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PRELIMINARY STUDY OF EFFECTS OF MILITARY OBSCURANT SMOKES ON FLORA AND FAUNA DURING FIELD AND LABORATORY EXPOSURES

FINAL REPORT

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The Army routinely uses various types of obscurant smokes for training under simulated battle conditions. Since continued routine use of the smokes could be detrimental to the native flora and fauna of training sites, a preliminary biological and chemical study of obscurant smokes was conducted to determine whether tests could be developed to demonstrate measurable changes in organisms exposed to smokes and to evaluate whether short exposures to smokes produced measurable changes in the organisms tested. Fog oil, hexachloroethane, and tank diesel smokes were tested in the field and chemically analyzed at distances from the source ranging from 15 to 150 m. *Tradescantia* clones 4430 and 03, the native plant *Ambrosia dumosa*, and the native rodents *Perognathus formosus; Peromyocus crinitus; Dipodomys deserti; Dipodomys merriami;* and *Neotoma lepeda*, were exposed to the smokes for 30 minutes. In addition, *Tradescantia* clone 4430 was exposed to tank diesel in the laboratory at concentration levels equivalent to exposure at 15 m or 50 m. (cont'd)

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Tradescantia clones were examined for mutagenic effects indicated by micronuclei induction in developing pollen and pink somatic mutations in stamen hairs. Photosynthetic perturbations were measured in *Tradescantia* and *Ambrosia dumosa* using variable fluorescence induction. Animals were examined for sister chromatid exchanges and chromosome aberrations. It was found that all of the smokes tested exerted varying degrees of physiological and mutagenic effects in one or several of the assay systems at one or more of the exposure distances. These studies

The studies reported here indicate that exposed ecological systems, or at least components of these systems, are at a higher risk than are control organisms for several types of damage attributed to obscurant smoke exposure. The tests developed were deemed adequate for indicating changes in the specimens caused by the smokes.

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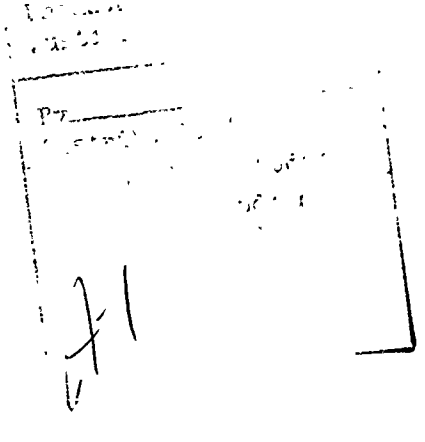

EXECUTIVE SUMMARY

The Army routinely uses various types of obscurant smokes for training under simulated battle conditions. Since continued routine use of the smokes could be detrimental to the native flora and fauna of training sites, a preliminary biological and chemical study of obscurant smokes was conducted to determine whether tests could be developed to demonstrate measurable changes in organisms exposed to smokes and to evaluate whether short exposures to smokes produced measurable changes in the organisms tested. Fog oil, hexachloroethane, and tank diesel smokes were tested in the field and chemically analyzed at distances from the source ranging from 15 to 150 m. *Tradescantia* clones 4430 and 03, the native plant *Ambrosia dumosa*, and the native rodents *Perognathus formosus*, *Peromyocus crinitus*, *Dipodomys deserti*, *Dipodomys merriami*, and *Neotoma lepeda* were exposed to the smokes for 30 minutes. In addition, *Tradescantia* clone 4430 was exposed to tank diesel in the laboratory at concentration levels equivalent to exposure at 15 m or 50 m.

Tradescantia clones were examined for mutagenic effects indicated by micronuclei induction in developing pollen and pink somatic mutations in stamen hairs. Photosynthetic perturbations were measured in *Tradescantia* and *Ambrosia dumosa* using variable fluorescence induction. Animals were examined for sister chromatid exchanges and chromosome aberrations. It was found that all of the smokes tested exerted varying degrees of physiological and mutagenic effects in one or several of the assay systems at one or more of the exposure distances. All of the smokes field-tested exerted varying degrees of lethal, physiological, and mutagenic effects in one or several of the assay systems at one or more of the exposure distances. In most cases, the high variability of the assays made it impossible to demonstrate an exposure (distance) dependence, although tank diesel (TD) smoke makes the dependence evident in *Tradescantia* clone 4430 micronuclei (MCN). These results suggest that the plants and animals exposed to smokes at Fort Irwin are at a toxicologically higher risk for several types of damage than control organisms. Direct effects found include decreased fertility, changes in energy production, and decreased survivability in both plants and animals, increased genotoxic damage in plants, and increased genotoxic damage in animals. If these effects are extensive in a species at Fort Irwin, they may be manifested as reductions in the target population, or they may propagate and affect ecosystem properties such as stability, resilience, and resistance. However, this study was not able to assess ecological significance from the effects reported here for individual organisms.

The laboratory studies with *Tradescantia* clone 4430 suggest that the revised MCN method offers a useful measure of response. Pollen abortion (PA), variable fluorescence (VF), and electron pool measurements also appear to be useful endpoints. The failure of MCN and PA to show exposure-related responses may result from a binary response mechanism (mutagenic or nonmutagenic), rather than an exposure-dependent one. Although the stamen hair pink mutation test has been used successfully for low-dose, low-dose-rate radiation exposures, it does not appear to have sufficient sensitivity for chemical studies.

A significant conclusion from this work is that genetic studies can be conducted in the field using native small mammals.

To deduce the general pattern of effects when several components act simultaneously, *Tradescantia* should be exposed to single substances and known compositions

of their mixtures. Effects on the same plant for each of the systems reported here should be obtained during these studies.

The total acreage available for training is finite, so land quality must be preserved indefinitely. Ecological systems subjected to chronically administered acute chemical insults (e.g., smokes) may take years or decades to exhibit manifestly obvious symptoms of toxic stress. However, by the time these symptoms are observable, the system may be damaged beyond repair. Thus, the use of biomonitors for long-term monitoring of active and reclaimed sites is more significant to Army programs than monitoring specific exposure events. The utility of *Tradescantia* for such monitoring is of interest. Such studies should examine the response of the various *Tradescantia* systems from whole plants grown in contaminated soils.

Some types of measurements of *Tradescantia*, such as VF, appear to apply to other plants. A systematic study of several plants native to each of the various biomes found in Army training areas is needed to develop short- and long-term in-situ biomonitors.

The work reported here has shown that it is possible to obtain usable cell cultures from native rodents. This work should be extended, and the requirements and procedures for consistently producing usable cell cultures from native species determined. Studies should also be done to determine whether there are significant changes in sister chromatid exchanges, chromosome abberations, or other measures of cytogenetic damage in native species exposed to smokes and obscurants.

PREFACE

This investigation was performed for the U. S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) under Intra-Army Order No. 83II3 U12, dated March 1983. The work was performed by the Environmental Division (EN), U.S. Army Construction Engineering Research Laboratory (USA-CERL). The USAMBRDL Technical Monitor was MAJ David Parmer.

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I. INTRODUCTION

BACKGROUND

Air pollutants affect biological components of terrestrial, aquatic, and groundwater ecological systems. Aside from limited field trials based on laboratory ecosystem models, there has been little opportunity to study the effects of large areal pollutant sources under controllable conditions. The Army trains with large amounts of large-area obscurant smokes on many training sites nationwide. Obscurants used in the largest quantities are (1) fog oil (FO), (2) tank diesel (TD), and (3) hexachloroethane (HC)-based smokes.

Two forms of evidence indicate that smokes could cause significant toxicological damage to ecological systems. First, some compounds in smokes are mutagenic or carcinogenic in standard assay systems. Other components and mixtures are neurological and renal toxins or produce other types of toxicological damage. Second, compounds similar to those contained in commonly used smokes (aromatic hydrocarbons and heavy metals) cause observed damage in ecosystems.

Native flora and fauna are chronically exposed to Army training smokes. Although the smokes are known to contain toxic compounds, no field studies have been made of the separate or combined biological/environmental effects of these smokes and smoke byproducts. Thus, quantitative data for readily measured effects in plants and animals are needed to identify the ecological system effects of smokes.

OBJECTIVE

The objectives of this study were to (1) determine whether tests could be developed that would demonstrate measurable changes in organisms exposed to smokes and obscurants in the field and (2) determine whether short, acute exposures to smokes and obscurants would produce measurable changes in the organisms tested. The results from the study would provide a basis for designing appropriate followup field and laboratory studies.

APPROACH

Field studies were designed and implemented at the National Training Center at Fort Irwin, CA. Studies were made of the effects of smokes on *Tradescantia* clone 03 (*Tradescantia paludosa* Anderson & Wood) and clone 4430 (*Tradescantia hirsutiflora* Bush X *Tradescantia subacaulis* Bush), plants used in laboratory and field bioassay studies, and a native plant species *Ambrosia* [*Franseria*] *dumosa* (Gray) W. Payne (Burrowbush). Genotoxic responses of *Tradescantia* exposed to TD, HC, FO, and HC + FO obscurant smokes were examined. Changes in the variable fluorescence of *Tradescantia* and *Ambrosia dumosa* exposed to these smokes were determined. Selected results from *Tradescantia* 4430 were later confirmed in a laboratory study with TD smoke.

Live-trapped small mammals (*Perognathus formosus, Peromyocus crinitus, Dipodomys deserti, Dipodomys merriami,* and *Neotoma lepeda*) (all rodents) were exposed

in the field to various smokes. Bone marrow cells of selected rodent species were examined for karyclogical irregularities.

Tradescantia was selected for field use because it was one of the few plant assay systems that had been extensively field-tested with air contaminants. To develop a basis for linking results in *Tradescantia* with possible ecological system effects, several parallel studies were conducted in the field using *Ambrosia dumosa*. From the same ecological perspective, chromosome spreads were taken from *Dipodomys* spp. live-trapped from a control area several miles away at Goldstone Arsenal and then actually exposed at the Fort Irwin training area. These results were then compared with chromosome spreads from *Dipodomys* spp. live-trapped in the training area at Fort Irwin. It was hypothesized that there would be statistically significant responses, with magnitudes varying with exposure.

Following collection of the data, the results were analyzed to determine the effects of the smokes on the specimens and to ascertain the effectiveness of the tests.

SCOPE

This study was designed to determine whether the same qualitative types of effects occurred in several species, but not to quantify effects on native species chronically exposed to smokes nor to demonstrate whether effects on native species were ecologically significant. Such a demonstration would require extensive data collection and analyses of ecosystem function (stability, connectance, etc.) and performance.

Bridging the gap between field and laboratory experimentation for research such as this study describes presents problems in experimental design, variability, sample size, repeatability, and data analysis and interpretation. However, achieving the goal of measuring the effects of human activity on the environment involves an initial attempt and the expectation of a certain amount of error. In this study, researchers learned a great deal more from the unforeseen problems that occurred throughout the testing than from actual data collected. Lessons learned from these pioneering studies indicate that higher-quality followup studies should be performed. However, it is still too early in this type of research to expect clean exposure/response and repeatability.

MODE OF TECHNOLOGY TRANSFER

It is anticipated that the results of this preliminary study will impact the methods used to determine the effects of chemicals on Army training areas, and on training area management, preservation, and restoration. The initial technology transfer will be through a technical report.

The findings and recommendations will lead to further research in biomonitoring technology, including: (1) a workshop to identify criteria for selecting biomonitoring test systems, (2) selection of biomonitoring test systems, (3) field studies with additional test systems, and (4) field and laboratory studies in laboratory clones and native varieties of *Tradescantia*. (Several populations of wild varieties of *Tradescantia* are found across the nation and exist on an estimated 80 percent of Army installations across the United States.)

II. PROCEDURE

FIELD TRIALS

Experimental Design for Chemical Field Studies

The sampling system collected particulates and vapors for chemical analysis. A collection approach similar to the U. S. Environmental Protection Agency's (USEPA) procedure for smoke stack sampling was used. Particulates were collected on 0.45- to 0.22-μm cellulose acetate or glass fiber filter traps for inorganic or organic analyses, respectively. Charcoal and silica gel adsorption tubes were used to sample organic vapors and hydrochloric acid. The adsorption tubes consisted of 15-cm x 4-mm inside diameter (i. d.) borosilicate glass tubes filled with two portions of adsorbent material (300 mg and 150 mg of 20/40 mesh JXC charcoal, or 20/40 mesh acid-washed silica gel grade 62 or GC 20/35 Tenax). The front portion was 8 cm long, and the back portion was 4 cm long; the two parts were separated with a small glass wool plug. Such an arrangement is routinely used to determine the breakthrough of air pollutants from the adsorption tubes. Various components of the sampling train, which included impingers for aerosol sampling, were interconnected by short pieces (about 4 cm) of clean polyethylene tubing. The sampling train was connected to a portable air-sampling pump (Fixt-Flo Model 1, Mine Safety Appliances Corp., Pittsburgh, PA) operating at about 1.5 L/min. The volume of air sampled was measured with a calibrated dry gas meter.

Workers wearing protective suits and gas masks were stationed in a circumferential pattern at 15 m, 25 m, 50 m, 100 m, and 150 m in front of a smoke generator, and held the sampling tubes in the smoke plume. One worker and tube were deployed at each distance. Analytical procedures used are described in the section Analytical Methods for Field and Laboratory Studies.

Field Exposures of *Tradescantia* and *Ambrosia dumosa*

Tradescantia plants were cultivated specifically for this study in a greenhouse in Columbia, MO. Inflorescence-bearing cuttings (flower stalks) were collected from all healthy plants and transported in open ice chests, 50 per glass jar (no statistical randomization), in Hoagland's solution; moist absorbent paper was placed over the cuttings. They arrived at the test site about 24 hr after collection.

The cuttings were removed from the ice chests at the test site and maintained by placing aerators in each jar; illumination under fluorescent fixtures was provided for 18 hr/day for 1 to 4 days. On test days, one jar of cuttings was selected arbitrarily for each clone. These cuttings were established as controls by placing them outside, several miles east of the testing site area. This site was chosen to prevent shifting winds from exposing control plants to test smokes.

An experimental design had originally been developed that would have exposed *Tradescantia* clones 03 and 4430 to smokes at 15, 25, 50, 100, and 150 m from the smoke source. The clones would have been exposed concurrently at various distances from the smoke. To provide a basis for comparing changes in photosynthesis in exposed *Tradescantia* with that of plants naturally present in the environment, changes in the photosynthesis of *Ambrosia dumosa* concurrently exposed to smokes were evaluated. However, unexpected plant mortality and the opportunity to test additional smoke

formulations required reevaluating the use of a limited stock of *Tradescantia* and revising the original design. Replicate trials were therefore replaced by limited trials of additional types of smokes. To maximize use of available stocks, both clones were often used in a given trial but at different distances. Branches from a single large native specimen of *Ambrosia dumosa* were collected and tagged immediately before exposure. Branches from the same bush were used in all experiments on a given day. Cuttings were exposed at the control and at 15, 25, 50, 100, and 150 m. Studies of the within-species homogeneity of *Ambrosia dumosa* were outside the scope of this study.

