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Monitoring Ambient Air for Mutagenicity Using the Higher
Plant Tradescantia

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Introduction

The theme of the conference on "Genotoxic Effects of Airborn Agents" concerned the state of the art of bioassay systems from cultured microbes to laboratory animals and their application to the assessment of human health effects of airborne environmental contaminants. The major emphasis for short-term bioassays has been placed on bacterial and mammalian cell lines. However, 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 (1, 2, 3, 4, 5) This paper will deal exclusively with somatic mutation in the Tradescantia stamen hair, describing the system briefly, demonstrating its relevance to environmental mutagen assessment 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 airborne environmental pollutants. The sources of these chemical environmental contaminants include automotive and industrial combustion products (6) pesticides (7, 8) and commercially used chemical additives, solvents or catalysts (9, 10). In the past few years there has been an accelerating effort to detect and identify the most hazardous pollutants (11, 12, 13, 14, 15, 16). 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 1, 17, 5, 18) 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 1 c, d). The pink events are essentially nonlethal; 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 (19, 20). 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 2a 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 (1, 17). 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 unrooted, fresh cuttings each with a young inflorescence containing 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 (1) and the flowers are analyzed each day as they bloom for approximately two 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 methods for calculating mutation rates are given elsewhere (1, 17, 18, 21).

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 (22, 1). Many data exist on induced

chromosome aberrations in both somatic and gametic tissues (23, 24). The classical linear, non-threshold, dose-response curves established for several endpoints in Tradescantia serve as models for chemical mutagen responses; these include prediction 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 (1).

Chemical exposures under laboratory conditions

The techniques developed for vapor phase chemical exposures in the laboratory have been described in detail elsewhere (17, 25) so comments here will be limited to a few pertinent observations.

Young inflorescences of Tradescantia clone 4430 were exposed to gaseous 1, 2 - dibromoethane (DBE), an alkylating agent used as a standard chemical mutagen in our studies. The mutation frequency increased linearly with both increasing chemical concentration (0.1 to 100 ppm) and duration of exposure (2-144 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 injury.

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, 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, the response is greater at low doses (Figures 3, 4, 6). Extrapolation from high to low levels of mutagen exposures would lead to an underestimate of the hazard (26, 27).

Although a large percentage of the effort of this group has been spent on the development of a 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 in 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 statistically significant mutagenic response.

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

Chemical exposures under field conditions (ambient air)

Laboratory studies with chemicals indicated that the stamen hair system was highly sensitive to gaseous chemicals (17, 28) and that the system should respond to in situ exposures to industrial pollution at ambient levels. The criteria for monitoring air pollution for mutagenicity in the field include: (1) a roadworthy vehicle to house test organisms during exposure; (2) exposure

of the test organisms under suitable culture conditions; (3) a constant flow of untempered ambient air; and (4) a chronic 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 (5, 18). The mobile laboratory 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 and the second is used for ambient air exposures. The chambers are designed to maintain any desired standard laboratory condition or, if desired, to simulate fluctuations in the temperature and relative humidity of the ambient air outside. Ambient air is drawn into the exposure 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 are accomplished in the following manner: fresh cuttings of Tradescantia clone 4430 are made from stock plants grown in controlled environment chambers at Brookhaven National Laboratory; they are hand carried to the test site by car or airplane; cuttings are placed in the chambers in glass containers filled with Hoagland's nutrient solution (Figure 1b); and the cuttings are exposed continuously 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 bloom each day. Exposures of a few hours to several weeks could be made but a ten-day period was chosen for the Tradescantia plants because it was long enough to maximize the sensitivity of the system and to simulate chronic exposures in the workplace, yet short

enough to permit analysis of a sufficient number of flowers over the peak response period 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 results in an observed rate for a given test site based on an average stamen hair population between 200,000 and 300,000. 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.

