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- 1.4
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- 1.8
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- 2.2
- 2.5
- 2.8
- 3.2
- 3.6
- 4.0
- 4.5
- 5.0

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**ACUTE ENVIRONMENTAL TOXICITY AND
PERSISTENCE OF METHYL SALICYLATE:
A CHEMICAL AGENT SIMULANT**

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FOREWORD

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PREFACE

The work described in this report was authorized under a Related Services contract with the U.S. Department of Energy, Contract DE-AC06-76RLO 1830. This work was started in March 1990 and completed in January 1991.

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The authors wish to thank Don C. Klopher and Barbara A. O'Brien for technical support during this project.

EXECUTIVE SUMMARY

Analysis of the results from the low and high air concentration generations indicates that air concentrations of methyl salicylate (MES) were a mixture of aerosol and vapor, with vapor dominating in the low-concentration exposures. The deposition velocity for MES to soil surfaces was 0.140 cm/sec. For foliar surfaces, deposition velocities ranged from 0.001 to 0.003 cm/sec at low air concentrations, and from 0.001 to 0.014 cm/sec for high-dose exposures. Collection efficiency was greatest for sagebrush.

The residence times or half-times of MES deposited to soils were 0.6 to 1 h for the first phase of depuration in Burbank and Palouse soils. The second depuration component had a half-time of 17 h in Burbank soil and 116 h in Palouse soil. The depuration of MES deposited to foliar surfaces also exhibited biphasic curves. The half-life of the short-residence-time component ranged from 1 to 2 h, while the longer-time component had a half-life of 5 to 31 h. Retention on foliar surfaces was greatest for sagebrush and lowest for tall fescue.

It was found that MES is phytotoxic at foliar mass-loading levels of less than 1 to 2 $\mu\text{g}/\text{cm}^2$, with sagebrush being more sensitive than grass or pine. At higher mass-loading rates, 4 to 46 $\mu\text{g}/\text{cm}^2$, severe damage was evident in both sagebrush and tall fescue. Regrowth of tall fescue indicated that the effects of MES are residual; growth rates were affected even after the second harvest, but only at the higher exposure concentrations. In vitro studies showed MES to adversely affect both photosynthesis and respiration; however, recovery occurred within 4 to 14 days as new growth was initiated.

Results from in vitro testing with MES indicated that concentrations below 100 $\mu\text{g}/\text{g}$ dry soil generally did not negatively impact soil microbial activity. The short-term effect of MES on soil dehydrogenase activity was more profound than the effect on soil phosphatase activity, indicating soil dehydrogenase is more susceptible to MES than soil phosphatase. However, soil enzyme activities increased, recovered, or were enhanced after 42 days of incubation, indicating the effect is transient, with a recovery trend. Results of an earthworm bioassay indicate survival to be 96% at soil doses of 56 μg MES/ cm^2 soil. At higher dose levels (331 μg MES/ cm^2 soil), survival decreased to 86% after 21 days of exposure.

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1.0 INTRODUCTION

Army Regulation (AR) 200-2 integrates environmental considerations into Army plans and programs. As implemented by the U.S. Army Materiel Command, this regulation requires environmental analyses and documentation for all items developed by subordinate research and development commands (Capt. S. Bennett, personal communication). The Chemical Research, Development, and Engineering Center (CRDEC) as the developer is responsible for research to generate environmental data on chemical simulants. The purpose of the research is to determine the environmental fate and effects of simulants in terrestrial and aquatic systems. These data are used to support environmental issues on simulant use in tests, trials, and training.

Chemical simulants are substances whose characteristics partially resemble selected physical and chemical properties of chemical agents. Simulants are used in tests or trials to determine the performance of equipment. Simulants are also used in training troops to operate equipment and to perform in a chemically contaminated environment. Only limited data on the chemistry and behavior of these materials are available (Capt. S. Bennett, personal communication).

The CRDEC maintains an Environmental Fate and Effects Data Base, which is a compilation of physical, chemical, toxicological, and environmental data on selected simulant agents. This data base also contains available data on chemicals employed for decontamination of simulant- and agent-contaminated equipment. The computerized data base can be accessed through the CRDEC Data Management Office. Reinbold et al. (1986) used this data base to conduct a hazard ranking. Based on these rankings, simulants requiring further laboratory evaluation to determine their environmental persistence and acute terrestrial and aquatic toxicity were selected from those shown in Table 1.1.

