pp. 13-23. In: "Radiobiological
Protection, First European Symposium on Rad-equivalence."
Commission of the European Community, Luxemburg, EUR 5725e.
17. Nauman, C. H., A. G. Underbrink, and A. H. Sparrow. 1975. Influence
of radiation dose rate on somatic mutation induction in
Tradescantia stamen hairs. Radiat. Res. 62:79-96.

18. Nix, C. E. and B. Brewen. 1978. The role of Drosophila in chemical mutagenesis testing. EPA-600/9-78-027:pp.111-123.
19. Neel, J. V. 1974. Developments in monitoring human populations for mutation rates. Mutat. Res. 26:319-328.
20. Oser, B. L. 1971. Toxicology of pesticides to establish proof of safety pp 411-456. In: "Pesticides in the Environment." Vol. 1, Part II. R. White-Stevens (Ed), Marcel Dekker, New York.
21. Plewa, M. J. 1978. Activation of chemicals into mutagens by green plants: A preliminary discussion. Environmental Health Perspectives 27:45-50.
22. Savage, J. R. K. 1975. Dose-response curves for chromosome type aberrations in Tradescantia. I. Preliminary considerations. Radiation Botany 15:87-140.
23. Schairer, L. A., J. Van't Hof, C. G. Hayes, R. M. Burton and F. J. de Serres. 1978. Exploratory monitoring of air pollutants for mutagenicity activity with Tradescantia stamen hair system. Environmental Health Perspectives 27:51-60.
24. Sparrow, A. H., K. P. Baetke, D. L. Shaver and V. Pond. 1968. The relationship of mutation rate per roentgen to DNA content per chromosome and to interphase chromosome volume. Genetics 59:65-78.
25. Sparrow, A. H., L. A. Schairer and R. Villalobos-Pietrini. 1974. Comparison of somatic mutation rates induced in Tradescantia by chemical and physical mutagens. Mutat. Res. 26:265-276.
26. Sparrow A. H., A. G. Underbrink and H. H. Rossi. 1972. Mutations induced in Tradescantia by small doses of x rays and neutrons: analysis of dose-response curves. Science 176:916-918.

27. Thompson, M. Emmerling- and M. M. Nawrocky. 1979. Genetic studies of flower color in Tradescantia. J. Heredity 70:115-122.
28. Thompson, M. Emmerling- and M. M. Nawrocky. Unpublished data.
29. Underbrink, A. G., L. A. Schairer and A. H. Sparrow. 1973a. The biophysical properties of 3.9-GeV nitrogen ions. V. Determination of relative biological effectiveness for somatic mutations in Tradescantia. Radiat. Res. 55:437-446.
30. Underbrink, A. G., L. A. Schairer and A. H. Sparrow. 1973b. Tradescantia stamen hairs: a radiobiological test system applicable to chemical mutagenesis pp. 171-207. In: "Chemical Mutagens: Principles and Methods for Their Detection." Vol. 3, A. Hollaender (Ed), Plenum Press, New York.
31. Underbrink, A. G. and A. H. Sparrow. 1974. The influence of experimental end points, dose, dose rate, neutron energy, nitrogen ions, hypoxia, chromosome volume and ploidy level on RBE in Tradescantia stamen hairs and pollen pp.185-214. In: "Biological Effects of Neutron Irradiation," International Atomic Energy Agency, Vienna.
32. Underbrink, A. G., A. H. Sparrow, D. Sautkulis and R. E. Mills. 1975. Oxygen enhancement ratios (OERs) for somatic mutations in Tradescantia stamen hairs. Radiat. Bot. 15:161-168.
33. Vogel, F. and G. Röhrborn (Eds). 1970. "Chemical Mutagenesis in Mammals and Man." Springer-Verlag, New York.
34. Vogel, E. and F. H. Sobels. 1976. The function of Drosophila in genetic toxicity testing pp.93-142. In "Chemical Mutagens: Principles and Methods for Their Detection." Vol. 4. A. Hollaender (Ed). Plenum Press, New York.