For a given trial (i.e., smoke/exposure scenario/day), jars of 50 cuttings each of *Tradescantia* clone 4430 or 03 in Hoagland's solution were selected arbitrarily from the available stock for both control and exposed groups. One jar was used at each distance, including the control, for the selected plant(s). To maintain exposure to the shifting smoke plume, the containers were placed in baskets carried by suitably protected personnel. However, exposures within some of the plumes were intermittent at times, and the density of smoke sometimes varied. Each exposure lasted 30 minutes. Table 1 gives details of the exposures.

Field Exposures of Native Rodents

To determine the effects of the smokes on animals, several species were live-trapped from a control area at Goldstone Arsenal for use as controls and in single acute exposures. More animals that presumably had been chronically exposed were collected from a smoke-impact site at Fort Irwin. Individually caged animals from the control site were arbitrarily selected for exposure at 15 m to a given smoke (FO, HC, TD, or HC + FO). Bone marrow cells from 25 animals (field-identified as: 18 *Perognathus formosus*, three *Peromyscus crinitus*, three *Dipodomys deserti*, and one *Neotoma lepeda*) were cultured onsite. Twenty-four other exposed and/or control animals (three *Perognathus formosus*, seven *D. deserti*, and 14 *D. merriami*) were transported to the University of Missouri-Columbia for additional studies of culturing methods. One control from each of *P. formosus*, *P. crinitus*, and *D. merriami*, one presumed chronically exposed *D. deserti*, and one fog-oil-exposed *P. formosus* died in the field. Two *D. merriami* (one control and one HC-exposed) died within 24 hr after arriving at the laboratory.

Results from cell-culturing studies of *D. merriami* were the most successful, so this species was selected for further study. This species was very abundant and generally provided an adequate number of cells. Only small numbers of *Perognathus formosus* were captured and due to the small size of *Perognathus formosus*, sufficient numbers of cells were not always available. Although *D. deserti* was one of the largest animals captured and yielded excellent cultures, their low trapping numbers were insufficient for adequate statistical analysis. During a second field trip, 27 unexposed *D. merriami* were collected from a control area at Goldstone Arsenal. Since the home range of this species is a few hundred meters, the three *D. merriami* trapped at the test site were likely to have been chronically exposed to smokes. (D. merriami may travel 500 to 1000 m, but their home ranges are probably much smaller than 2 to 4 ha in the Mojave. The home range of *D. merriami* in New Mexico is 1.6 ha.) Eight of these 30 animals were exposed to smokes/mixtures that are not considered in this report. Three more either died or failed to give usable chromosome spreads.

TABLE 1. MEASUREMENTS IN FIELD-EXPOSED PLANTS AT 15 TO 150 M

Distance (m)	*Tradescantia* 4430					*Tradescantia* 03			*Ambrosia dumosa*	
	MCN	SH	VF	EP	FP	MCN	VF	EP	VF	EP
FO										
Control	3.07	0.90	26.4	10.1	100	2.28	28.6	17.1	43.5	28.8
15	13.2	2.5	38.7	14.3	19.0					
25	NS	2.7	40.4	NS	48.1				NS	NS
50						NS	34.4	21.2	35.1	21.2
100						NS	NS	NS	37.5	NS
150						NS	34.5	NS	35.6	23.9
HC										
Control	3.07	0.90	26.4	10.1	100	2.28	28.6	17.1	43.5	28.8
15						NS	35.8	NS		
25						NS	NS	NS	39.8	17.2
50	12.7	NS	38.4	16.5	31.4				NS	21.5
100						NS	34.3	NS	NS	NS
150						NS	NS	NS	40.2	23.3
HC + FO										
Control	3.07	0.90	26.4	10.1	100	2.28	28.6	17.1	43.5	28.8
15	30.1	1.8	39.2	NS	37.4					
25	40.1	1.6	38.5	13.3	47.8				39.0	17.9
50						4.0	NS	NS	37.8	18.0
100						3.8	NS	NS	NS	20.8
150						NS	NS	NS	NS	19.7
TD										
Control	3.07	0.90	26.8	6.8	100	2.28	25.1	10.5	33.3	16.6
15	40.2	3.6	33.2	10.4	NS				NS	NS
25	29.8	3.6	33.2	9.6	126.9				NS	NS
50	NS	2.7	34.1	10.2	68.4				NS	NS
100						NS	30.0	14.2	NS	13.7

MCN = micronuclei per 100 tetrads.
SH = pink events per 1000 hairs.
VF = variable fluorescence--a dimensionless ratio of slope (mm):total (mm).
EP = electron pool--the area bounded by the two intersecting lines given by the total slope and a horizontal line at the maximum inflection of the graph, in arbitrary stripchart units.
FP = flower production--the number of flowers per cutting in exposed plants as a percentage of flowers per cutting in control plants.
NS = not statistically significant at $p \geq 0.90$.

LABORATORY STUDIES WITH TANK DIESEL SMOKE

Generation of Diesel Smoke

A test atmosphere generation system[2] was used to generate known concentrations of tank diesel smoke under controlled conditions for chemical characterization and the biological exposure studies. The system, which had been used to generate known concentrations of gaseous and liquid substances, was modified to generate aerosols. The primary dilution module of the system was replaced with a constant-flow-rate reciprocating piston pump (Milton Roy Co. mini pump). Diesel fuel was sprayed into a heated stainless steel tube placed in a stainless steel block maintained at a predetermined temperature (400, 475, 500, or 595°C, within ± 5°C) by an electronic temperature controller. Preheated clean air was introduced into the manifold at a controlled flowrate of 10 L/min. Provision was made to dilute the generated smoke by mixing clean air downstream. Condensed aerosols were trapped in the dilution chamber prior to chemical sampling and introduction into the biological exposure chamber.

Sampling of Diesel Smoke During Laboratory Exposures

Before the actual exposure studies, the smoke generation, sampling, and analytical methodologies were validated with model compounds selected from those detected in diesel smoke produced in the field study. To ensure a constant generation of diesel smoke constituents during an exposure study, samples were taken periodically from the diesel smoke screen and analyzed by gas chromatography, and concentrations of major aliphatic hydrocarbon constituents monitored. Diesel smoke aerosols and vapors from the biological exposure chambers were sampled with a sampling train consisting of a glass fiber filter, Tenax adsorbent tube (6 cm x 0.4 cm i.d.) and a microprocessor-controlled, constant rate pump. The volume of air sampled was measured by a calibrated dry gas meter.

Laboratory Exposures of *Tradescantia*

Tradescantia clone 4430 was exposed to tank diesel smoke in the laboratory to determine: (1) the effects of smoke generation temperature on the smoke's chemistry, (2) the effects of smoke generation temperature on the biological responses exhibited by *Tradescantia*, (3) the reproducibility of the chemical composition of, and biological effects produced by, smokes generated at a given temperature, (4) additional biological endpoints not considered in the Fort Irwin study. Another purpose was to confirm or deny observations made on *Tradescantia* in the field study of tank diesel.

Laboratory studies examined several genotoxic endpoints: induction of micronuclei (MCN); induction of pink mutations in stamen hairs (SH); and pollen abortion (PA). Also studied were the effects on the electron transport processes of photosynthesis (electron pool [EP] and variable fluorescence [VF]) in *Tradescantia* clone 4430. These endpoints, and the methods used to measure them, are described subsequently.

The chamber, constructed of lucite or glass, was equipped with external fluorescent lighting and maintained at room temperature. Tank diesel smoke was generated at 400, 475, 500, and 595°C and cooled to room temperature before introduction into the exposure chamber. Exposure concentrations were the same (by chemical analysis) as the field concentrations measured at 15 or 50 m. After equilibrium of the smoke concentration was attained, fresh unrooted flower stalks and arbitrarily selected

attached leaves of *Tradescantia* clone 4430, maintained in groups of 50 in beakers in half-strength Hoagland's nutrient solution, were introduced into the exposure chamber. The equilibrium in the exposure chamber was disturbed as little as possible while the plants were introduced. Exposures lasted 30 minutes to 4 hours. Stem packing density allowed very little surface area of the nutrient solution to be exposed to the generated smokes. Table 2 gives details of the exposures (temperature, time).

Following exposure, the lower portions of the cuttings were removed, rinsed, put into fresh beakers containing half-strength Hoagland's solution, and placed in a growth chamber. Perturbations of the electron transport system during photosynthesis were measured on arbitrarily selected leaves taken immediately after exposure and then dark-adapted for 30 minutes.

ANALYTICAL METHODS FOR FIELD AND LABORATORY STUDIES

Chemical Analysis of Smokes

The USEPA master analytical scheme,[3] as reported by Vogt, et al.,[4] was used to chemically analyze the smokes. The glass fiber filters used for particulates were extracted in microsoxhlet extractors with 25 mL of methylene chloride for 12 hr. Extracts were concentrated to 2 mL under a gentle stream of prepurified nitrogen; they were then separated into aromatic and aliphatic fractions by extraction with dimethyl sulfoxide and subsequent back extraction with benzene. As a control for the extraction procedure, filters were spiked with known amounts of naphthalene D-8 and anthracene D-10 immediately after sampling.

Extracts were screened by high-pressure liquid chromatography using gradient elution (40 to 100 percent acetonitrile/water) and a column containing octadecyl siloxane bonded to 5-μm silica gel particles. The extracts were also characterized using a gas chromatograph/mass spectrometer. The gas chromatograph was equipped with a 30-m x 0.25-mm (i. d.) fused silica capillary column DB-5 (J & H Scientific, Inc.). Helium was used as the carrier gas (30 cm/sec). The inlet temperature was maintained at 256°C, and the column oven temperature was programmed from 60 to 260°C at 5°C per min. The mass spectrometer was operated in electron impact mode, using an ionization energy of 60 eV and a scan rate of 1 sec/scan from 45 to 400 amu. The system was tuned and calibrated daily to meet the spectral specifications for decafluorotriphenyl phosphine. Calibration standards for polynuclear aromatics and aliphatic hydrocarbons were run once every 8 hr during experimentation.

All the major aliphatic and aromatic components of the original tank diesel and the condensates collected in the trap at different temperatures were identified and quantitated. Quantitation was performed by normalizing the concentrations of the major components against the analytical concentrations of the perdeuterated naphthalene and anthracene spiked onto the filters. Laboratory and field exposures were correlated by comparing the normalized concentrations of hexadecane and octadecane.

Volatile organics were collected on Tenax resin and heat desorbed at 200°C using nitrogen at a gasflow rate of 40 mL/min. Analysis was by a gas chromatograph equipped with a flame ionization detector and a 150-cm x 0.25-mm (i. d.) borosilicate glass tube packed with 1 percent SP-1000 on Carbopak B-60/80 mesh. The inlet temperature was

TABLE 2. CHROMOSOME ABERRATIONS IN FIELD-EXPOSED *D. MERRIAMI*

Exposure	Animal I. D.	CA/Cell	No. Metaphases	z	p
Controls	40	0.016	244		
	33	0	8		
	25	0.053	456		
	11	0.017	237		
	All	0.034	945		
FO	1	0.015	198		
	41	0.018	166		
	30	0.037	164		
	All	0.023	528	-1.24	0.185
HC + FO	19	0.018	54		
	42	0.018	276		
	43	0.017	951		
	All	0.017	1281	-2.31	0.028
Chronic	22	0.031	129		
	44	0.016	1696		
	All	0.018	1825	-2.40	0.022
All exposed		0.018	3634	-2.48	0.018

maintained at 200°C, column oven temperature was programmed from 80 to 200°C, and the detector temperature was kept at 240°C.

Analysis of Plants for Various Effects

Experimental methods for analyzing MCN,[5-7] SH,[8] FP, and PA[9] have been published for *Tradescantia*. Published methods for EP and VF (Kautsky effect) measured in leaf tissue[10-12] were applied to *Tradescantia* and *Ambrosia dumosa*.

Micronuclei (MCN)

The MCN method was devised by Ma, et al.,[5-7] using clone 03. Twenty-six hours after exposure, inflorescences from both clones 4430 and 03 were fixed for MCN determination in 3:1 alcohol:acetic acid for 24 hours, followed by two changes in 70 percent ethanol. MCN were determined microscopically under 400x high, dry magnification.

Because of unexpected variations found in the scoring of MCN during the laboratory exposures of clone 4430, each procedural step involved in the MCN technique developed for *Tradescantia* clone 03 was evaluated along with clone 4430. Plants of clone 4430 were irradiated with 28.4 rads of gamma rays. Scoring of a portion of the irradiated material (Prof. T. Ma, Western Illinois University) revealed that there was difficulty in visually recognizing the correct tetrad stages suitable for scoring. However, the proper stage was found by heating the stained slide on a hot plate at 80°C twice (5 sec each) and then gently pressing on the cover slip with the palm of the hand. If the four cells comprising the tetrad fell apart, they were not in the correct stage (i. e., they came from a population of cells that was too developed and, in effect, not at risk at the time of exposure). These results led to the development of correction factors for MCN counts for control and treated plants described in the following section.

Statistical Correction Factor for MCN Counts From Laboratory Study

Correction factors for laboratory plants exposed to TD were developed using a confidence interval approach. The mean and one standard error on the mean of irradiated cells scored by the old method (without the 5-sec heating steps) were 3.59 $\pm$ 0.45 (3.14, 4.04)* MCN (N = 6) for controls and 5.59 $\pm$ 2.37 (3.22, 7.96) MCN (N = 5) for exposed. The mean and one standard error on the mean of irradiated cells scored by the new method were 2.48 $\pm$ 0.35 (2.13, 2.83) MCN (N = 13) for controls. Two experimenters scored the exposed group using the new method. The results were 22.52 $\pm$ 5.82 MCN (N = 8) and 24.20 $\pm$ 3.96 MCN (N = 6). The confidence interval for the exposed cells scored by the new method is obtained by pooling these two results.

The pooled standard error is obtained as:

$S_1^2 = 5.822^2 (8) = 270.9792$

$S_2^2 = 3.962^2 (6) = 94.0896$

*Parenthetical numbers indicate one standard error upper and lower boundaries on the mean.

$$S_W^2 = [(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2] / (N_1 + N_2 - 2)$$
$$= [(8 - 1)(270.9792) + (6 - 1)(94.0896)] / (8 + 6 - 2) = 197.2752$$

$$SE_{pool} = S_W/(N_1 + N_2)^{1/2} = 14.04569/(14)^{1/2} = 3.75.$$

The pooled mean is obtained as:

$$X_W = [8(22.52) + 6(24.20)]/14 = 23.24.$$

Hence, the new interval for the exposed group is $23.24 \pm 3.75 = (19.49, 26.99)$.

Correction factors for MCN counts are obtained from the intervals as:

Treated: (26.99/7.96, 19.49/3.22) = (3.39, 6.05); mean = 4.72.
Control: (2.13/3.14, 2.83/4.04) = (0.678, 0.700); mean = 0.689.

In the subsequent statistical analysis, these mean correction factors were applied to MCN counts from laboratory-exposed plants. Because more than 1 year had elapsed since the field study, it was deemed inappropriate to apply these corrections to field-exposed plants.