This report, one of a series, addresses just one of the chemical simulants listed in Table 1.1, namely methyl salicylate (MES). The objective of these studies is to determine the potential acute environmental effects and persistence of various classes of agent simulants. Previous reports (Van Voris et al. 1987; Cataldo et al. 1988, 1990, 1991) have described the environmental effects and chemical fate of several other simulants: diisopropyl methyl phosphonate and diisopropyl fluorophosphate,

TABLE 1.1. Chemical Agent Simulants and Decontaminants Currently in Use by the U.S. Army

<u>Chemical Material</u>	<u>CAS Number</u>
Simulant Agents	
Bis (2-ethylhexyl) 2-ethylhexyl phosphonate	126-63-6
Bis-(2-ethylhexyl)-phosphonate (BIS)	3658-48-8
n-butyl mercaptan (BUSH)	109-79-5
2-chloroethyl ethyl sulfide (CEES)	693-07-2
Diethyl adipate	141-28-6
Di (2-ethylhexyl) phthalate (DOP)	117-81-7
Diethyl hydrogen phosphonate (DEHP)	762-04-9
Diethyl malonate (DEM)	105-53-3
Diethyl phthalate (DEP)	84-66-2
Diethyl pimelate	2050-20-6
Diethyl sebacate (DES)	110-40-7
Diisopropyl fluorophosphate (DFP)	55-91-4
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethyl adipate (DMA)	627-93-0
Dimethyl hydrogen phosphonate (DMHP)	868-85-9
Dimethyl methylphosphonate (DMMP)	756-79-6
Dipropylene glycol monomethyl ether (DPGME)	34590-94-8
Ethanol	64-17-5
Ethyl chloroacetate (ECA)	105-39-5
Diethyl mercaptosuccinate, 0,0-dimethyl phosphorodithioate (Malathion)	121-75-5
Methyl salicylate	119-36-8
Diethyl p-nitrophenyl phosphate (Paraoxon)	311-45-5
Diethyl p-nitrophenyl thiophosphate (Parathion)	56-38-2
Polyethylene glycol 200 (PEG 200)	25322-68-3
Triethyl phosphate (TEP)	78-40-0
Trimethyl phosphate (TMP)	512-56-1
Decontaminants	
Diethylenetriamine	111-40-0
Perchloroethylene	127-18-4
Phenol	108-95-2
Chlorobenzene	108-90-7

2-chloroethylethyl sulfide, bis-(2-ethylhexyl)-phosphonate, triisopropyl phosphite, and diethyl malonate.

1.1 CHEMICAL PROPERTIES

The simulant MES is used to perform entry/exit tests for collective protection and nuclear, biological and chemical shelters. While a relatively limited data base exists on the fate and effects of MES, the document by Howard et al. (1985) contains what limited information is available concerning its chemical characteristics, stability, and toxicity.

Methyl salicylate, the chemical formula for which is $C_6H_4(OH)(COOCH_3)$, is an organic ester. It is liquid at room temperature and has a water solubility of 0.07% at 20°C. Its important physical properties include a boiling point of 223°C, a volatility of 7.57×10^2 mg/m³, and a vapor pressure of 1 mm Hg at 54°C.

1.2 ENVIRONMENTAL FATE AND EFFECTS

The environmental persistence of MES is dependent on its chemical half-life, which is affected by chemical hydrolysis and biotic decomposition. Methyl salicylate can undergo both acidic and alkaline hydrolysis of the ester to produce methyl alcohol and salicylic acid or a salicylic acid salt. Both MES and salicylic acid can undergo biodegradation by aerobic and anaerobic microorganisms.

1.3 OBJECTIVES

The purpose of this study was to provide information about the environmental behavior and effects of the agent simulant MES. The scope was restricted to assessment of impacts on a limited number of terrestrial organisms based on contact toxicity and assessment of the chemical persistence of the simulant in soils and on vegetative surfaces. The study included only cursory evaluation of major decomposition products.

2.0 MATERIALS AND METHODS

The following methodology and experimental protocols were used in evaluating acute environmental effects and chemical fate of methyl salicylate (MES) applied as an aerosol. Exposure tests were performed under dynamic conditions, using a sealed chamber supplied with filtered air and equipped with a mixing fan. Plants and soils were exposed to MES aerosols in the chamber. The MES aerosol mass concentration and deposition velocities to suspended coupons and test surfaces were measured. Exposed plants and soils were sampled periodically following each test and results were analyzed to provide dose, persistence, and effects information. A study of the influence of MES on soil microorganisms was performed by amending soils with liquid MES.

2.1 EXPOSURE SYSTEM

Aerosol exposures of plants and soils to MES were conducted in a sealed exposure chamber at the Pacific Northwest Laboratory^(a) Aerosol Wind Tunnel Research Facility. The exposure chamber test system used was identical to the system used to conduct tests of diethyl malonate aerosols (Cataldo et al. 1990) and was an improved version of that used to test diisopropyl methylphosphonate and diisopropyl fluorophosphate (Van Voris et al. 1987), 2-chloroethyl ethyl sulfide (Cataldo et al. 1988), and bis-(2-ethylhexyl)-phosphonate (Cataldo et al. 1991). Modifications to the test system since the earlier tests included the addition of a second aerosol generator and four aerosol sampling ports and the reconfiguration of the exhaust scrubber and filters.