35. Waters, M. D., S. Nesnow, J. L. Huisinoh, S. S. Sandhu and L Claxton (Eds). 1978. In: "Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures." Plenum Press, New York.
36. White-Stevens, R. (Ed). 1971. "Pesticides in the Environment." Vol. 1, Part II. Marcel Dekker, New York.

Table 1. A list of chemicals with which Tradescantia was treated, the conditions of the treatment and their mutagenic effect given in terms of the level of statistical significance (%).

No.	Chemical	Phase	Exposure Time (hr)	Minimum ^a conc	Maximum ^b conc for effect	Statistical Signific. %
1	Ozone	Vapor	6	5 ppm		2
2	Sulfur Dioxide	Vapor	6	40 ppm		1
3	Nitrogen Dioxide	Vapor	6	50 ppm		5
4	Nitrous Oxide	Vapor	6	250 ppm		1
5	Ethyl Methanesulfonate	Vapor	6	5 ppm		1
6	1,2-dibromoethane	Vapor	6	0.14 ppm		1
	"	"	144	0.14 ppm		1
7	Trimethylphosphate	Vapor	13	2 ppm		2
8	Trichloroethylene	Vapor	6	0.5 ppm		1
9	Vinyl Chloride	Vapor	6	75 ppm		2
10	Vinylidene Chloride	Vapor	6	—	1288 ppm	Insig.
		Vapor	24	22 ppm		5
11	Vinyl Bromide	Vapor	24	50 ppm		1
12	2-bromoethanol	Vapor	6	24 ppm		2
13	Dichlorodifluoromethane (Freon-12)	Vapor	6	—	392 ppm	Insig.
14	Chlorodifluoromethane (Freon-22)	Vapor	6	194 ppm		2
15	Hexamethylphosphoramide	Vapor	6	Saturated		1
16	Benzene	Vapor	6	4000 ppm		1
17	Caffeine	Liquid	Chronic	10 ⁻³ M		Insig.
18	Atrazine	Liquid	Chronic	0.045 gm/pot		Insig.
19	Sodium Azide	Liquid	3	10 ⁻⁴ M		1
20	1,1-dibromoethane	Vapor	6		250 ppm	Insig.
21	Dimethylamine Hydrochloride	Liquid	2	10 ⁻² M		10
22	Ethyl Alcohol	Vapor	24	1000 ppm		1
23	Hydrazoic Acid	Vapor	6	3.5 ppm		1
24	N-methyl-N-nitro-N-nitroso- guanidine	Liquid	1.5	10 ⁻³ M		1
25	Safrrole	Liquid	24	6.17x10 ⁻⁶ M		1
26	3,3 ¹ ,5,5 ¹ -tetramethyl benzidine	Liquid	24	4.16x10 ⁻⁶ M		1
27	Alpha-naphthylamine	Liquid	24	6.75x10 ⁻⁵ M		1
28	Beta-naphthylamine	Liquid	24	6.98x10 ⁻⁵ M		1
29	Benzidine	Liquid	24	5.43x10 ⁻⁷ M		1
30	Hexamethylphosphoramide	Liquid	24	5.58x10 ⁻⁵ M		1
31	Pyrene	Liquid	24	4.94x10 ⁻⁶ M		1
32	Diethylstilbestrol	Liquid	24	3.73x10 ⁻⁴ M		1
33	Benzo (a) pyrene	Liquid	24	3.96x10 ⁻⁵ M		1
34	N,N ¹ -ethylenethiourea (2-Imidazolidinethione)	Liquid	24	9.79x10 ⁻⁵ M		1

a - the lowest concentration at which an effect was observed

b - the highest concentration at which no effect was observed

Table 2.