Statistical Method Used To Determine Significance of Plant Responses

The choice of a statistical test to determine the significance of responses must be based on (1) the hypothesis being tested, (2) the nature or properties of the data, and (3) the robustness of the test statistic to departures from strict adherence to the statistical assumptions. Generally, treatment groups for this study were compared with controls using a z-test based on a pooled variance. The z-test is sufficiently robust when sample sizes are large (N>30). For most of the endpoints considered here, sample sizes are statistically large e. g., thousands of stamen hairs examined for pink mutants in *Tradescantia*). For most of the plant studies, a single observation is actually a mean value of several days' observation. The primary concern is differences in response between each exposed group in an experiment and the response of the control group. Correction for multiple comparisons e. g., of each exposure group with the control[13-16], which is necessary when the simultaneous confidence interval is of concern (the usual circumstance), is not needed here. This is because interest is only in whether individual comparisons are significant at a predetermined level (here, $p \geq 0.9$). In this regard, Williams[14] has stated that where the experimenter's objective is simply to determine whether there has been a (significant) response based "solely on comparison between each dose mean and the control mean," either t tests or multiple comparison tests can be used "according to his taste in error rates."

The z-test is:

$$z = (TREAT - CNTL)/SE_{pool}$$

where:

CNTL = response in control group

TREAT = response in treatment group.

SE_{pool} is obtained as:

Pooled variance = VARPOOL =

$$[(N_{cntl}-1)(N_{cntl})SE_{cntl}^2+(N_{treat}-1)(N_{treat})SE_{treat}^2]/[N_{cntl}+N_{treat}-2]$$

SE_{pool} = pooled SE = $[VAR_{pool}/(N_{cntl} + N_{treat})]^{1/2}$

where:

SE_{cntl} = standard error, controls

SE_{treat} = standard error, treatment

N_{cntl} = number in control group

N_{treat} = number in treatment group.

Stamen Hair Pink Mutations (SH)

Stamen hair mutation frequencies increase to a maximum several days after mutagen exposure, and then decrease. Kudrirka and Van't Hof have discussed a rationale for time differences between the appearance of maximum mutation frequencies in clone 4430.[17] After remaining in the G1 growth stage for up to several days, the cells in G1 then shift into the G2 stage of the cell cycle before gene expression in terminally differentiating systems such as SH. The shift from G1 to G2 does not require cell division, but it does require DNA replication. This shift makes the cells more vulnerable to mutagens that are more effective during DNA replication. Other mutagens such as ionizing radiation, which breaks chromosomes, require a mitosis to segregate the fragments. Thus, one might predict that mutation frequencies produced by certain chemical mutagens found in smokes would be seen in older buds (appearing early in the scoring period) that are undergoing the shift from G1 to G2 at the time of exposure. Other mutagens in these smokes could produce the highest mutation frequencies in younger buds undergoing mitosis and appear during later scoring periods. Therefore, mean mutation frequencies for pink mutations in stamen hairs were derived from daily values scored from days 7 through 12 or 11 through 15 after treatment.

The average number of stamen hairs used as the scaling factor for the experiment was determined 6 days after exposure. All old and newly bloomed flowers were removed the previous day to determine flower production on the sixth day. On each of days 6 through 12 (or beyond), flowers were picked after they were fully opened, between 4 and 6 hours after the start of the light period. (After 6 hours, the pollen begins to dehisce and the stamen hairs begin to shrivel. If a partially unopened flower is picked, it will not open any further.) Flowers from each exposure and the control were placed in separate, labeled, covered petri dishes containing a piece of filter paper moistened lightly with water to prevent desiccation. The covered petri dishes were placed in a refrigerator and stored for no longer than 2 days.

The average number of stamen hairs was determined from six flowers (total of 36 stamens) selected arbitrarily from both treated and control plants. This number was used to calculate the denominator for the stamen hair mutation frequencies independently for each experiment. The following procedure was used each day to determine the average number of stamen hairs used as the denominator for that day's scoring.

Paraffin oil (Seybolt viscosity 125/135) was placed in the center of a 7.5- x 5-cm glass plate and spread into a thin layer with a dissecting needle. Six flowers were removed at random from the control and treated specimens. Taking the flowers one at a time, the petals were pressed back; six stamens were removed from each flower with a pair of jeweler's forceps. The stamens were placed in the paraffin oil, with the anther end oriented toward the top of the slide, making sure that a thin layer of paraffin oil covered all of the stamens; more oil was added as needed. Three rows of stamens, with two or three flowers per row, were put on a slide. The slide was then placed on an 11-cm^2 white glazed ceramic tile under a dissecting microscope, and both the slide and tile were illuminated. The hairs on each stamen were combed and counted using a dissecting needle; stamen hair counts were recorded for each of the six stamens of each flower.

Mutation frequencies were determined beginning on day 7 and proceeding through day 12 or longer. At 4 to 6 hours after the start of the light period, flowers were picked and placed in separate, labeled, covered petri dishes (containing a moistened piece of filter paper) for each exposure and control. On the same day (preferably) the flowers were picked, the stamen hairs were prepared for scoring pink mutations. Stamens were removed and placed on a 7.5- x 5.0-cm slide as described above. Pink mutations were scored with a dissecting microscope at 25x or suitable magnification to ensure good resolution. A dissecting needle was used to comb the hairs and count the number of pink mutant events in the blue stamen hairs. A pink mutation event for each stamen hair was the number of individual pink cells or run of pink (P) cells separated by one or more blue (B) cells; for example, B, P, B, P, B, constituted two pink mutation events; B, P, P, B, constituted one pink mutation. An entire pink hair was also considered to be a single mutation event. The number of pink mutations from all six stamens of each flower constituted an observation.

Every day, the mutation frequency was determined for each treatment and each control. The numerator was the total number of observed pink mutation events of all flowers at each exposure and control. The denominator was the average number of stamen hairs determined on day six, multiplied by the number of flowers for each concentration and control. For example, if six stamens on each of seven flowers were scored for pink events at exposure R on day eight, and the average number of hairs per stamen was 60.2, then 7 x 6 x 60.2 = 2528.4 is the denominator. If the total number of pink events in the seven flowers was 31, then 31/2528.4 = 0.01226 was the mutation frequency for that day. The average mutation frequency from days 7 through 12 was used to calculate the points on the exposure-effect curve. The frequency of mutations was expressed as events per 1000 hairs (e. g., a frequency of 12.26 means 12.26 x 10^{-3} events/hair).

Flower Production (FP)

Tradescantia produces a determinate number of flowers (i. e., an inflorescence will not continue to produce indefinitely, but a well-developed inflorescence may produce 30 or more flowers). An average inflorescence will produce a flower every day or every other day, sometimes every third day, or occasionally two blooms on the same day.

Flowers were picked on each day of the experiment, and data were recorded cumulatively according to the following procedure. On day -1 (the day before exposure), the number of flower stalks and the number of flowers that had bloomed on each flower stalk were counted and recorded. On day 0 (the day of exposure), the number of flowers that had bloomed on each flower stalk since day -1 were counted and recorded. This was repeated on each subsequent day, 1, 2,..., n. Information on the condition of flowers and buds was also recorded during the scoring. This information included the loss of a flower stalk due to accident, etc., and the occurrence and number of "blasted" flower buds or inflorescences that occurred during the experiment. (Blasted buds appear brown and frequently necrotic, and were recorded as the number blasted and designated by a "B.") The final tabulation of data for each day for each exposure and control included the total number of flowers bloomed, the total number of flower stalks, and the ratio of the two as the average number of flowers per flower stalk. The ratio for each day from day 1, 2,..., n, but not days -1 and 0, were used as the observations.

Pollen Abortion (PA)

Microscope slides of 7.5 x 2.5 cm were prepared by placing the necessary information (date, experiment number, treatment, code number) on the frosted edge. A small drop of lacto-phenol cotton blue stain was placed in the center of each slide. The cotton blue stain was prepared by mixing equal parts of lactic acid, phenol, glycerine, and water, in which 0.08 percent to 1 percent cotton blue powder (acid blue 93; Taylor Chemical Co., St. Louis, MO) was dissolved.

Three or more anthers were removed from each flower and dropped into the stain. One method was to hold the flower by the pedicel in the left hand and, with a jeweler's forceps in the right hand, pluck or scrape the anther portion of the stamens off into the drop of stain. The anthers were stirred in the stain for about 30 sec with the forceps or a dissecting needle. Anther debris was removed and the stained pollen covered very carefully with a 22- x 22-mm cover slip. Slides may be stored indefinitely or scored immediately. Pollen abortion was scored using a compound microscope at high dry (400 to 600x) magnification.

Scoring was begun on one side near the middle of the portion of the slide covered by the cover slip. The slide was scanned all the way from one side to the other, including the edges. Since correction for edge effects is difficult,[18] the edges and area between were included in the count to compensate for any tendency for the aborted, lighter pollen grains to move differentially to the edges when the cover slip was placed on the slide. Nonaborted pollen grains took up the cotton blue phenol stain and appeared as solid blue round or oblong objects. The aborted cells were empty hyaline shells without the blue contents and assumed a variety of shapes, ranging from nearly the same shape as the nonaborted pollen grains to crumpled and very distorted structures. Aborted cells generally contained a small droplet of yellow pollen pigment, which helped distinguish them from debris. Some instruction and experience was needed to distinguish aborted cells from debris. The data were expressed as percent pollen abortion for each slide scored (i. e., the number aborted divided by the total number of aborted and nonaborted). A minimum of 300 pollen grains was scored per slide and a minimum of 5 slides was scored for each concentration or treatment. The count from each slide is a datum point.

Electron Pool and Variable Fluorescence (EP and VF)

Following exposure, arbitrarily selected leaves of each type of plant were taken to determine electron pool (EP) and variable fluorescence (VF). Leaf samples were put in sequentially numbered plastic bags containing moist filter paper and placed in the dark until fluorometer readings could be made. Readings were made in sequence: 1, then 2, etc., and back to 1 until at least 10 recordings had been done for each species, both for treated and controls. This ensured that readings from any group of samples were spread over time.

VF and EP were determined using a model SF-10 Plant Productivity Fluorometer. For each reading, a 2-cm segment of dark-adapted leaf was placed in the instrument's leaf holder, with the top of the leaf facing up. Segments were taken from leaves of about the same age, except for *Ambrosia dumosa*, for which the entire leaf was used. The fluorometer probe was inserted on top of the leaf segment, and the leaf was illuminated at 670 nm. Fluorescence at wavelengths greater than 710 nm was simultaneously detected over 10 or more seconds[12] on a strip chart recorder.

Analysis for Chromosomal Effects in Mohave *Dipodomys* spp.

Several cell types were cultured initially, including blood, bone marrow, testes, and lung. Since the bone marrow gave the best cultures, it was used in subsequent experiments.

Bone marrow cells from 25 *D. merriami* collected/exposed during the two trips were cultured and analyzed, using standard procedures[19,20] for sister chromatid exchanges (SCE). Cells were placed in Dulbecco modified Eagle's culture medium with 10 percent fetal bovine serum, phytohemagglutinin (1:100 dilution of Gibco stock), and 10^{-5} M BrdU, and then cultured in complete darkness. Three hours before cell harvest, Colcemid was added to the culture at the rate of 0.1 µg/mL. Chromosomes were prepared and stained with 0.5 µg/mL Hoechst 33258 to reveal SCE under a fluorescence microscope as reciprocal exchanges of bright and dark segments along each of the two sister chromatids. Slides were also treated with ultraviolet light (UV) and stained with Giemsa to reveal SCE differential staining similar to fluorescence patterns, but with dark and bright regions reversed.

Slides were stained with aceto-orcein and examined under phase-contrast microscopy for chromosome aberrations (CA). Aberrations were scored as deletions, exchanges, gaps, dicentrics, translocations, acro-telocentrics, and dot chromosomes.

III. RESULTS

FIELD EXPOSURES

Biological Responses

Results obtained with field-exposed *Tradescantia* and *Ambrosia dumosa* are detailed in Appendix A and summarized in Table 1. The table shows the actual values for results for each exposure versus controls significant at $p \geq 0.90$ using the z-test; results that were not significant are indicated as "ns." Appendix A gives results using other

statistical tests. FO, HC, HC + FO, and TD gave elevated responses at one or more distances for at least one endpoint in each plant system. As shown in the table, VF and EP responses in exposed *Tradescantia* are greater than those of the controls, and responses in *Ambrosia dumosa* are decreased. The relative sensitivity of the plants, judged by both the number of positive responses and number of distance-response sequences, is *Tradescantia* 4430 > *Ambrosia dumosa* > *Tradescantia* 03.

Collectively from the two field trips (p 10), 30 *D. merriami* exposed to HC, FO, TD, or HC + FD gave usable chromosome spreads. Of these, 19 individually caged animals had been acutely exposed for 30 minutes to an individual smoke (HC, FO, TD) or to a mixture of HC + FO smoke at 15 m from the source. Four animals had presumably been chronically exposed at the test site. Seven unexposed animals from Goldstone Arsenal were retained as controls. Tables 2 and 3 give details of the exposures.

Table 3 gives the results of SCE analyses for *D. merriami* exposed in the field. The table gives the identifying number of the animal (rat), its sex, frequencies of cells with indicated SCE counts, the number of cells scored (CELL), the total number of SCE counted (SCE), the mean SCE count (MEAN), its variance (VAR), and a statistic H′, defined as VAR/MEAN. As discussed on p 25, a Kruskal-Wallis test on the H′ values by the method of Margolin and Shelby[21] showed that the SCE rates of the exposed animals did not differ significantly from those of the controls.

Table 2 summarizes the results for chromosome aberration (CA) frequencies in each animal. A z-test showed several exposed groups of *D. merriami* where CA rates were much less than those of the controls. Five animals did not yield successful chromosome spreads because of death before marrow could be cultured, loss of the cell culture, or lack of mitotic cells. Also, some of the animals yielded a low number of cells that failed to divide rapidly as well, and consequently were lacking in mitotic cells. This was likely an age-related problem, with young animals having numerous cells, of which many divide rapidly, and old animals having a lesser number of cells, of which few divide rapidly.

Chemical Analysis

Hexachloroethane (HC) smoke mixtures consist of approximately equal proportions by weight of zinc oxide and hexachloroethane and about 6 percent aluminum metal. During the reaction that forms the smoke, zinc chloride, aluminum oxide, carbon monoxide, and carbon are released, as well as tetrachloroethylene (3 to 17 percent yield), carbon tetrachloride (1 to 3 percent), hexachloroethane, hexachlorobenzene, and smaller quantities of phosgene, cadmium chloride, lead chloride, and arsenic chloride.[22] The specifications for types SGF-1 and SGF-2 FO are given in Military Specification MIL-F-12070A. These oils have characteristics that make them similar to TD, No. 1 and No. 2 fuel oils, and lubricating oils. Analyses of samples of SGF-2[23] showed 42.4 to 59.7 percent aliphatics and substituted indanes, naphthalenes, tetrahydronaphthalenes, biphenyls, and multialkyl polycyclic hydrocarbons. The resolution of the gas chromatographic method they used[23] was inadequate for identifying and confirming individual chemical species. Figure 1 shows the compounds separated, identified, and confirmed in the aromatic fraction of the TD smoke during the current study.