Results of in situ exposures to ambient air

The first field trials for the MMV were conducted in the summer of 1976 in Elizabeth, N. J. Over the next four years a total of 17 additional sites throughout the United States were monitored in a preliminary study. This study had two objectives: (1) to demonstrate the adaptability of the stamen hair system to ambient air monitoring and (2) if mutagenicity were observed, to look for causative agents common to positive sites. (Fig. 8). 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. Two exceptions were the sites at Grand Canyon, AZ, and Pittsboro, NC. 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 ambient air 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., the weighted mean for four replicated ambient air samples ($3.35 \pm 0.09/10^3$ hairs) was determined and this mutation frequency was established as the standard for comparison for all other field sites monitored.* The results of mutagenicity monitoring at all 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 many industrial sites. The most consistent mutagenic response was that associated with processing of petroleum products. Mutation rate varied not only from site to site but also with repeated exposures at the same site. The induced mutation frequency ranged from 16.6% at Elizabeth, NJ in January to 90.6% in September. Some of this variation in response was undoubtedly due to seasonal change in effluent production 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. It is important to note that atmospheric monitoring at fixed sites is very dependent upon wind direction and speed. Although trailer locations were selected

*In January, 1980 a second clean air site was selected at Pittsboro, NC and the results were in good agreement with those of the Grand Canyon, AZ study.

downwind from the desired source of pollution, a false negative may result from unpredicted wind changes, extended periods of rain or other environmental factors.

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 mammalian cell and tissue cultures are short-term assays used most frequently 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 trichlorethylene), 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 complicated experimental procedures 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.

The high sensitivity and versatility 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. Preliminary results from many sites showed a significant increase in mutation rate with one (Elizabeth, NJ) approaching a background doubling. The environment most consistently 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 (29). 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. Fractionation 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 (27), dose rate effects (30, 31), oxygen enhancement ratios in tissue (32) and nuclear factors influencing sensitivity (33). 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 mutagen agents is of equal importance to earlier studies related to nuclear fallout and its impact on ecology in general but food chain in particular.

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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	Statistical Signific. for effect %
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	Safrole	Liquid	24	$6.17 \times 10^{-6}M$		1
26	3,3',5,5'-tetramethyl benzidine	Liquid	24	$4.16 \times 10^{-6}M$		1
27	Alpha-naphthylamine	Liquid	24	$6.75 \times 10^{-5}M$		1
28	Beta-naphthylamine	Liquid	24	$6.98 \times 10^{-5}M$		1
29	Benzidine	Liquid	24	$5.43 \times 10^{-7}M$		1
30	Hexamethylphosphoramide	Liquid	24	$5.58 \times 10^{-5}M$		1
31	Pyrene	Liquid	24	$4.94 \times 10^{-6}M$		1
32	Diethylstilbestrol	Liquid	24	$3.73 \times 10^{-4}M$		1
33	Benzo (a) pyrene	Liquid	24	$3.96 \times 10^{-5}M$		1
34	N,N'-ethylenethiourea (2-Imidazolidinethione)	Liquid	24	$9.79 \times 10^{-5}M$		1
35	2-nitrofluorene	Liquid	24	$4.7 \times 10^{-6}M$		2
36	Tetrachlorethylene	Vapor	6	1035 ppm		5
37	Dillate	Vapor	24	800 ppm		1

a - the lowest concentration at which an effect was observed

b - the highest concentration at which no effect was observed

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 indicated by dotted area, 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, indicated by dotted area.

Figure 2 (a) Enlargement of filament showing several stunted stamen hairs typical of those seen after exposure to radiation or chemical mutagens. (b) 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. [From Schairer et al. 1978 (5)]

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. [From Schairer 1981 (34)]

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. [From Schairer et al. 1978 (18)]

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

4-692-76

(a)

2-337-77

(b)

(c)

(d)

FIGURE 1

FIGURE 2

1-775-80

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6.

FIGURE 7

FIGURE 8