The exposure chamber is shown in Figure 2.1. The volume of the exposure chamber was 0.37 m³. The floor area of the chamber was 0.40 m², with the height varying between 0.6 and 0.9 m. A small mixing fan was mounted above the grated floor to provide slow horizontal mixing within the chamber. Mixing velocities were estimated to be <1 m/sec. Lighting to promote plant respiration during tests was provided by an incandescent lamp mounted at the back window of the chamber.

(a) Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under Contract DE-AC06-76RLO 1830.

FIGURE 2.1. Acute-Dose Aerosol Exposure Chamber for Methyl Salicylate Tests

To contain MES aerosols, the exposure chamber was operated at a negative pressure of 0.8 cm H₂O during both test and purge periods. High-efficiency particulate air-filtered room air was supplied to the chamber at a flow rate of 76 ± 1 Lpm. The air was first passed through the aerosol generator chamber attached to the side of the exposure chamber (Figure 2.1) and was used as a carrier flow to transport the generated aerosol into the exposure chamber. Aerosol was introduced via an inlet tube located near the bottom of the chamber, below the level of the grated floor panel. Exhaust was drawn from the top of the chamber and passed through a dual-stage liquid scrubber, filters, and flowmeter, to the facility's exhaust system. In addition to the carrier

air flow, 12 or 13 Lpm was introduced at the aerosol generator to operate a pair of nebulizers. The leak rate of room air into the chamber during test periods was determined from vacuum tests to be 0.04 ± 0.02 Lpm, a level less than 0.1% of the rate of aerosol flow through the chamber.

2.2 EXPOSURE PROTOCOL

Four exposure tests with MES aerosols were performed. MES-00 was a trial test performed to obtain a baseline relationship between aerosol generation procedures and resulting average aerosol concentration within the exposure chamber. Aerosol characterization and analytical procedures were also tested during MES-00, and two plants were exposed and observed for gross effects only. MES-01 was performed as a low-dose test with both plants and soils. MES-02 was performed as a high-dose test with both plants and soils. MES-03 was performed with a target aerosol concentration of 85% of that actually present during MES-02, exposing plants for a depuration study.

Plants, soils, and deposition coupons were placed in the exposure chamber before each test. Plants and soils were placed on the grated floor panel above the aerosol inlet. At least one-half of the metal grating of the floor, which had a porosity of 90%, was left uncovered to ensure good aerosol mixing and transport between the lower and upper regions of the chamber. Deposition coupons, 47-mm flat glass fiber substrate, were suspended horizontally within the plant canopy. Rubber access doors on both the exposure and generation chambers were sealed before each test using clamps; sealant grease was not required. The lamp and mixing fan were turned on and the carrier air flow was established just before each test. The MES aerosols were generated by applying compressed air to nebulizers containing neat MES. The exposure tests (MES-01, -02, and -03) were 60 min long, beginning with the initiation of aerosol generation. Purge periods, between cessation of aerosol generation and accessing the chamber to remove samples, were 10 min long. Trial test MES-00 was 45 min with a 10-min purge.

Aerosols of MES were produced by nebulizing the chemical. The procedure was validated during MES-00, and aerosol concentrations during subsequent tests were generated using the same procedure to provide aerosols having target concentrations between 100 and 1000 mg/m³. Samples of test aerosols were obtained during each test using bubblers and a gas syringe to trap the MES present in known quantities of

chamber atmosphere. Because difficulties were associated with the gas syringe procedure, aerosol concentrations were determined from the bubbler data.

Plants, soils, and deposition coupons were removed from the test chamber at the end of the purge period. The initial samples were then obtained. After the initial sampling, plants were placed in a growth chamber for maintenance until subsequent sampling periods. Deposition coupons were contacted with solvent (acetonitrile) either after the purge period or at specific periods after the end of the test to provide data on deposition velocity and depuration rate.

The exposure system was not cleaned between tests because consideration of the maximum potential influence of previous tests on subsequent ones indicated that it was not warranted. The low-dose test (MES-01) was performed 19 days after the trial test (MES-00); tests MES-01, -02, and -03 were performed within a 2-day period. The chamber was ventilated between tests. Increased aerosol concentration resulting from a prior test was most likely to have affected MES-03, which was performed 1 day after MES-02; both tests were of high-concentration aerosols. Such an increase in aerosol concentration was not resolved analytically, because the actual concentration (750 mg/m³) during MES-03 was nearly equal to the target concentration (760 mg/m³).

Actual test conditions are listed in Table 2.1. Temperature and relative humidity were measured at the air-flow inlet to the exposure chamber. Flow rates of aerosols through the exposure system were determined as the sum of the inlet carrier air and the compressed air used in the nebulizers. The small leak rate (0.04 ± 0.02 Lpm) did not significantly influence air flow in the exposure system and was not included in the table.