Standard assay systems have shown many of the compounds identified in these smokes to be mutagenic or carcinogenic (Appendix B). Histopathologic examination of male Fischer 344 rats exposed in a 90-day continuous inhalation study of diesel fuel marine (DFM) showed changes entirely consistent with chronic progressive nephrosis.

TABLE 3. SCE FREQUENCY PROFILES FOR FIELD-EXPOSED *D. MERRIAMI*

Rat I.D.	Sex	Frequencies of Cells With Indicated SCE Counts 2	3	4	5	6	7	8	9	0	CELLS SCORED	SCE	MEAN (SCE/cell)	VAR	H'
Control															
8	F	0	1	2	2	5	4	1	0	1	16	98	6.13	3.45	0.56
11	M	0	0	1	1	0	0	0	0	0	2	9	4.50	0.50	0.11
12	M	1	0	0	2	0	0	0	0	0	3	12	4.00	3.00	0.75
24	M	3	18	10	12	8	1	0	0	0	52	215	4.13	1.61	0.39
25	F	3	15	13	26	8	4	3	0	0	73	334	4.58	2.14	0.47
33	F	0	0	0	0	0	0	1	2	6	9	111	12.33	14.75	1.20
Pooled											155	779	5.03	2.77	0.55
Standard deviation among subjects: 3.22															
Chronic															
4	M	0	0	1	1	0	0	0	0	0	2	9	4.50	0.50	0.11
9	M	2	7	1	2	0	0	0	0	0	12	39	3.25	0.93	0.29
22	M	0	3	6	7	5	4	5	0	2	32	188	5.88	4.31	0.73
Pooled											46	236	5.13	3.36	0.65
Standard deviation among subjects: 1.32															
FO															
1	M	3	9	9	7	1	1	1	0	0	31	125	4.03	1.90	0.47
2	F	0	0	1	2	0	2	1	1	0	7	45	6.43	3.29	0.51
21	M	0	0	0	1	0	0	0	0	0	1	5	5.00	0.00	0.00
30	F	3	9	10	4	3	2	0	0	0	31	125	4.03	1.83	0.45
Pooled											70	300	4.29	2.00	0.47
Standard deviation among subjects: 1.13															
HC															
6	F	0	1	1	2	2	0	0	0	0	6	29	4.83	1.37	0.28
20	F	0	11	12	9	8	9	2	2	0	53	271	5.11	2.87	0.56
29	M	3	6	3	7	1	1	2	0	2	26	121	4.65	5.60	1.20
Pooled											85	421	4.95	3.61	0.73
Standard deviation among subjects: 0.23															
TD															
7	F	0	1	1	2	0	0	0	0	0	4	17	4.25	0.92	0.22
23	M	3	8	12	15	4	9	4	3	0	58	299	5.16	3.40	0.66
34	M	0	5	5	9	9	4	3	0	0	35	186	5.31	2.16	0.41
38	M	0	0	0	5	0	1	0	1	1	8	53	6.63	6.84	1.03
39	M	0	1	3	0	2	1	0	1	4	12	100	8.33	29.33	3.52
Pooled											117	655	5.60	5.72	1.02
Standard deviation among subjects: 1.59															
HC + FO															
3	M	7	8	7	8	5	1	1	0	0	37	151	4.08	2.47	0.60
5	M	0	0	4	8	4	3	1	1	2	23	151	6.57	12.08	1.84
19	M	5	3	2	8	3	1	0	0	0	22	92	4.18	2.44	0.58
31	M	2	5	5	6	3	3	1	0	1	26	130	5.00	5.92	1.18
Pooled											108	524	4.85	5.33	1.10
Standard deviation among subjects: 1.15															

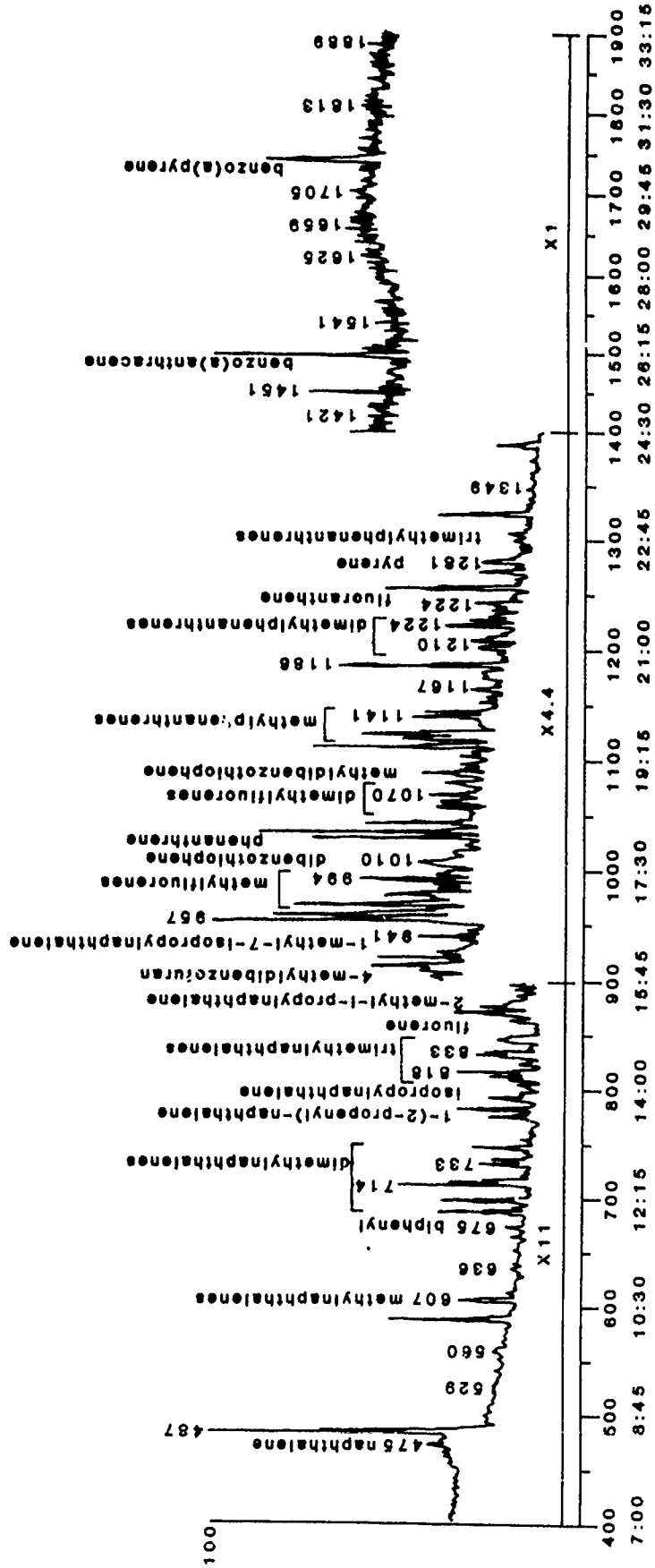


Figure 1. Chromatographic Profile of Aromatic Fraction of Tank Diesel Smoke.

However, no significant increases in renal cell tumors were recorded in male or female rats when exposures were limited to 90 days.[24] The inhalation of oil mists of SGF-1 fog oil caused lung tumors in mice and stomach tumors in monkeys. Laboratory animals exposed to lubricating oils like SGF-2 have developed skin and lung tumors. In addition, these oils have sometimes resulted in pneumonia and adverse effects on liver, spleen, kidneys, colon, skin, and heart.[25] In contrast to fog oils, "it may be tentatively concluded that diesel fuels meeting federal specifications are not carcinogenic after topical application... However, certain paraffins, olefins and alkylderivatives...are capable of accelerating the induction of skin cancer in C3H mice."[26] Renal cell tumors were recorded in male or female rats when exposures were limited to 90 days.[24,27]

In the field study, relative concentrations of smoke at each distance were obtained from the mass spectra using an appropriate standard (Table 4). The exact figures are extremely difficult to determine. As shown in the table, the rates follow an exponential die-off relationship.

Little or no chemical transformation of FO or TD was observed when the smoke was generated (Figure 2). Comparison of Figures 1 and 2 shows that the chemical composition of each smoke was essentially like that of the original material. Little or no chemical interaction between HC and FO smokes was observed when the smokes were generated together.

LABORATORY EXPOSURES

The rate of diesel introduced into the generation system was highly reproducible (300 $\pm$ 5 μL/min) between 300 and 595°C; changes in viscosity related to temperature caused little or no variation. The efficiency of aerosolization, which depends on the generation temperature, increased smoothly. Efficiencies were: 50 percent (330°C), 70 percent (400°C), 80 percent (460°C), 85 percent (520°C), and 95 percent (595°C). Table 5 summarizes the chemical composition.

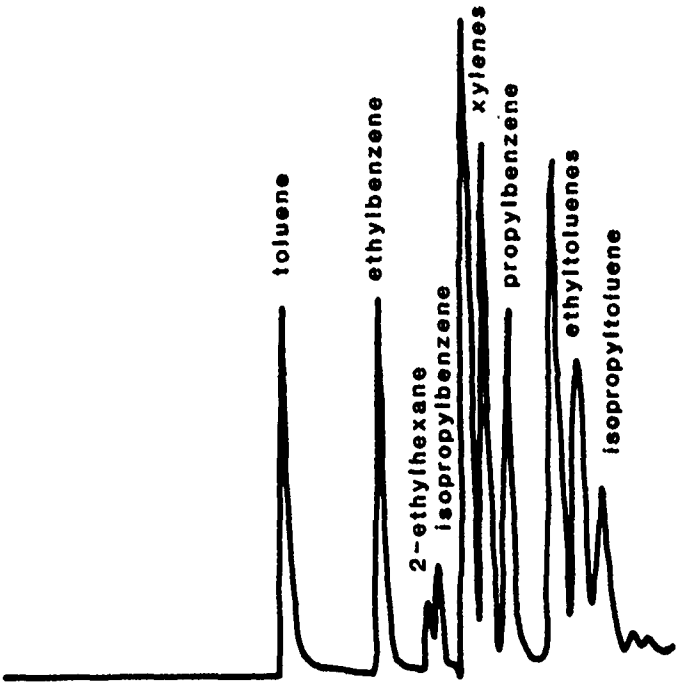
Visual examination of the condensates showed that they resembled the original tank diesel, except that the condensate obtained at 595°C was much darker and contained large amounts of carbonaceous matter. Small variations were found in the relative concentrations of the major components; however, no significant changes in the major aliphatic or aromatic moieties (Table 5) were found up to 520°C. Similar compositions were found by Parr.[28] However, a major component of the smoke generated at 595°C was oxygenated molecules, such as benzaldehyde (probably formed by partial oxidation of toluene) (Figure 2). A number of heterocyclic organics were also observed in the smoke generated at 595°C.

TABLE 4. RELATIVE CONCENTRATION OF SMOKES AT VARIOUS DISTANCES: FIELD STUDY

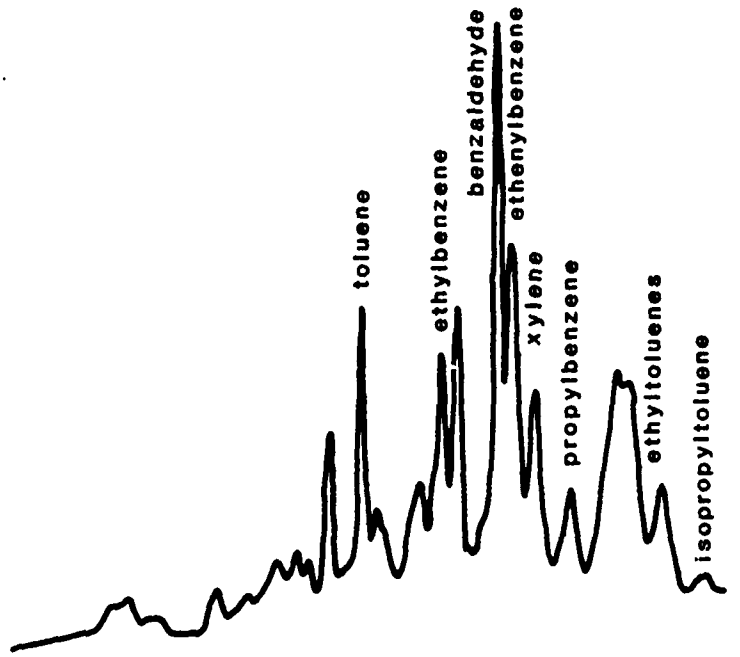
Distance (m)	Abundance of Mass 238[1]	Concentration Relative to 100 m	
		Measured Die-off	Exponential Die-off
Fog Oil			
15	9779	53.7	53.7
25	2438	13.4	19.3
50	745	4.1	8.4
100	182	1	1.2
HC			
Distance (m)	Conc. of $ZnCl_2$[a] (g/m^3)	Concentration Relative to 100 m	
		Measured Die-off	Exponential Die-off
15	72924	14.3	14.3
25	31145	6.3	5.1
50	15961	3.1	2.3
100	5078	1	0.3
Tank Diesel			
Distance (m)	Conc. of Octadecane[b] (g/m^3)	Concentration Relative to 100 m	
		Measured Die-off	Exponential Die-off
15	1474	13.5	13.6
25	539	13.4	4.9
50	108	1	2.7

[a]Data taken from experiments involving simultaneous exposure to HC smoke and oil-fog.

[b]Most abundant constituent of TD.



a) Smoke Generated at 400°



b) Smoke Generated at 595°

Figure 2. Chromatographic Profiles of Volatile Fractions of Tank Diesel Smoke Generated at 400° and 595°C Identified by GC-MS.

TABLE 5. COMPOUNDS IDENTIFIED IN TANK DIESEL

	Compound	Concentration Relative to Hexadecane
Aromatic		
	Methylnaphthalene (two isomers)	5
	Dimethylnaphthalene (six isomers)	7
	Methylbiphenyl	2
	Trimethylnaphthalene (three isomers)	7
	Fluorene	8.5
	Methylfluorene	4
	Phenanthrene	4
Aliphatic		
	n-Nonane	5.6
	n-Decane	20
	n-Undecane	32
	n-Dodecane	48
	n-Tridecane	67
	n-Tetradecane	72
	n-Pentadecane	97
	n-Hexadecane	100
	n-Heptadecane	68
	n-Octadecane	56
	n-Nonadecane	29
	n-Eicosane	17
	n-Heneicosane	8
	n-Docosane	3.2
	n-Tricosane	1.6

IV. ANALYSIS OF BIOLOGICAL RESPONSES

ANALYSIS OF *TRADESCANTIA* DATA FROM FIELD STUDIES

Concentrations of all components of obscurant smokes (HC, FO, TD) decreased with increasing distance from the smoke source, as shown for abundance of mass 238 in Table 4. This can be explained by inverse-square-law die-off.[29] The results show that there was a distance-dependent concentration gradient during plant exposures. However, the actual quantity of smoke reaching a plant cannot be determined from these data, because the chemical air sampling used active collection methods, whereas the biological exposures were passive. Appreciable heterogeneity in the visual density of the plume was observed at plant sampling locations. Since concentration gradients within the plume at the point of exposure were not well averaged during passive exposure, dosimetric analysis for plant exposures is, at best, semi-quantitative. This concentration heterogeneity may be the major factor causing the patchy response found in the exposed plants (Table 1).