**Mutagenicity of Ambient Air at Various Industrial Sites
as Measured by *Tradescantia* Stamen Hairs**

Mixed Industrials

Site Location	Date of Exposure	Pollution Source	Pollution-induced* Events/ 10^3 Hairs $\pm$ S.E.	Increase (% of Control)
Charleston, WV	Mar. 1977	Chemicals	1.70 ± 0.19	51.4
El Dorado, AR	May 1978	Brominated compounds	1.00 ± 0.20	30.2
	June 1978	Brominated compounds	0.47 ± 0.19	14.2
Lake Charles, LA	Feb. 1979	Industrial complex	0.49 ± 0.20	14.8
Houston, TX	June 1979	Industrial complex	0.14 ± 0.21	4.2
Birmingham, AL	Apr. 1977	Steel	1.54 ± 0.21	45.5
Magna, UT	Oct. 1977	Copper	0.88 ± 0.17	26.6
El Paso, TX	May 1978	Lead/Arsenic	0.16 ± 0.19	4.8
Upland, CA	Aug. 1977	Automotive & Photochemical	0.45 ± 0.18	13.6
	Sept. 1977	Automotive & Photochemical	0.88 ± 0.17	26.6

Petroleum Products

Elizabeth, NJ	July 1976	Petroleum & Automotive	1.85 ± 0.19	55.9
	Oct. 1976	Petroleum & Automotive	1.72 ± 0.21	51.9
	Jan. 1977	Petroleum & Automotive	0.55 ± 0.17	16.6
Baton Rouge, LA	May 1977	Petroleum	1.37 ± 0.21	41.4
Houston, TX	July 1977	Petroleum	1.39 ± 0.18	41.9
El Paso, TX	Apr. 1978	Petroleum	1.04 ± 0.18	31.4
Elizabeth, NJ	Sept. 1978	Petroleum & Automotive	3.00 ± 0.23	90.6
	Nov. 1978	Petroleum & Automotive	1.86 ± 0.22	56.2
Beaumont, TX	Apr. 1979	Petroleum	0.88 ± 0.18	26.6

*Grand Canyon, AZ	Nov. 1977	Clean Air Control	3.31 ± 0.14
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Figure Legends

Figure 1 (a). Normal stock plant of Tradescantia clone 4430 showing several mature inflorescences. (b) Floral cuttings are shown prepared for exposure. Sixty cuttings per dish are grown in aerated Hoagland's nutrient solution during and after treatment. (c) Somatic mutation at the blue color locus results in expression of the recessive pink allele in petal sectors; the large size of the sector shown indicates induction early in the development of the bud. (d) Enlargement of stamen hairs with pink mutant cells.

Figure 2 (a,b). Enlargement of stamen hairs showing several types and combinations of abnormal cells typical of those seen after exposure to radiation or chemical mutagens. Meaning of letter codes is indicated in the insert in (a). (c) Mutagen-induced pollen abortion in Tradescantia as indicated by unstained (cotton blue) or collapsed grains.

Figure 3. Neutron and x ray dose-response curves for pink mutant events in stamen hairs of Tradescantia clones 4430 and 02. Note slope change from plus 1 below 6 rads x rays to a 2-hit component resulting in 1.22 and 1.41 slopes at higher doses.

Figure 4. Neutron and x ray dose-response curves for colorless mutant events in stamen hairs of Tradescantia clones 4430 and 02.

Figure 5. DBE-induced mutation response plotted against total dose (ppm X hours of exposure) results in a linear response curve. The standard x-ray curve is shown for comparison.

Figure 6. Response curves for pink stamen hair mutations in Tradescantia clone 4430 show the results of 6- and 144-hour exposures to DBE. Slopes less than +1 were obtained at low doses. Open and closed symbols (6-hr) represent separate experiments.

Figure 7. View of the front of the Mobile Monitoring Vehicle (MMV) which shows remote mounting of the air conditioning and filter train for the three heat exchangers for the growth chambers. Lower right, 220 volt 100 amp electrical power cable.

Figure 8. The mutagenicity of ambient air as measured by Tradescantia in the MMV is summarized for fourteen test sites visited.

0 5 10 cm

(a)

(b)

(c)

(d)

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8