TABLE 2.1. Conditions During Methyl Salicylate Aerosol Exposure Tests

Test	Date	Temperature (°C)	Relative Humidity (%)	Duration		Flow Rate	
				Test (min)	Purge (min)	Test (Lpm)	Purge (Lpm)
MES-00	3/08/90	NR ^(a)	NR ^(a)	45	10	88	191
MES-01	3/27/90	21	40	60	10	89	76
MES-02	3/27/90	22	35	60	10	90	77
MES-03	3/28/90	22	35	60	10	86	76

(a) Environmental data were not recorded during MES-00, but all test conditions were similar to those during other tests

2.3 AEROSOL GENERATION

The MES aerosols were generated using two ABCO No. 535092 polyethylene nebulizers. The pair of nebulizers were operated continuously during all tests except MES-01. During MES-01, the nebulizers were operated in a pulsed, intermittent mode to produce a lower aerosol concentration in the exposure chamber. Neat MES was poured into the 25-mL reservoirs of the nebulizers before operation. The nebulizers were placed in the aerosol generation chamber, connected to 0- to 30-psi compressed-air tubes, and monitored through a window during each test. Aerosol generation was controlled by the supply of compressed air to the nebulizers. A toggle valve was used to rapidly start/stop aerosol generation, and pressure was set using a metering valve. In addition to monitoring operating pressure, the flow rate of compressed air was measured using a flowmeter. Nebulizer operation was steady throughout all MES tests.

Nebulizers were selected because they provided an easily controlled steady output of pure MES droplets in the micrometer size range. In addition, the generation process could be started or stopped within <0.5 sec, required no stabilization period, and remained unaffected by repeated intermittent periods of use (MES-01). Within each nebulizer, neat liquid MES was aspirated from a lower reservoir by the flow of an expanding compressed-air jet across the top of a feed tube. The liquid then spread out as a film on a spherical obstruction in the air jet, was atomized by shear forces in the air-flow separation region at the edge of the sphere, and dispersed in the turbulent air flow downstream of the sphere. Only small droplets were produced; large droplets impacted on the outlet tube of each nebulizer and drained back into the reservoir. Because neat MES was generated, the aerosol supplied to the exposure chamber included only air-suspended droplets and vapor.

The rate of aerosol production using nebulizers is a function of the fluid properties of the liquid simulants, operating conditions (primarily air pressure), and the specific geometry of the nebulizer. Table 2.2 lists the known properties and aerosol generation rates for six liquid simulants and three other compounds. These generation rates were measured in the process of performing exposure tests similar to the current MES study. Although the pressure of compressed air was varied from test to test, each compound was generated during at least one test using a pressure of 10 psi. The compounds are compared in the table at that operating pressure. Use of Nebulizer #2 was limited to DEM and MES (results are included in footnotes to the table); Nebulizer

#2 usually produced aerosol generation rates about 1.3 times greater than those produced by Nebulizer #1 (the difference was attributed to possible differences in nebulizer geometry). Generation rates for the nine tested compounds varied between 0.11 and 0.56 mL/min. No single fluid property was found to be related to the differences in aerosol generation rates. However, viscosities were not available for most of the compounds. Viscosity, or a combination of viscosity and vapor pressure, may act to control generation rate.

Aerosol generation rates and nebulizer operating conditions during tests MES-00 through MES-03 are shown in Table 2.3. Aerosol generation was performed by continuous operation of both nebulizers during three of the tests. During MES-01, however, the nebulizers were energized intermittently, using the toggle valve on the compressed-air line. The mode of operation for MES-01 was 6 sec on, followed by 54 sec off. The total time the nebulizers were operated during the 60-min test was 6.25 min (the extra 0.25 min of generation were inadvertently added when, three times during the test, the toggle valve was not closed on time). Nebulizer air flow rates were measured at the operating pressure and corrected for standard conditions; they represent the total flow rate of compressed air delivered to both nebulizers. Aerosol generation rates are listed in g/min in Table 2.3; they can be converted to volume rates by dividing the values by 1.184 g/mL, the density of MES. Listed generation rates are also the sum of the rates of both nebulizers (Nebulizer #1 produced aerosol at a slightly lower rate than did Nebulizer #2, as described above). During MES-00, for example, the combined aerosol generation rate was 0.312 g/min, equal to the test-average rate as the nebulizers were operated continuously; this rate is equivalent to a volume rate of 0.263 mL/min. Generation rates of 0.108 and 0.155 mL/min were produced by Nebulizers #1 and #2, respectively.

TABLE 2.2. Aerosol Generation Rates for Neat Chemical Agent Simulants and Other Liquids Using an ABCO No. 535092 Nebulizer (#1) at 10 psi and ~STP

Compound	Molecular Weight	Density (g/mL)	Vapor Pressure (mm-Hg)	Viscosity (centistokes)	Rate of Generation (mL/min)
Methanol	32	0.79	~100		0.56 ± 0.02
Ethanol	46	0.79	~45		0.52 ± 0.03
DFP	184	1.055	0.58		0.4(a)
CEES	125	1.07	3.4		0.40 ± 0.03
DIMP	180	0.98	0.17		0.3(a)
BIS	~308	0.94	5.8 x 10 ⁻⁵	6.6	0.19 ± 0.01
DEM	160	1.055	0.25	~1.36	0.17(b)
MES	152	1.18	1 @ 54°C		0.11(c)
H ₂ O	18	1.00	17.5	~1.00	0.11 ± 0.02

(a) Approximate rate.