The chemical composition of the smokes revealed many compounds known to individually affect genetic and other target (tissues) in animals and plants (Appendix B). Based on this knowledge and the published results of diesel engine, petroleum refinery, and other industrial air emission studies using *Tradescantia*,[5,8,30,31] it was anticipated that *Tradescantia* exposed to smokes would show exposure-related responses.

FO, HC, and HC + FO affected the variable fluorescence and electron pool of *Ambrosia dumosa* (Table 1). Normally, leaves absorb sunlight, which triggers photosynthesis. However, pollutants can reverse this metabolic process, making some leaves emit a faint light. Ellenson[32,33] has found that the size, shape, and rate of appearance of light emissions from leaves correlates with the source and extent of a plant's injury. Although *Ambrosia dumosa* was used at the longer distances (> 15 m) as was *Tradescantia* clone 03, there were too few responses to determine quantitative agreement. Qualitatively, of seven VF response pairs, three were significant (z-test) in both species, two were not significant in *Tradescantia* but were significant in *Ambrosia dumosa*, one was significant in *Tradescantia* but not in *Ambrosia dumosa*, and one was not significant in either. For the five EP response pairs, two were significant in both and three were not significant in *Tradescantia* but were significant in *Ambrosia dumosa*. These data suggest that *Ambrosia dumosa* may have been more responsive than *Tradescantia* clone 03.

What may be more important than small differences in sensitivity is the difference in the direction of response in the two species. In both *Tradescantia* clones, exposure increased both VF and EP over the response in the controls, whereas these responses decreased in *Ambrosia dumosa* (z-tests). One explanation for this difference would involve fundamentally different mechanisms of action. Laboratory studies with FO and *Tradescantia* 4430 suggest a different explanation (see p 30).

Evidence from two plant species showed that several mutation and electron transport system responses were affected by exposure to HC, FO, HC + FO, and TD; however, these results cannot be used to directly address the question of ecological significance, because there is no theory that relates these responses to long-term effects on the viability of exposed plants. Nonetheless, since fundamental genetic and nongenetic plant functions are affected, these changes provide a basis for identifying

ecologically deleterious effects (for example, decreased flower production, which is related to plant viability and organism fitness).

Flower production (FP) was severely reduced ($p > 0.90$) in *Tradescantia* 4430 exposed to FO, HC, and HC + FO at 15, 25, and 50 m, and TD at 50 m (Table 6). The response to TD at 15 m is not significant at $p = 0.9$, although the response at 25 m is significantly above that of the controls. These latter responses are likely artifacts (of unknown origin), since FP in the study area control used as a reference was depressed relative to FP in the Columbia, MO, control (16 percent vs. 35 percent).

Changes in the numbers of flowers produced during the critical scoring period, when mutation frequency reaches its maximum, are important for understanding exposure-related changes in mutation frequency. These data show that FP was reduced during this critical scoring period. This reduction could account for the erratic mutagenicity data, since the components lethal to the developing flower buds could also mask mutagenicity. Furthermore, within 24 hr of exposure to HC smoke at 50 m, the clone 4430 cuttings showed foliage damage. Although the leaves appeared to be dry and withered at the tips and along the leaf margins, they did not die during the scoring period. By the end of the scoring period, cuttings from all exposures except the TD smoke were severely damaged, and many had died. Cuttings from the three TD smoke exposures were comparable to control cuttings. Since these smoke cuttings remained viable, it is likely that exposure to the other smokes had a lethal effect that was not manifested until about 2 weeks after exposure.

TABLE 6. FLOWER PRODUCTION IN FIELD-EXPOSED *TRADESCANTIA* 4430 DURING SCORING PERIODS OF DAYS 7-12 AND 11-15

Treatment	Scoring Days	No. Flowers	No. Cuttings	% Control[a]
Control	11-15	81	91	
FO-15 m	11-15	12	71	19.0
FO-25 m	11-15	39	91	48.1
HC+FO-15 m	11-15	29	87	37.4
HC+FO-25 m	11-15	48	113	47.8
HC-50 m	11-15	21	75	31.4
Control	7-12	62	91	
TD-15 m	7-12	60	101	87.2#
TD-25 m	7-12	83	96	126.9
TD-50 m	7-12	41	88	68.4

[a]Corrected for differences in numbers of cuttings in control and exposed. All responses, except those noted by a #, differ significantly ($p \geq 0.9$) from controls.

ANALYSIS OF *TRADESCANTIA* DATA FROM LABORATORY EXPOSURES

Table 7 gives data for MCN, SH, PA, EP, and VF for *Tradescantia* exposed in the laboratory. Experiments simulating 15 m from the source and 400 to 500°C, showed no significant increases in the frequency of stamen hair (SH) mutations, although a few comparisons had statistical significance (z-tests).

MCN values were later corrected for statistical analysis. Table 8 gives the corrected values. All comparisons of corrected MCN in treatment groups with MCN in controls were significant at $p \geq 0.9$ (z-tests). Comparisons between treatment groups (i. e., between hours) were also significant, with the following exceptions: 1 hr vs. 2 hr, 12/21/83, 500°C; 0.5 hr vs. 4 hr, 1 hr vs. 2 hr, 11/30/84, 595°C.

Corrected mean MCN counts did not follow smooth time-response or temperature-response relationships Table 9). Furthermore, in support of these findings and in contrast with the field studies, coefficients of variation varied randomly--that is, independently of temperature or time (Table 8). (Several statistical methods, including analysis of variance and regression were used on the net scores and on their corresponding ranks.)

The percentages of pollen abortion were determined in three experiments on day 7 through day 15 after exposure. Daily comparisons of controls and exposed groups, or of exposed groups with each other, gave results of variable statistical significance. No obvious patterns of significance emerged. However, when total counts for each treatment were compared (z-test) with each other or with total counts for controls, differences were found for all comparisons for exposures at 15 m, both at 500°C and 595°C. Exposures at 50 m were not significant (Table 10).

Changes in the electron transport system and photosynthesis in a plant are described by the values of R (rise, which measures the initial fluorescent response); S (slope, which measures the transient fluorescent response); TR (total response = R + S); and electron pool (EP). The significance of these measures varied randomly in the laboratory studies, and no patterns were evident.

Variable fluorescence is defined as the ratio of the slope to the total response, VF = S/TR. z-tests between control and experimental groups for both VF and EP differed for most combinations of time, temperature, and distance. In Table 11, a "+" means that the control value exceeded the value in the exposed group; a "-", when comparing two exposed groups, means that VF or EP decreased as exposure time increased. At 15 m, EP and VF increased significantly to 0.5 hr (only at 595°C), dropped to background at 1 hr, and decreased significantly below background at 2 hr. At 50 m, EP and VF increased significantly to 1 hr, dropped to background at 2 hr, and then decreased significantly below background. Unlike the results with MCN and PA, VF and EP mean responses in an experiment appear to increase and decrease in a consistent, exposure-dependent manner. Similarly, the response time (rise above, return to, and drop below baseline) seems to increase with distance in a manner consistent with exposure-related changes.

These results suggest another explanation for the differences observed in EP and VF measurements made on field-exposed *Tradescantia* clones and *Ambrosia dumosa*. In the laboratory studies, statistically significant increases in VF and EP were produced in *Tradescantia* clone 4430 at relatively short (< 1 hr) exposures. Within 2 hr, responses decreased to background and after 4 hr of exposure at a level equivalent to 15 m, the

TABLE 7. RAW VALUES FOR LABORATORY-EXPOSED *TRADESCANTIA* 4430

	MCN	MCN SE	N	SH	SH SE	N	PA	PA SE	N	EP	EP SE	N	VF	VF SE	N
11/1/83 400°C, 15 m															
Control	2.63	0.54	10	1.14	0.21	6									
1 h	2.85	0.76	11	(1.34)	0.33	6									
2 h	6.23	1.48	10												
11/16/83 475°C, 15 m															
Control	1.91	0.51	11	1.14	0.29	5									
0.5 h	2.46	0.87	8												
1 h	6.88	1.21	17	(0.72)	0.22	6									
2 h	1.65	0.26	10												
11/30/83 500°C, 15 m															
Control	0.86	0.20	10												
1 h	2.12	1.00	11												
2 h	2.07	0.67	10												
4 h	2.84	0.76	10												
1/5/84 500°C, 15 m															
Control	0.93	0.22	10	1.34	0.30	6	13.34	1.18	47	32.25	4.47	10	33.49	0.76	10
1 h	1.68	0.98	10	(1.88)	0.50	6	11.11	1.39	43	34.00	3.62	10	34.28	0.56	10
2 h	2.04	0.65	10	(1.68)	0.18	6	18.76	1.63	45	39.65	3.04	10	(34.01)	0.54	10
4 h	3.87	1.25	10	(0.82)	0.22	6	15.43	1.23	45	23.55	2.27	10	32.46	0.57	10
3/8/84 500°C, 15 m															
Control	1.12	0.32	7												
1 h	2.84	0.63	10												
2 h	2.47	0.47	10												
4 h	2.20	0.70	10												
8/29/84 595°C, 50 m															
Control	1.87	0.50	10	1.16	0.27	6	8.94	0.62	48	18.5	2.7	15	39.1	3.5	15
0.5 h	3.42	1.14	9	(1.23)	0.17	6	(8.98)	0.56	50	26.9	4.9	11	44.9	3.1	11
1 h	1.49	0.39	11	(1.05)	0.26	6	(9.38)	0.43	50	28.6	2.6	13	49.4	1.3	13
2 h	1.20	0.39	9	(1.48)	0.34	6	(8.88)	0.48	50	(17.7)	3.3	10	(39.3)	4.1	10
4 h	2.26	0.78	9	3.19	0.54	6	(9.10)	0.52	50	8.5	1.9	12	24.4	3.8	12
11/30/84 595°C, 15 m															
Control	5.27	1.66	7	1.11	0.26		6.54	0.45	30	12.12	2.30	8	16.6	1.91	8
0.5 h	6.98	2.73	6	(1.17)	0.27		13.93	1.60	35	23.64	4.07	11	26.3	2.34	11
1 h	5.24	0.92	10	(0.62)	0.12		9.44	1.17	35	(12.09)	2.33	11	15.6	1.96	11
2 h	5.23	1.10	6	(0.67)	0.17		8.27	0.42	35	4.50	4.50	11	7.0	1.37	11
4 h	7.36	1.22	6	plants moribund			10.44	1.37	15	0.80	0.28	10	1.8	0.59	10

MCN = micronuclei/100 tetrads; SH = pink events per 1000 hairs; VF = variable fluorescence--a dimensionless ratio of slope (mm): total (mm); EP = electron pool--area bounded by the two intersecting lines given by the total slope and a horizontal line at the maximum inflection of the graph, in arbitrary stripchart units; PA = pollen abortion expressed as the percentage of pollen aborted: total number scored, per slide. Values in parentheses are not statistically significant at $p \geq 0.9$.

TABLE 8. SIGNIFICANCE OF MCN IN *TRADESCANTIA* CLONE 4430 EXPOSED TO TD: LAB STUDY

Exposure (hr)	Corrected MCN/100 Tetrads		z-Value		CV
	Gross	Net	Raw	Corrected	
11/1/83, 400°C, 15 m					
1 hr	13.45	11.61	0.464	24.489*	18.7
2 hr	29.40	27.56	4.570*	35.000*	12.8
1 vs. 2 hr	15.95	15.95	4.182*	19.738*	
11/16/83, 475°C, 15 m					
0.5 hr	11.61	10.27	1.173	21.922*	19.9
1 hr	32.47	31.14	6.491*	40.668*	13.0
2 hr	7.78	6.45	-0.882	21.890*	20.9
0.5 vs. 1 hr	20.86	20.86	5.049*	23.832*	
0.5 vs. 2 hr	-3.82	-3.82	-1.974*	-9.319*	
1 vs. 2 hr	-24.68	-24.68	-6.758*	-31.896*	
11/30/83, 500°C, 15 m					
0.5 hr	4.05	3.34	-0.623	13.028*	33.5
1 hr	16.37	15.66	6.337*	40.524*	11.0
2 hr	13.02	12.31	2.942*	20.820*	21.5
0.5 vs. 1 hr	12.31	12.31	7.224*	34.097*	
0.5 vs. 2 hr	8.96	8.96	3.405*	16.071*	
1 vs. 2 hr	-3.35	-3.35	-1.190	-5.615*	
12/21/83, 500°C, 15 m					
1 hr	10.00	9.40	2.361*	17.628*	26.0
2 hr	9.77	9.17	3.461*	26.230*	17.1
4 hr	13.40	12.80	5.039*	32.587*	13.7
1 vs. 2 hr	-0.23	-0.23	-0.081	-0.384	
1 vs. 4 hr	3.39	3.39	1.130	5.334*	
2 vs. 4 hr	3.63	3.63	1.520	7.174*	
1/5/84, 500°C, 15 m					
1 hr	7.92	7.28	1.493	14.497*	30.9
2 hr	9.62	8.97	3.235*	26.171*	17.1
4 hr	18.26	17.61	4.633*	27.761*	16.1
1 vs. 2 hr	1.69	1.69	0.612	2.890*	
1 vs. 4 hr	10.33	10.33	2.758*	13.016*	
2 vs. 4 hr	8.63	8.63	2.598*	12.261*	

* = Significant at $p \geq 0.9$
CV = coefficient of variation, %

TABLE 8 (CONT'D)

Exposure (hr)		Corrected MCN/100 Tetrads Gross	Net	z-Value Raw	Corrected	CV
3/8/84, 500°C, 15 m						
1	hr	13.40	12.62	4.342*	31.863*	12.9
2	hr	11.65	10.87	4.384*	35.320*	11.7
4	hr	10.38	9.60	2.479*	22.040*	18.7
1 vs. 2	hr	-1.74	-1.74	-0.941	-4.444*	
1 vs. 4	hr	-3.02	-3.02	-1.359	-6.415*	
2 vs. 4	hr	-1.27	-1.27	-0.640	-3.023*	
8/29/84, 595°C, 50 m						
0.5	hr	16.142	14.837	2.586*	24.751*	17.6
1	hr	7.03	5.72	-1.212	18.266*	25.1
2	hr	5.66	4.35	-2.082*	13.544*	32.2
4	hr	10.66	9.36	0.861	20.663*	21.1
0.5 vs. 1	hr	-9.10	-9.10	-3.487*	16.457*	
0.5 vs. 2	hr	-10.47	-10.47	-3.685*	-17.393*	
0.5 vs. 4	hr	-5.75	-5.75	-1.869*	-8.820*	
1 vs. 2	hr	-1.36	-1.36	-1.046	-4.936*	
1 vs. 4	hr	3.63	3.63	1.878*	8.863*	
2 vs. 4	hr	5.00	5.00	2.431*	11.474*	
11/30/84, 595°C, 15 m						
0.5	hr	32.94	29.26	1.110	18.999*	19.0
1	hr	24.73	21.05	-0.035	24.270*	17.0
2	hr	24.68	21.00	-0.039	20.373*	17.7
4	hr	34.73	31.06	1.973*	29.329*	12.3
0.5 vs. 1	hr	-8.21	-8.21	-1.504	-7.099*	
0.5 vs. 2	hr	-8.26	-8.26	-1.189	-5.613*	
0.5 vs. 4	hr	1.79	1.79	0.254	1.200	
1 vs. 2	hr	-0.04	-0.04	-0.014	-0.066	
1 vs. 4	hr	.0010.00	2.887*	13.624*		
2 vs. 4	hr	.0510.05	2.593*	12.240*		

* = Significant at $p \geq 0.9$
CV = coefficient of variation, %

TABLE 9. AVERAGE NET MCN SCORES IN *TRADESCANTIA* CLONE 4430 EXPOSED TO TD: LAB STUDY

	Temperature, °C			
Time, hr	400	475	500	595
0.5	---	10.2	3.34	29.26
1.0	11.61		31.14	11.24	21.05
2.0	27.56		6.45	15.65	21.00
4.0	---	--	13.34	31.06

*Corrected for underscoring and controls. All results differ significantly from corresponding controls ($p \geq 0.90$).

TABLE 10. POLLEN ABORTION IN *TRADESCANTIA* CLONE 4430 EXPOSED TO TD: LAB STUDY

		z-test	
Comparison	15 m, 500°C	15 m, 595°C	50 m, 595°C
Cntl vs. 0.5 hr	----	8.33	ns
Cntl vs. 1 hr	-2.46	4.37	ns
Cntl vs. 2 hr	5.42	5.63	ns
Cntl vs. 4 hr	2.45	7.18	ns

ns = not significant ($p < 0.9$).

TABLE 11. VF AND EP IN *TRADESCANTIA* CLONE 4430 FOR VARIOUS TD GENERATION TEMPERATURES

Comparison	z-test					
	15 m, 500°C 1/5/84		15 m, 595°C 11/30/84		50 m, 595°C 8/29/84	
	VF	EP	VF	EP	VF	EP
cntl vs. 0.5 hr			+	+	+	+
cntl vs. 1 hr	+	ns	ns	ns	+	+
cntl vs. 2 hr	ns	+	-	-	ns	ns
cntl vs. 4 hr	-	-	-	-	-	-
0.5 vs. 1 hr			-	-	-	-
0.5 vs. 2 hr			-	-	-	-
0.5 vs. 4 hr			-	-	-	-
1 hr vs. 2 hr	ns	+	-	-	-	-
1 hr vs. 4 hr	-	-	-	-	-	-
2 hr vs. 4 hr	-	-	-	-	-	-

ns = not significant ($p < 0.9$); +(-) = significant increase (decrease) ($p \geq 0.9$).

decrease in VP and EP below controls became statistically significant. These results are consistent with a metabolic explanation. Low (short-duration) exposures probably act like phenols, in that they stimulate certain enzyme systems. The results suggest that stimulation of enzyme activity peaked between 1 and 2 hr and then decreased below background (at 2 hr) as enzyme systems became saturated. Changes in enzyme availability and activity would be detected as decreases in response. If similar sequences of excitation-inhibition are primarily responsible for the EP and VF responses observed in *Tradescantia* and *Ambrosia dumosa*, then *Ambrosia dumosa* was more sensitive than either *Tradescantia* clone, since the effect was observed at shorter exposure times (e. g., 0.5 hr vs. 4 hr) and lower exposures (e. g., > 100 m vs. 15 m).

OBSERVATIONS ON EXPOSURE-RESPONSE, TIME-RESPONSE, AND TEMPERATURE-RESPONSE RELATIONSHIPS IN FIELD AND LABORATORY EXPOSURES OF *TRADESCANTIA*

Most of the *Tradescantia* systems used in this study had previously been reported to give probit-like response curves[14] when exposed to varying concentrations of single pure chemicals.[35] In contrast, no distance-response (field and laboratory study) or exposure-response (laboratory study) relationships were generally found for any of the *Tradescantia* effects from exposures to the obscurant smoke mixtures. There are several possible explanations for this, including sampling, statistical, chemical, physiological, and physical effects. The common features of these types of effects are the distinction between, and the relationship of, the exposure level to the toxicant dose adsorbed by the plant.

The simplest explanation for the erratic responses observed in the field is poor control of the exposures. Workers tried to hold plants in the smoke plume, but uniform exposures were not possible because the height and density of the plume varied erratically at a given distance and between distances. Although the general pattern of decreasing concentration with increasing distance would be maintained, the effective concentration experienced by plants passively exposed at a given distance would not be the same, quantitatively, as the concentrations determined by drawing portions of the plume through adsorbents during chemical sampling. This explanation is tenable for field exposures, but it does not account for similar patterns of erratic responses in the laboratory exposure studies. It is probable that other factors, common to both field and laboratory, were also important.

Whether or not mean responses in exposed plants differed significantly from those of the controls, the standard error (SE) of measurement in exposed plants was several times that of the controls. For example, the SE for control MCN responses in clone 4430 was 0.79 (n = 6), but was 18.4 (n = 4), 11.0 (n = 9), and 24.8 (n = 4) for plants exposed in the field to TD at 15, 25, and 50 m, respectively. However, plants exposed under laboratory simulation conditions were no more variable than concurrent controls. These high (relative to control) and distance-dependent variabilities reduce the ability to detect real differences in means. They also reduce the sensitivity of tests between control and exposed plant responses.

Another factor that may affect the expression of a distance-response relationship is repeatability. In one series of experiments reported by Ma, et al. (1983), MCN/100 tetrads of clone 4430 were 3.35 $\pm$ 0.69 (SE, n = 7), 5.30 $\pm$ 1.86 (n = 6), and 5.71 $\pm$ 0.73 (n = 7) for laboratory controls. Replicate results for plants exposed for 80 minutes to the exhaust of an engine burning fuel-soybean oil at 1:1 ratio and dilution of exhaust to air of 1:50 were 11.03 $\pm$ 2.55 (n = 10) and 18.23 $\pm$ 5.41 (n = 6) MCN/100 tetrads. The first replicate gave responses that increased smoothly with exposure duration. In the second replicate, responses at 60 and 80 minutes dropped below responses at 40 and 20 minutes, while the response at 100 minutes more than doubled the 80-minute response. Laboratory exposures simulating exposure to TD at 15 m for periods of up to 4 hr showed similar variation in replication studies. In a replication experiment using 1,2-dibromoethane (DBE), in which MCN were scored only from early tetrads, replicated responses differed by about 1.5 for exposures at 40, 80, and 160 ppm.[36] The authors also noted the "great variance denoted by the standard errors. ." Smaller standard errors were obtained in another trial using more samples per group. However, the "magnitude of the variance was in proportion to the dosages of DBE applied," a result that is exactly opposite of what was described above for TD smoke.

Visual observations of plants exposed in the field and in the laboratory showed a buildup of residue on the leaf surface. If this residue physically blocked the stomata and epidermal leaf surfaces, highly variable results, expressed as high plant-to-plant coefficients of variation and inconsistent distance (or exposure time) relationships would result because the absorbed dose was no longer proportional to the length of exposure.

The smoke residue on the leaf could produce independent physiological effects. For example, the adsorptivity of the leaf surface might be affected by solvent-stripping of the leaf cuticle by residue components or by reactions between the smoke and waxy leaf surface components. In addition, although the stomata appeared to be open when examined under a microscope, it is possible that they were functionally closed, thereby reducing the respiration rate.

The response of the plant to mixtures of known composition has not been studied. It is assumed for discussion that the effects of several components are generally additive and proportional to the molar concentration of the component in the mixture.[37] Because differences in volatility of individual compounds would cause the loss of certain components with distance, the plants exposed at various distances in the field would be affected by smokes of different composition, and hence toxicity, at each distance.

Another factor very recently identified by W. R. Lower, which future studies may show to be overriding, is the fact that whole plants respond with more sensitivity than the cuttings used in these experiments. Cuttings have been used successfully for several decades in radiation experiments. However, exposure and dose for radiation are generally indistinguishable because radiation directly penetrates, and may thereby directly affect, all parts of the plant. With chemical exposures, the effective dose is governed by physical and physiological factors, and is usually an unknown (but presumably small) fraction of the total exposure. The whole plant may be expected to respond differently, and with more sensitivity, than any of its individual organs to chemical exposures, because more routes of intake are operating and because the physiological requirements of the entire plant differ from those of individual organs.

EFFECTS OF SMOKES ON *DIPODOMYS MERRIAMI*

The 30 *D. merriami* exposed to various concentrations of smoke treatments at 15 m gave usable chromosome spreads. Results for other species examined in preliminary trials are not presented because too few animals were studied.

Sister-Chromatid Exchanges (SCE)

In detecting evidence of SCE effects due to chemical exposure, Carrano and Moore[38] have suggested that the existence and number of a subject's cells having unusually high SCE counts may be as important as the subject's mean SCE level. Margolin and Shelby[21] also motivated by the possibility that the cells being scored are a mixture of subpopulations that are distinguished by longevity, sensitivity to mutagens, or some other unknown biological consideration, proposed an adjunct to the analysis of subject mean SCE levels. The method is based on the expectation that SCEs from a single subject will distribute in a Poisson manner if all the lymphocytes are homogeneous. Several authors have concluded that the Poisson model is an inadequate description for intrasubject SCE counts in that it fits some subjects' data but not others.[21,39,40] These findings support the contention that lymphocytes within a single subject are heterogeneous, so that SCE counts are drawn from a mixture of Poisson distributions.[39] The standard test for a single homogeneous Poisson distribution versus a mixture of Poisson distributions is the dispersion test.[41] The test statistic is the product (n-1) H', where n is the number of cells scored and H' is the ratio of the unbiased sample variance to the sample mean. Table 3 gives frequency profiles for control and exposed *D. merriami*, number of cells scored, total SCE, and values of the sample mean (SCE/cell), sample variance, and H'. The quantity H' is actually a heterogeneity index of SCE cell counts within an individual, suitably normalized to adjust for the individual's mean SCE level. As such, H' is particularly sensitive to the presence of Poisson distribution mixtures.[42]

A Kruskall-Wallis analysis of the 25 values of the heterogeneity index H' indicated no exposure differences among groups. Kruskall-Wallis analysis of the control group versus the TD group or the HC + FO group were also not significant. Thus, there is no

evidence from these data that SCE counts were affected by exposures to smokes. This may signify a true lack of response. Alternatively, the single exposures used may have been inadequate to produce a significant effect, although this would not explain the absence of an effect in chronically exposed animals.

It is also possible that, as in the human studies examined by Margolin and Shelby,[21] the numbers of cells scored did not give adequate statistical power. The median inter-subject standard deviation of 1.36 SCE/cell for *D. merriami* (Table 3) is virtually identical with the median value of 1.30 for humans.[21] Therefore, for the sample sizes used here, probabilities of detecting differences of 0.5, 1.0, and 2.0 SCE/cell between the control group and an exposed group are about 0.09, 0.23, and 0.67. The conclusion is that sample sizes were inadequate. This is shown more clearly in Table 12, which gives the sample size needed to detect a given difference between the control and experimental groups with a stated (two-sided) probability of detection.

Chromosome Aberrations (CA)

Mean CA frequencies for each treatment group, P_t, were compared with the control group frequency, $P_c = 0.034$, using a z-test:

$$z = (P_t - P_c)/[(P_t(1 - P_t)/n_t + P_c(1 - P_c)/n_c]^{1/2}$$

Mean CA frequencies from *D. merriami* chronically exposed to HC + FO, and a pooled group chronically exposed to FO, HC + FO were significantly ($p > 0.95$) depressed in comparison to unexposed *D. merriami*. Preston[43] has noted that the lymphocyte assay is not at all reliable for indicating chronic chemical exposures. This is because during the chronic exposure, repair can be taking place in the G0 lymphocyte, resulting in less damage than during the in-vitro S phase. There will also be a further reduction in aberration frequency due to lymphocyte turnover. "Consequently, the induced aberration frequency will be very low."[43] Besides these difficulties, the bone marrow assay used in this study is affected by the fact that bone marrow cells are a cycling population. Thus, during an acute exposure, cells will be present in all stages of the cycle, and if some time elapses between exposure and sampling (longer than about 24 hr in humans), the analyzed cell population will be in its second or subsequent division after exposure. Following chemical exposure, only chromatid-type aberrations would be expected to be induced, irrespective of treated cell cycle stage. The consequence is that cells scored after the first mitosis after exposure "would be produced from the induced chromatid aberrations, and cell killing would result from division of aberrant cells, resulting in a decreased aberration frequency."[43]

V. CONCLUSIONS AND RECOMMENDATIONS

Tests were developed to demonstrate changes in organisms exposed to smokes and obscurants in the field. All of the smokes field-tested exerted varying degrees of lethal, physiological, and mutagenic effects in one or several of the assay systems at one or more of the exposure distances. In most cases, the high variability of the assays made it impossible to demonstrate an exposure (distance) dependence, although tank diesel (TD) smoke makes the dependence evident in *Tradescantia* clone 4430 micronuclei (MCN) (Table 1). These results suggest that the plants and animals exposed to smokes at Fort Irwin are at a toxicologically higher risk for several types of damage than control

TABLE 12. APPROXIMATE NUMBER OF ANIMALS REQUIRED TO DETECT A DIFFERENCE IN SCE/CELL BETWEEN THE CONTROL AND AN EXPOSED GROUP

Difference SCE/Cell	Probability of Detection (1-β) 0.80	0.90	0.95	0.99
0.5	50[a]	66	82	115
1.0	22	30	37	52
1.5	8	11	13	19
2.0	5	6	7	10

[a]This number of animals is required in each group.

organisms. Direct effects found include decreased fertility, changes in energy production, and decreased survivability in both plants and animals, increased genotoxic damage in plants, and increased genotoxic damage in animals. If these effects are extensive in a species at Fort Irwin, they may be manifested as reductions in the target population, or they may propagate and affect ecosystem properties,[44] such as stability, resilience, and resistance. However, this study was not able to assess ecological significance from the effects reported here for individual organisms.

The laboratory studies with *Tradescantia* clone 4430 suggest that the revised MCN method offers a useful measure of response. Pollen abortion (PA), variable fluorescence (VF), and electron pool measurements also appear to be useful endpoints. The failure of MCN and PA to show exposure-related responses may result from a binary response mechanism (mutagenic or nonmutagenic), rather than an exposure-dependent one. Although the stamen hair pink mutation test has been used successfully for low-dose, low-dose-rate radiation exposures, it does not appear to have sufficient sensitivity for chemical studies.

A significant conclusion from this work is that genetic studies can be conducted in the field using native small mammals.

To deduce the general pattern of effects when several components act simultaneously, *Tradescantia* should be exposed to single substances and known compositions of their mixtures. Effects on the same plant for each of the systems reported here should be obtained during these studies.