(b) Only one test (DEM-01) was performed at 10 psi. At 14.5 psi, Nebulizers #1 and #2, respectively, provided aerosol generation rates of 0.224 ± 0.002 and 0.29 ± 0.4 mL/min.

(c) Only MES-00 was performed at 10 psi; the generation rate was 0.16 mL/min using Nebulizer #2. Nebulizers #1 and #2, respectively, provided generation rates of 0.121 and 0.183 mL/min during MES-03 at an air pressure of 10.5 psi, and rates of 0.122 ± 0.003 and 0.187 ± 0.015 mL/min during MES-01 and MES-02 at an air pressure of 12 psi.

TABLE 2.3. Aerosol Generation Rates During Methyl Salicylate Exposure Tests

Test	Dose	Generation Period	No. of Nebulizers	Operating Pressure (psi)	Air Flow Rate (sLpm)	Aerosol Generation ^(a) Rate	
						Rate (g/min)	Test-Avg. (g/min)
MES-00	Trial	Continuous	2	10	12.0	0.312	0.312
MES-01	Low	Intermittent ^(b)	2	12	12.8	0.345	0.036(c)
MES-02	High	Continuous	2	12	12.8	0.386	0.386
MES-03	High	Continuous	2	10.5	12.0	0.360	0.360

(a) Aerosol generation rates are equal to the sum of the rates of both nebulizers.

(b) Intermittent generation consisted of operating the nebulizers for 6 sec once each minute for 60 min.

(c) The test-average aerosol generation rate was obtained by multiplying the continuous-operation generation rate by the ratio of the time the nebulizers were operating to the total duration of the test (6.25 and 60 min).

2.4 AEROSOL CHARACTERIZATION

Target test-average aerosol concentrations were 100 and 1000 mg/m³ for tests MES-01 and MES-02, respectively. A 750-mg/m³ target concentration was later selected for MES-03, the final test. The MES aerosol was generated at an intermediate rate during trial test MES-00, which was performed primarily to test aerosol generation and characterization procedures. Aerosol characterization measurements provided information on aerosol concentrations in the test chamber and on deposition and depuration rates. The primary method of measuring aerosol concentrations was the use of smog bubblers. Gas-sampling syringe measurements, the second of two independent aerosol concentration measurements, were not successful because of an undetected failure of the syringe's gas-tight gaskets. The velocity of aerosol MES deposition to glass fiber coupons suspended in the exposed plant canopy was measured. Depuration measurements were made both from a glass cover slip and from the exposed glass fiber coupons. Particle size distribution of MES aerosols was not measured; however, based on generation procedures, median particle size was expected to be <2 µm.

2.4.1 Bubbler Samples - Aerosol Concentration

Two Ace Glass Co. No. 7529 smog bubblers were used to obtain time-averaged samples of MES aerosol concentration over four intervals during each test. Aerosol samples were drawn through a single bubbler after measurements made using both bubblers in series during MES-00 revealed that the first bubbler captured more than 99.8% of the MES aerosol. Samples were obtained at a flow rate of about 0.5 Lpm and typically for 10 min. A porosity C frit was used, and the bubblers were operated with about 50 mL of acetonitrile. Sample flow rate and total sample volume were monitored by passing the sample exhaust through an American Meter Co. Singer Dry Test Meter Model 115 (± 0.02 Lpm). The volume of solvent remaining after each sample was measured gravimetrically.

Bubbler samples were submitted for chemical analysis and results reported in ppm, or mg/L, of MES per volume of solvent. The total mass of MES present in each sample was determined by comparison with the total volume of solvent present in the bubbler at the end of each sample. Aerosol mass concentration was determined by comparing the total mass of MES with the total volume of aerosol sampled. Ten-minute

bubbler samples were typically obtained 10, 25, 40, and 55 min after the beginning of each 60-min test. Average aerosol concentration during each test was determined as the average of these samples.

2.4.2 Deposition Coupons - Deposition Velocity

Eight to twelve deposition coupons were located within the plant canopy during the exposure tests. The coupons were 47-mm glass fiber filters (Gelman type A/E) that were suspended horizontally on springs. The total area of the coupons available for deposition of MES was 34.0 cm², including both top and bottom and excluding the area covered by the supporting spring. The coupons were removed from the chamber immediately after each test (at the beginning of the chamber purge period) and used to obtain data on both deposition velocity and depuration rate. The glass fiber substrate was also a surrogate deposition surface for comparison with the plant and soil surfaces.

Pairs of deposition coupons were contacted with 10.0 mL of acetonitrile solvent at intervals between 1 min and 4 h after the coupons were removed from the exposure chamber. Two coupons were selected randomly at each sampling period. In addition to deposition and depuration information, the data provided an indication of MES application uniformity within the chamber. Deposition velocity of MES to the coupons was determined using the first pair of coupons sampled after each test. To determine the deposition velocity in cm/sec, the mass of MES on each coupon, determined chemically, was compared with the product of the average aerosol concentration, test duration, and coupon surface area. Results for the first and each succeeding pair of deposition coupons sampled also provided information on the persistence or depuration of MES (Section 2.4.3).