The total acreage available for training is finite, so land quality must be preserved indefinitely. Ecological systems subjected to chronically administered acute chemical insults (e. g., smokes) may take years or decades to exhibit manifestly obvious symptoms of toxic stress. However, by the time these symptoms are observable, the system may be damaged beyond repair. Thus, the use of biomonitors for long-term monitoring of active and reclaimed sites is more significant to Army programs than monitoring specific exposure events. The utility of *Tradescantia* for such monitoring is of interest. Such

studies should examine the response of the various *Tradescantia* systems from whole plants grown in contaminated soils.

Some types of measurements of *Tradescantia*, such as VF, appear to apply to other plants. A systematic study of several plants native to each of the various biomes found in Army training areas is needed to develop short- and long-term in-situ biomonitors.

The work reported here has shown that it is possible to obtain usable cell cultures from native rodents. This work should be extended, and the requirements and procedures for consistently producing usable cell cultures from native species determined. Studies should also be done to determine whether there are significant changes in sister chromatid exchanges, chromosome aberrations, or other measures of cytogenetic damage in native species exposed to smokes and obscurants.

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LIST OF ABBREVIATIONS

amu:	Atomic mass unit
CA:	Chromosome aberration
CV:	Coefficient of variation
DBE:	1,2-Dibromoethane
DFM:	Diesel fuel marine
eV:	Electron volt(s)
EP:	Electron pool
FO:	Fog oil
FP:	Flower production
HC:	Hexachloroethane smoke
HC + FO:	Hexachloroethane and fog oil smoke generated simultaneously
i.d.:	Internal diameter
MCN:	Micronucleus test or micronuclei (depends on context)
ns:	Not statistically significant
PA:	Pollen abortion
SCE:	Sister chromatid exchange(s)
SH:	Stamen hair test
TD:	Tank diesel smoke (includes exhaust fumes)
UV:	Ultraviolet
VF:	Variable fluorescence

APPENDIX A: RESULTS OF PLANT EXPOSURES AT FORT IRWIN

The following tables give all the raw plant data obtained during the Fort Irwin study. These tables expand data given in the text. The following symbols are used in all the tables:

Coef. var. = coefficient of variation = mean/standard deviation (%)

Obs = observed

Corr = corrected for controls

$ = z is significant at 90 percent two-sided confidence level

* = z is significant at 95 percent two-sided confidence level

= t' (unequal variances assumed) is significant at 95 percent two-sided confidence level

+ = Wilcoxon test, one tail ($p \geq 0.90$)

TABLE A-1. MICRONUCLEI, *TRADESCANTIA* CLONE 4430, FORT IRWIN

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		3.07	0.79	6				
FO	15	13.15	4.08	8	10.08	4.16	2.43*#+	89.39
	25	11.14	7.82	6	8.07	7.86	1.03	172.82
HC	50	12.68	4.86	10	9.61	4.92	1.95$+	122.79
FO/	15	30.05	13.69	8	26.98	13.71	1.97*+	129.07
HC	25	40.06	15.44	8	36.99	15.46	2.39*#+	109.16
TD	15	40.23	18.36	4	37.16	18.38	2.02*+	91.36
	25	29.83	10.95	9	26.76	10.98	2.44*#+	110.41
	50	36.69	24.75	4	33.62	24.76	1.36+	134.98

TABLE A-2. MICRONUCLEI, *TRADESCANTIA* CLONE 03, FORT IRWIN

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		2.28	0.39	25				
FO	15	2.49	0.47	6	0.21	0.61	0.34	60.08
	100	1.60	0.33	6	-0.68	0.51	-1.33	78.21
	150	2.29	0.80	6	0.01	0.89	0.01	95.20
HC	15	2.76	1.09	6	0.48	1.15	0.41	102.74
	25	3.36	0.56	6	1.08	0.68	1.58+	49.75
	100	1.92	0.79	6	-0.36	0.88	-0.41	112.40
	150	3.02	0.64	6	0.74	0.75	0.99	60.79
FO/	50	4.02	0.48	7	1.74	0.62	2.81*#+	40.70
HC	100	3.79	0.79	10	1.51	0.88	1.71$+	73.51
	150	3.02	0.58	7	0.74	0.79	1.06	61.23
TD	100	2.42	0.86	6	0.14	0.94	0.15	95.58

TABLE A-3. PINK EVENTS/HAIR, DAYS 11-15, FORT IRWIN, *TRADESCANTIA* CLONE 4430

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		0.902	0.195	5				
FO	15	2.52	0.84	5	1.62	0.86	1.88$+	76.52
	25	2.67	0.84	5	1.77	0.86	2.05*+	72.22
HC	50	0.67	0.35	5	-0.23	0.40	-0.58	133.92
HC/	15	1.79	0.32	5	0.89	0.38	2.37*+	46.81
FO	25	1.57	0.24	5	0.67	0.31	2.16*+	44.04

TABLE A-4. PINK EVENTS/HAIR, DAYS 7-12, FORT IRWIN, *TRADESCANTIA* CLONE 4430

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		1.170	0.160	6				
TD	5	3.590	0.780	6	2.420	0.796	3.04*#+	54.33
	25	3.570	0.780	6	2.400	0.796	3.01*#+	54.63
		2.690	0.450	6	1.520	0.478	3.18*#+	43.49

TABLE A-5. VARIABLE FLUORESCENCE, *TRADESCANTIA* CLONE 4430, FORT IRWIN

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		26.40	2.20	13				
FO	5	8.70	2.90	1	12.30	3.64	3.38*#+	31.20
	25	0.40	1.20	10	14.00	2.51	5.59*#+	19.62
HC	0	8.40	1.50	10	12.00	2.66	4.51*#+	21.93
FO/	5	9.20	1.00	10	12.80	2.42	5.30*#+	19.49
HC	5	8.50	1.40	10	12.10	2.61	4.64*#+	21.42
TD								
CONTROL		6.80	1.50	13				
	5	3.20	1.30	10	6.40	1.99	3.22*#+	18.91
	25	33.20	1.10	10	6.40	1.86	3.44*#+	17.72
	50	34.10	1.30	10	7.30	1.99	3.68*#+	18.41

TABLE A-6. VARIABLE FLUORESCENCE, *TRADESCANTIA* CLONE 03, FORT IRWIN

	OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL	28.60	2.40	12				
FO 50	34.40	1.30	10	5.80	2.73	2.12*+	25.09
100	31.30	1.20	10	2.70	2.68	1.01	27.11
150	34.50	2.00	10	5.90	3.12	1.89$+	28.64
HC 15	35.80	1.60	10	7.20	2.88	2.50*#+	25.48
25	30.20	1.10	10	1.60	2.64	0.61	27.64
100	34.30	1.90	10	5.70	3.06	1.86$	28.22
150	29.60	1.40	10	1.00	2.78	0.36	29.68
FO/ 50	28.90	1.70	10	0.30	2.94	0.10	32.18
HC 100	32.00	2.40	10	3.40	3.39	1.00	33.54
150	31.60	1.00	10	3.00	2.60	1.15	26.02
TD CONTROL	25.10	2.00	12				
100	30.00	1.40	10	4.90	2.44	2.01*+	25.73

TABLE A-7. VARIABLE FLUORESCENCE, *AMBROSIA DUMOSA*

	OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL	43.50	1.40	15				
FO 25	41.60	2.00	10	-1.90	2.44	-0.78	18.56
50	35.10	1.00	10	-8.40	1.72	-4.88*#+	15.50
100	37.50	1.70	10	-6.00	2.20	-2.72*#+	18.57
150	35.60	2.30	10	-7.90	2.66	-2.93*#+	23.92
HC 25	39.80	1.70	10	-3.70	2.20	-1.68$	17.50
50	41.80	1.90	10	-1.70	2.36	-0.72	17.85
100	42.90	2.20	10	-0.60	2.62	-0.23	19.22
150	40.20	0.90	10	-3.30	1.66	-1.98*	13.09
FO/ 25	39.00	1.20	9	-4.50	1.84	-2.44*#+	14.18
HC 50	37.80	2.10	10	-5.70	2.52	-2.26*#+	21.11
100	42.70	3.40	10	-0.80	3.68	-0.22	27.23
150	43.60	3.10	10	0.10	3.40	0.03	24.67
TD CONTROL	33.30	1.90	13				
15	37.60	2.10	10	4.30	2.83	1.52	23.82
25	37.30	2.20	10	4.00	2.91	1.38	24.64
50	34.30	2.40	10	1.00	3.06	0.33	28.22
100	32.20	1.20	10	-1.10	2.25	-0.49	22.07

TABLE A-8. ELECTRON POOL, *TRADESCANTIA* CLONE 4430, FORT IRWIN

	OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL	10.10	1.20	13				
FO 15	14.30	1.50	11	4.20	1.92	2.19*+	44.55
25	12.70	1.20	9	2.60	1.70	1.53+	40.09
HC 50	16.50	1.00	9	6.40	1.56	4.10*#+	28.40
FO/ 15	11.70	0.80	10	1.60	1.44	1.11	38.98
HC 25	13.30	1.30	10	3.20	1.77	1.81$+	42.06
TD							
CONTROL	6.80	0.90	13				
15	10.40	0.80	10	3.60	1.20	2.99*#+	36.61
25	9.60	0.80	10	2.80	1.20	2.33*#	39.67
50	10.20	1.20	10	3.40	1.50	2.27*#	46.50

TABLE A-9. ELECTRON POOL, *TRADESCANTIA* CLONE 03, FORT IRWIN

	OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL	17.10	2.00	12				
FO 50	21.20	1.10	10	4.10	2.28	1.80$+	34.05
100	16.10	0.90	10	-1.00	2.19	-0.46	43.08
150	17.20	1.50	10	0.10	2.50	0.04	45.96
HC 15	15.50	1.40	10	-1.60	2.44	-0.66	49.81
25	17.10	0.70	10	0.00	2.12	0.00	39.19
100	18.50	1.00	10	1.40	2.24	0.63	38.22
150	14.60	1.10	10	-2.50	2.28	-1.10	49.44
FO/ 50	14.60	1.10	10	-2.50	2.28	-1.10	49.44
HC 100	19.50	1.90	10	2.40	2.76	0.87	44.74
150	17.20	1.20	10	0.10	2.33	0.04	42.88
TD							
CONTROL	10.500	0.700	12				
100	14.200	1.200	10	3.700	1.38?	2.66*#+	30.94

TABLE A-10. ELECTRON POOL, *AMBROSIA DUMOSA*, FORT IRWIN

		OBS MEAN	OBS SE	N	CORR MEAN	CORR SE	z	COEF. VAR. Corr., %
CONTROL		28.77	2.13	15				
FO	25	31.75	3.08	10	2.98	3.74	0.80	37.30
	50	21.15	1.43	10	-7.62	2.57	-2.97*#+	38.36
	100	24.35	3.07	10	-4.42	3.74	-1.18	48.53
	150	23.85	2.01	10	-4.92	2.93	-1.68	38.83
HC	25	17.15	1.61	10	-11.62	2.67	-4.35*#+	49.23
	50	21.50	1.87	10	-7.27	2.83	-2.56*#+	41.69
	100	24.40	1.62	10	-4.37	2.68	-1.63	34.68
	150	23.30	1.94	10	-5.47	2.88	-1.90	39.10
FO/	25	17.94	1.01	9	-10.83	2.36	-4.59*#+	39.42
HC	50	18.00	1.91	9	-10.77	2.86	-3.76*#+	47.68
	100	20.80	1.53	10	-7.97	2.62	-3.04*#+	39.87
	150	19.68	0.83	10	-9.09	2.29	-3.98*#+	36.73
TD CONTROL		16.58	1.36	13				
	15	18.950	1.62	10	2.37	2.11	1.12	35.30
	25	16.150	0.75	10	-0.43	1.55	-0.28	30.41
	50	17.150	1.41	10	0.57	1.96	0.29	36.12
	100	13.700	1.00	10	-2.88	1.70	-1.71	38.96

APPENDIX B: SUMMARY OF TOXICITY AND GENOTOXICITY OF SMOKE AND OBSCURANT CONSTITUENTS

Organism	Concentration	Effects	Reference (See p. 76)
		NAPHTHALENE	
		Toxicity	
Algae			
Chlorella vulgaris	33 ppm	50% reduction of numbers vs. controls after 1 hr	1
Chlamydomonas angulosa	80000 pmole/mL	50% reduction in photosynthesis	2
Fish			
mosquito fish	150 ppm 220 ppm	24 hr TLm 96 hr TLm	3
sunfish	4 ppm	lethal after 1 hr	4
perch	20 ppm	lethal	4
minnow	11-15 ppm	lethal after 6 hrs	4
Mammal			
rat	1780 mg/kg 590 mg/kg	LD50 (orally) LD50 (ipr)	5 6
dog	400 mg/kg	LDLo (orally)	7
cat	1000 mg/kg	LDLo (orally)	7
child	100 mg/kg	LDLo (orally)	8
man	74 mg/kg	LDLo	9
		Mutagenicity	
Bacteria			
Salmonella	100 µg/plate	negative (<70 revertant colonies)	10
Mammal			
mouse	200 mg/kg	positive (ipr)	11

Organism	Concentration	Effects	Reference (See p. 76)
		Tumorigenicity	
Mammal rat	3500 mg/kg	positive	12
		Miscellaneous	
Mammal rabbit	495 mg	mild skin irritation	13
	ALKYLNAPHTHALENES		
1-methylnaphthalene			
		Toxicity	
Algae *Chlamydomonas angulosa*	10000 pmole/mL	50% reduction in photosynthesis	2
Fish fathead minnows	39 ppm 9 ppm	1 hr LC50 96 hr LC50	14
brown trout yearlings	8.4 ppm	48 hr LC50	15
Mammal rat	5000 mg/kg	LDLo	16
		Mutagenicity	
Salmonella	6 mmol/L	positive	17
2-methylnaphthalene			
		Toxicity	
Algae *Chalmydomonas angulosa*	30000 pmole/mL	50% reduction in photosynthesis	2
Mammal rat	5000 mg/kg	LDLo (orally)	16

Organism	Concentration	Effects	Reference (See p. 76)
1,6-dimethylnaphthalene			
		Toxicity	
Mammal			
rat	5000 mg/kg	LDLo (orally)	18
2,6-dimethylnaphthalene			
		Toxicity	
Marine			
Neanthes arnaceodentata	2 ppm	96 hr TLm	19
1-ethylnaphthalene			
		Toxicity	
Mammal			
rat	5000 mg/kg	LDLo (orally)	16
2-ethylnaphthalene			
		Toxicity	
Mammal			
rat	5000 mg/kg	LDLo (orally)	16
2,3,6-trimethylnaphthalene			
		Toxicity	
Marine			
Neanthes arenaceodentata	2 ppm	96 hr TLm	19