2.4.3 Depuration from Surrogate Surfaces

The depuration of MES drops and particles deposited from test aerosols was measured. The rate of MES evaporation from a glass microscope cover slip was measured gravimetrically before the aerosol tests were performed. Because it was recognized that relatively large droplets placed on a glass slide would not experience the same evaporation kinetics as fine droplets collected on glass fiber substrate, the post-test interval for sampling coupons exposed during the aerosol tests was selected assuming a more rapid evaporation rate. The depuration of MES particles deposited to

glass fiber deposition coupons during the actual exposure tests was measured chemically.

Three drops of MES, initially weighing a total of 45.5 mg, were placed on a glass cover slip and measured over a 30-h period. The measurements were performed under laboratory conditions, and between measurements the cover slip was maintained in a dust shield that permitted gas exchange with the laboratory. All measurements were made on a semi-micro mass balance (± 0.05 mg). Results were determined as the percentage of the original mass remaining as a function of time. No chemical analysis of the residual material was performed.

The depuration rate of MES from glass fiber coupons suspended in the plant canopy during the exposure tests was determined from the chemical analysis of pairs of coupons that were exposed, removed from the chamber, and contacted at post-test intervals in pairs, as described in Section 2.4.2. The total mass of MES present on each sample was determined. The minimum mass of MES that could be resolved on the coupons was 1 μg per 10 mL of acetonitrile solvent (0.1 ppm). Depuration results allowed determination of the approximate half-life of MES aerosol deposits on the surrogate coupons.

2.5 SIMULANT CHEMISTRY

2.5.1 Chromatographic Determination of Salicylate and Methyl Salicylate

Methyl salicylate and salicylate in sample extracts were determined by reversed-phase chromatography on a C-18 column. Acetic acid was added to the mobile phase to suppress the ionization of salicylic acid and the salicylic acid esters resulting in increased retention times for these species. The mobile phase chosen for this study was 70:30 methanol:1.5% aqueous acetic acid. The isocratic mobile phase was delivered by a Waters 600E system controller and pump to a 5- μm Ultrasphere octadecyl silica column (25 cm x 4.5 mm ID) at a flow rate of 1.0 mL/min. Sample aliquots (20 μL) were introduced to the column by a WISP automatic injector. The separated components were detected by their ultraviolet absorption at 304 nm by a Waters 490E detector operated at a sensitivity of 0.008 a.u.f.s. Integrated peak areas, provided by a Hewlett-Packard model 3390A integrator, formed the basis for quantification.

The internal-standard method was used to quantitate MES and salicylate contained in the sample extracts. Several compounds were evaluated for their suitability as internal standards. The retention times of a series of salicylate esters were determined to find an internal standard that was easily resolved from MES and that eluted within a reasonable retention window of the simulant. Higher homologues of the ester series were targeted as possible internal standards due to their chemical similarity to MES. The tentative internal standards were ethyl (Chem Service, West Chester, Pennsylvania), n-butyl, and isopentyl (Pfaltz & Bauer, Stamford, Connecticut) esters of salicylic acid. Ethyl salicylate was found to best meet the retention and resolution criteria and was chosen as the internal standard for this study.

A concentrated stock solution of internal standard was prepared by bringing 200 μL of ethyl salicylate to a final volume of 50.0 mL with acetonitrile. Next, a series of MES standards (>99.9%; Aldrich, Milwaukee, Wisconsin), ranging from 0.117 to 117.4 ppm, were prepared in acetonitrile. Aliquots (10.0 mL) of each of the standards were removed and spiked with 25.0 μL of internal standard solution, resulting in an ethyl salicylate concentration of 11.3 ppm. These solutions were analyzed under the chromatographic conditions described above. A chromatogram of the 1.17-ppm standard is presented in Figure 2.2. Methyl salicylate and ethyl salicylate have retention times of 7.18 and 10.51 min, respectively, under the chromatographic conditions employed. Detection of salicylate was also possible under these conditions; this compound eluted with a retention time of 4.00 min. By plotting the ratio of the integrated peak area of MES to that of the internal standard as a function of MES concentration, a standard calibration curve was obtained. This curve is presented in Figure 2.3. The detection limit for MES under these conditions was approximately 0.1 ppm.

2.5.2 Chemical Analysis of Samples

All plant tissue, soil, deposition coupon, and syringe samples generated during this study were placed into 20-mL liquid scintillation vials containing approximately 10 mL of acetonitrile. The vials were then sealed with Teflon-lined screw caps and the samples immediately transported to the chemical-analysis laboratory. Each sample was then spiked with 25 μL of internal-standard solution. After thorough mixing, a 3-mL aliquot of each sample was removed and analyzed for salicylate and MES by the high-performance liquid chromatography described above. For bubbler samples, the exact

FIGURE 2.2. Chromatogram of a 1.17-ppm Standard of Methyl Salicylate

FIGURE 2.3. Standard Calibration Curve for Methyl Salicylate

volumes of acetonitrile remaining after sampling were recorded and the samples placed in 100-mL bottles for transport. Aliquots (10.0 mL) were removed, spiked with 25 μ L of internal standard solution, and otherwise analyzed as described above.

With the exception of sagebrush foliage, all deposition coupon, soil, plant tissue, and syringe sample blanks produced chromatograms devoid of components absorbing at 304 nm. Sagebrush blank extracts contained at least four compounds that were poorly retained on the C-18 column (Figure 2.4). The most highly retained of these indigenous plant components (retention time of 5.23 min) eluted well before the retention time of MES (7.18 min).

Representative chromatograms of soil and plant extracts are presented in Figure 2.5. Both chromatograms are from extracts performed after significant depletion of MES had occurred. The top chromatogram is from a 2-h post-exposure extract of Burbank soil, whereas the bottom chromatogram is from an extract of short-needle pine performed 24 h after MES exposure. Given that soils and plant tissues contain considerable amounts of moisture, it is significant that salicylate, the hydrolysis product of MES, was absent from all the chromatographic profiles generated from these samples. Analysis of the scrubber waste, on the other hand, indicated that MES had undergone complete hydrolysis in the aqueous sodium hydroxide solution (pH = 10 to 12).

FIGURE 2.4. Chromatogram of an Acetonitrile Extract of Sagebrush Foliage Blank

FIGURE 2.5. Representative Chromatograms of Methyl Salicylate-Exposed Burbank Soil (top) and a Short-Needle Pine Plant Extract (bottom)

2.6 ESTIMATION OF DOSE TO PLANT AND SOIL SURFACES

Following extraction, the tissues were removed from the vials and leaf areas were measured using a Licor LI-3000 leaf area meter. The foliar mass loading was calculated as nanograms contaminant per square centimeter of leaf surface.

Using a cork borer, three subsamples of each soil sample were removed from Petri dishes exposed to aerosols (sample area was $3 \times 0.95 \text{ cm}^2$) and extracted as described in Section 2.5. The solvent was transferred to a clean vial for analysis, and the soil samples were air-dried for 16 h, then dried at 60°C for 8 h. Tared tubes were reweighed

to obtain the dry weight of the soil, and mass loading was calculated (ng contaminant/g soil). All sample extracts from the tests were kept frozen at -80°C before analysis.

2.7 PLANT EFFECTS

The effects of MES on vegetation were explored by studying evidence of phytotoxicity and metabolic effects in three species: the short-needle pine (*Pinus echinata*), tall fescue (*Festuca arundinacea*, 'K-13') and sagebrush (*Artemisia tridentata vaseyana*).

2.7.1 Gross Phytotoxicity

Assessments of phytotoxicity resulting from foliar contamination with MES were based on the development of visual toxicity symptoms. These symptoms included leaf burn, blade die-back, necrotic spotting, and chlorosis. Quantitation of these effects is based on a Modified Daubenmire Rating Scale (Daubenmire 1959), a discussion of which is included in Section 3 of this report.

2.7.2 Metabolic Effects

The basis of the observed phytotoxicity of MES on whole plants was further investigated in two ways: 1) the effects of the simulant on photosynthesis (oxygen evolution) and dark respiration (oxygen uptake) in intact leaf segments were studied; and 2) the effects of the simulant on specific photochemical reactions and electron transport chains in isolated chloroplasts were examined. Methods have been described previously (Cataldo et al. 1988).

2.8 SOIL MICROBIAL ASSAYS

Two soils were used to evaluate the effects of MES on soil microbiological properties. The soils were Burbank sandy loam soil (sandy, skeletal, mixed, xeric, Torriorthent), found in the Hanford area in southeastern Washington State, and Palouse silt loam soil (fine-silty, mixed, mesic, Pachic Ultic Haploxerolls), a soil typical of eastern Washington State agricultural areas. Selected physical and chemical properties for these two soils are listed in Table 2.4. Soils were amended with MES (Aldrich Cat. no. 24082-6, Lot no. 02030CV, 99% pure) to final concentrations ranging from 0 to

2500 µg/g dry soil. Soil moisture was adjusted to 26 and 23% for the Palouse and Burbank soils, respectively. The MES-amended soil was then incubated at 22°C in the dark. The effect of MES on soil microbial activities was then evaluated in vitro by measuring the activity of two soil enzymes, soil dehydrogenase and soil acid phosphatase.