Organism	Concentration	Effects	Reference (See p. 76)
		BIPHENYL	
		Toxicity	
Algae			
Chlamydomonas angulosa	8000 pmole/mL	50% reduction in photosynthesis	2
rat	3280 mg/kg	LD50 (orally)	20
	4000 mg/kg	LD50 (skin absorption)	21
human	4400 $\mu g/m^3$	TCLo (inhalation)	22
		Mutagenicity	
Mammal			
hamster	100 µmol/L	positive	23
		Tumorigenicity	
Mammal			
mouse	56 g/kg	positive (orally)	24
		FLUORENE	
		Toxicity	
Aquatic			
Neanthes arenaceodentata	1 ppm	96 hr TLm	19
grass shrimp	0.32 ppm	LC50	19
sheepshead minnow	1.7 ppm	LC50	19
		Mutagenicity	
Bacteria			
Salmonella	1000 µg/plate (negative)	< 70 revertants/plate	10

Organism	Concentration	Effects	Reference (See p. 76)
		ACENPATHENE	
		Mutagenicity	
Bacteria			
Salmonella	1000 µg/plate	positive	25
		PHENANTHRENE	
		Toxicity	
Algae			
Chlamydomonas angulosa	5000 pmole/mL	50% reduction in photosynthesis	5
Fish			
mosquito fish	150 ppm	96 hr LC50	19
rainbow trout and bluegill sunfish	5 ppm	24 hr lethal	4
Marine			
Neanthes arenaceodentata	0.6 ppm	96 hr LC50	19
grass shrimp	0.4 ppm	24 hr LC50	19
Mammal			
mouse	700 mg/kg	LD50 (orally)	26
		Mutagenicity	
Bacteria			
Salmonella	100 µg/plate	positive	27
Mammal			
rat (liver)	3 µmol/L	DNA damage, positive	28
hamster			
kidney	5 mg/L	DNA damage, positive	29
lung	40 mg/L/27 hr	cell damage, positive	30
fibroblast	10 µmol/L	SCE positive	29

Organism	Concentration	Effects	Reference (See p. 76)
		Tumorigenicity	
Mammal mouse (skin)	71 mg/kg	TDLo	31
		ALKYLPHENANTHRENES	
1-methylphenanthrene			
		Toxicity	
Marine neanthes arenaceodentata	0.3 ppm	96 hr TLm	19
		TETRAHYDRONAPHTHALENE	
		Toxicity	
Marine brine shrimp	78 ppm	24 hr TLm	32
Mammal rat	2860 mg/kg	LD50 (orally)	33
		Miscellaneous	
Mammal rabbit	500 mg	severe skin and eye irritation	33
		QUINOLINE	
		Toxicity	
Fish sunfish	52-56 ppm	1 hr TLm	34
perch	30-50 ppm	1 hr TLm	34
bluegill	5 ppm	4 hr TLm	34

Organism	Concentration	Effects	Reference (See p. 76)
Mammal			
rat	460 mg/kg	LD50 (orally)	35
		Mutagenicity	
Bacteria			
Salmonella	1 μmol/plate	positive	36
E. coli	30 μmol/L	positive	37
Mammal			
rat	1 mmol/L	DNA damage, positive	28
hamster (lung)	150 mg/L/27 hr	cell damage, positive	30
		Tumorigenicity	
Mammal			
rat	7770 mg/kg	TDLo	38
mouse	50 g/kg	TDLo	39
		Miscellaneous	
Mammal			
rabbit	10 mg/24 hr	mild skin irritation	33
	ALKYLQUINOLINES		
2-methylquinoline			
		Toxicity	
Mammal			
rat	1230 mg/kg	LD50 (orally)	33
		Miscellaneous	
rabbit	10 mg/24 hr	mild skin irritation	33

Organism	Concentration	Effects	Reference (See p. 76)
		5,6-BENZOQUINOLINE	
		Mutagenicity	
Bacteria			
Salmonella	100 nmol/plate	positive	35
		INDAN	
		Toxicity	
Mammal			
rat	5000 mg/kg	LDLo	8
		ACETONITRILE	
		Toxicity	
Mammal			
rat	3800 mg/kg	LD50 (orally)	40
	8000 ppm/4 hr	LCLo (inhalation)	41
guinea pig	177 mg/kg	LD50 (orally)	42
	16000 ppm/4 hr	LCLo (inhalation)	42
dog	16000 ppm/4 hr	LCLo (inhalation)	42
human	570 mg/kg	TDLo (orally)	43
		Miscellaneous	
Mammal			
rabbit	10 mg/24 hr	skin irritation	40
	20 mg	severe eye irritation	
		ACRYLONITRILE	
		Toxicity	
Mammal			
rat	82 mg/kg	LD50 (orally)	44
	500 ppm/ 4 hr	LDLo (inhalation)	

Organism	Concentration	Effects	Reference (See p. 76)
mouse	27 mg/kg	LD50 (orally)	45
	900 mg/m^3/60 min	LCLo (inhalation)	41
dog	110 ppm/4 hr	LCLo (inhalation)	46
cat	600 ppm/4 hr	LCLo (inhalation)	46
human	16 ppm/20 min	TCLo (inhalation)	47
		Mutagenicity	
Bacteria			
Salmonella	57 ppm	positive	48
		Tumorigenicity	
Mammal			
rat	3640 mg/kg	TDLo (orally)	49
	5 ppm/50 wks (intermit.)	TCLo	50
		Carcinogenicity	
human		suspected	51
animal		positive	51
		Miscellaneous	
rabbit	10 mg/24 hr	skin irritation	40
	20 mg	severe eye irritation	40
human	500 mg	skin irritation	47
	1-AMINOANTHRAQUINONE		
		Tumorigenicity	
Mammal			
rat	2400 mg/kg	TDLo (orally)	52

Organism	Concentration	Effects	Reference (See p. 76)
		Miscellaneous	
rabbit	100 mg/24 hr	severe eye irritation	53
		2-ANTHRACENAMINE	
		Mutagenicity	
Bacteria			
Salmonella	6 nmol/plate	positive	54
E. coli	30 µmol/L	DNA damage, positive	39
	100 mg/L	DNA repair	55
Mammal			
rat			
liver	30 µmol/L	DNA damage, positive	30
oral	100 mg/kg	sister chromatid exchange	56
skin	100 mg/kg	sister chromatid exchange	56
hamster			
kidney	80 ug/L	cell damage, positive	57
lung	100 mg/L	cell damage, positive	58
		Tumorigenicity	
rat			
oral	45 mg/kg/30 days	TDLo	59
skin	260 µg/kg	TDLo	60
mouse (skin)	62 mg/kg/2 yrs	TDLo	24
hamster (skin)	1200 mg/kg	TDLo	61
		AZOBENZENE	
		Toxicity	
Mammal			
rat	1000 mg/kg	LD50 (orally)	62

Organism	Concentration	Effects	Reference (See p. 76)
		Mutagencicity	
Bacteria			
Salmonella	50 µg/plate	positive	10
		Tumorigenicity	
Mammal			
rat	7350 mg/kg	TDLo	63
		Carcinogenicity	
animal		positive	64
rat		positive	63
mouse		negative	63
	7H-BENZ(de)ANTHRACENE-7-ONE		
		Toxicity	
Mammal			
rat	1500 mg/kg	LD50 (ipr)	65
mouse	290 mg/kg	LD50 (ipr)	65
		Mutagenicity	
Bacteria			
Salmonella	100 µmol/L	positive	17
		Miscellaneous	
Mammal			
rabbit	500 mg/24 hr 100 mg/24 hr	moderate skin irritation severe eye irritation	66

Organism	Concentration	Effects	Reference (See p. 76)
		BENZAMIDE	
		Toxicity	
Mammal			
mouse	1160 mg/kg	LD50 (orally)	67
		1,3-BENZENEDICARBONITRILE	
		Toxicity	
Mammal			
rat	1860 mg/kg	LD50 (orally)	68
mouse	178 mg/kg	LD50 (orally)	67
		1,4-BENZENEDICARBONITRILE	
		Toxicity	
Mammal			
rat	21 g/kg	LD50 (orally)	66
		Miscellaneous	
Mammal			
rabbit	500 mg/24 hr	moderate eye irritation	66
		BENZONITRILE	
		Toxicity	
Mammal			
rat	720 mg/kg	LDLo (orally)	69
	950 ppm/9 hr	LCLo (inhalation)	69
	1200 mg/kg	LD50 (skin)	69
cat	800 mg/kg	LD50 (orally)	70
rabbit	800 mg/kg	LD50 (orally)	70

Organism	Concentration	Effects	Reference (See p. 76)
		Miscellaneous	
Mammal			
rabbit	500 mg/24 hr	moderate skin irritation	70
		2-BIPHENYLAMINE	
		Toxicity	
Mammal			
rat	2340 mg/kg	LD50 (orally)	71
rabbit	1020 mg/kg	LD50 (orally)	71
		Mutagenicity	
Bacteria			
Salmonella	200 µg/plate	positive	10
E. Coli	250 mg/L	positive	72
Mammal			
rat (liver)	3 mmol/L	DNA damage, positive	28
hamster (kidney)	80 µg/L	cell damage, pcsitive	57
		4-BIPHENYLAMINE	
		Toxicity	
Mammal			
rat	500 mg/kg	LD50 (orally)	71
dog	25 mg/kg	LDLo (orally)	73
rabbit	690 mg/kg	LD50 (orally)	71
		Mutagencity	
Bacteria			
Salmonella	10 µg/plate	positive	74

Organism	Concentration	Effects	Reference (See p. 76)
E. coli	900 µg/L	positive	10
Mammal			
rat (liver)	30 µmol/L/2 hrs	positive	28
hamster			
kidney	80 µg/L	cell damage, positive	57
lung	300 µmol/L/2 hrs	cell damage, positive	75
oral	50 mg/kg	sister chromatid exchange	76
human (fibrocyte)	800 mg/L	unscheduled DNA synthesis	77
		Tumorigenicity	
Mammal			
rat	3850 mg/kg	TDLo (orally)	78
mouse	216 mg/kg/3 d	TDLo (orally)	79
		Carcinogenicity	
human		suspected	80
animal		positive	80
	HEXACHLOROBENZENE		
		Toxicity	
rabbit	10000 mg/kg	LD50	2
man	220 mg/kg	LDLo	52
		Mutagenicity	
Bacteria			
E. coli	20 µmol/L	positive	37

Organism	Concentration	Effects	Reference (See p. 76)
Yeast			
S. cereviseae	100 ppm	positive	81
		Tumorigenicity	
Mammal			
rat	1050 mg/kg	TDLo (orally)	82
mouse	6972 mg/kg	TDLo (orally)	83
hamster	1000 mg/kg	TDLo (orally)	84
		Carcinogenicity	
human		suspected	85
animal		positive	85
	HEXACHLORO-1,3-BUTADIENE		
		Toxicity	
Mammal			
rat	90 mg/kg	LD50 (orally)	86
mouse	110 mg/kg	LD50 (orally)	87
guinea pig	90 mg/kg	LD50 (orally)	88
		Mutagenicity	
Bacteria			
Salmonella	1 mg/plate	positive	89
Mammal			
rat (oral)	77 g/kg	unscheduled DNA synthesis	90
		Tumorigenicity	
Mammal			
rat	15 g/kg	TDLo (orally)	91

Organism	Concentration	Effects	Reference (See p. 76)
		Miscellaneous	
Mammal			
rabbit	810 mg/24 hr 162 mg	moderate skin irritation mild eye irritation	92
		Carcinogenicity	
animal		suspected	93
	HEXACHLOROETHANE		
		Toxicity	
Mammal			
rat	6000 mg/kg	LD50 (orally)	94
guinea pig	4970 mg/kg	LD50 (orally)	95
		Tumorigenicity	
Mammal			
mouse (78 wks)	230 g/kg	TDLo (orally)	96
		Carcinogenicity	
animal		suspected	97
mouse		positive	96
rat		negative	96
	1-NAPHTHYLAMINE		
		Toxicity	
Mammal			
rat	779 mg/kg	LD50 (orally)	98
mammal	4000 mg/kg	LDLo	99

Organism	Concentration	Effects	Reference (See p. 76)
		Mutagenicity	
Bacteria			
Salmonella	100 µg/plate	positive	10
E. Coli	25 mg/L	DNA repair, positive	55
Mammal			
rat (liver)	3 µmol/L	DNA damage, positive	28
hamster (fibrocyte)	60 mg/L/48 hr	cell damage, positive	100
human (fibrocyte)	20 mg/L	unscheduled DNA synthesis	77
		Carcinogenicity	
human		suspected	101
animal		indefinite	101
	NITROBENZENE		
		Toxicity	
Mammal			
rat	640 mg/kg	LD50 (orally)	102
	2100 mg/kg	LD50 (skin)	103
dog	750 mg/kg	LDLo (orally)	7
cat	2000 mg/kg	LDLo (orally)	104
rabbit	700 mg/kg	LDLo (orally)	105
woman	200 mg/kg	TDLo (orally)	106
man	35 mg/kg	LDLo	107
		Mutagenicity	
Yeast			
S. cereviseae	10 mmol/tube	positive	108

Organism	Concentration	Effects	Reference (See p. 76)
		Miscellaneous	
Mammal			
rabbit	500 mg/24 hr	moderate skin irritation mild eye irritation	109
		PHENYLACETONITRILE	
		Toxicity	
Mammal			
rat	270 mg/kg	LD50 (orally)	110
	430 mg/m^3/2 hr	LC50 (inhalation)	111
	2 g/kg	LD50 (skin)	112
mouse	78 mg/kg	LD50 (orally)	111
	100 mg/m^3	LCLo (inhalation)	110
rabbit	270 mg/kg	LD50 (skin)	112
		Miscellaneous	
rabbit	500 mg/24 hr	mild skin irritation	112
		PHTHALONITRILE	
		Toxicity	
Mammal			
mouse	65 mg/kg	LD50 (orally)	113
		Tumorigenicity	
Mammal			
rat	7425 mg/kg	TDLo (orally)	114
mouse	21 g/kg	TDLo (orally)	114
	813 mg/kg	TDLo (skin)	114

Organism	Concentration	Effects	Reference (See p. 76)
		PROPIONITRILE	
		Toxicity	
Mammal			
rat	39 mg/kg	LD50 (orally)	33
	500 ppm/4 hr	LCLo (inhalation)	33
rabbit	164 mg/kg	LD50 (skin)	33
		Miscellaneous	
Mammal			
rabbit	20 mg	eye irritation	33
		TETRACHLOROETHYLENE	
		Toxicity	
Mammal			
rat	8850 mg/kg	LD50 (orally)	115
	4000 ppm/4 hr	LCLo (inhalation)	116
dog	4000 mg/kg	LDLo (orally)	117
human	96 ppm/7 hr	TCLo	118
	280 ppm/2 hr	TCLo	119
	600 ppm/10 min	TCLo	119
		Mutagenicity	
Bacteria			
Salmonella	50 μL/plate	positive	120
		Tumorigenicity	
Mammal			
mouse	195 g/kg	TDLo (orally)	121
		Carcinogenicity	
animal		suspected	122

Organism	Concentration	Effects	Reference (See p 76)
mouse		positive	63
rat		negative	63
		Miscellaneous	
Mammal			
rabbit	810 mg/24 hr	severe skin irritation	92
	162 mg	mild eye irritation	

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