At 1 and 42 days after the MES amendment, soil samples were assayed for dehydrogenase activity by a modification of the method of Tabatabai (1982). Moisture loss from soils during the course of the incubation was negligible, and therefore soil moisture was not readjusted before assay. An aliquot of soil (2.0 g wet weight) was first mixed with 0.3 mL of CaCO₃ (3%, w/v), 0.15 mL of glucose (3%, w/v), and 0.375 mL of the substrate, 2,3,5-triphenyltetrazolium chloride (3% w/v). After incubation at 22°C for 24 h, 10 mL of methanol was added to the soil to extract the product, 2,3,5-triphenylformazan (TPF). The solution was mixed thoroughly and centrifuged at 12,000 x g for 10 min, and then the absorbance of the supernatant was determined at 485 nm with a spectrophotometer. Soil dehydrogenase activity, expressed as micrograms of TPF produced per gram of dry soil per 24 h, was determined by comparing absorbance values to a standard curve prepared with reagent-grade TPF and methanol.

TABLE 2.4. Selected Properties of Soils Used in the Methyl Salicylate Studies

Soil Property	Burbank Sandy Loam	Palouse Silt Loam
Sand (%)	45.1	1.1
Silt (%)	51.4	77.5
Clay (%)	4.0	21.4
Ash (%)	98.0	93.8
pH (100% field capacity)	7.4	5.4
Organic carbon (%)	0.5	1.7
Sulfur (%)	0.053	0.043
Nitrogen (%)	0.061	0.16
Total P (µg/g)	2400	3770
Phosphate-P (µg/g)	4.8	5.8
Carbonate/Bicarbonate (%)	<0.1	<0.1
Ammonium-N (µg/g)	6.1	18.3
CEC (meq/100 g)	5.5	23.8

The activity of soil acid phosphatase was also measured at 1 and 42 days after the addition of MES. A modified procedure of Tabatabai (1982) was used for this study. One gram of soil (wet weight) was placed in 15-mL centrifuge tubes with 4 mL of modified universal buffer (MUB) added. The MUB consisted of tris(hydroxymethyl)amino methane, 2.42 g; maleic acid, 2.32 g; citric acid, 2.80 g; boric acid, 1.256 g; and 1M NaOH, 97.6 mL; 1000 mL final volume with the pH adjusted to 6.5 with 0.1M HCl. One milliliter of para-nitrophenol phosphate (0.025M prepared with MUB) was added to each tube. The tubes were stoppered, vortexed, and incubated for 1 h at 37°C. One milliliter of 0.5M CaCl₂ and 4 mL of 0.5M NaOH were then added to stop the reaction. The mixtures were centrifuged at 12,000 x g for 10 min and the supernatant absorbance was measured at 400 nm with a spectrophotometer. Phosphatase activity was determined by comparing these values to a standard curve prepared with reagent-grade para-nitrophenol and expressed as micrograms of para-nitrophenol produced per gram of soil per hour.

All dehydrogenase and phosphatase activities were measured in triplicate and mean values were compared with those of the control soil (which was not MES-treated) and expressed as a percent of the control.

2.9 SOIL INVERTEBRATE MEASUREMENTS

An earthworm (*Eisenia fetida*) bioassay system was used to elucidate the toxicity of the MES. An artificial soil containing 350 g sand, 100 g kaolin, and 50 g dried peat moss (adjusted to pH 6.5 with CaCO₃) was used for the earthworm exposures. Twice weekly, worms were fed with fermented alfalfa and soil moisture was adjusted to 35% of dry weight. Each of these soil tests used 70 g of the artificial soil (placed in 100- x 25-mm Petri plates) containing 6 worms. Five replicate plates were used for each test series. The tests were terminated after 12 days and effects were observed over this period. The effects noted included both earthworm mortality and activity. Mass loading or dose was determined on similar soil plates without worms.

3.0 RESULTS AND DISCUSSION

3.1 AEROSOL CHARACTERIZATION

Average concentrations of methyl salicylate (MES) aerosols, measured during each exposure test, were in close agreement with target values. Results of deposition velocity measurements of MES aerosols to surrogate surfaces indicated that rates increased with increasing concentration and were possibly influenced by changing particle/vapor partition characteristics. In addition, uniformity of deposition to surrogate surfaces was good, indicating that the chamber was well-mixed. Depuration rate measurements from the suspended coupons showed MES half-lives to be <5 min.

3.1.1 Aerosol Concentration

Results of bubbler samples and aerosol concentration calculations are shown in Table 3.1. The total mass of MES per sample was the product of the MES concentration in the bubbler and the solvent volume remaining at the end of each sampling period. The aerosol concentration was determined as the total MES mass in each sample divided by the total sample volume. Back-up bubblers used during trial test MES-00 indicated virtually no carry-through of MES. Because the detection limit of the analytical method was 0.1 mg/L, the maximum possible carry-through from the first bubbler was 0.2% (based on bubbler sample 2a, the least concentrated sample). For the remaining tests, only a single bubbler was operated at a time. Test-average MES aerosol mass concentrations for exposure tests MES-01, MES-02, and MES-03 were 190, 890, and 750 mg/m³, respectively.

Aerosol concentration histories for all tests are shown in Figure 3.1. The observed increase in concentration during each test was not caused by the build-up of concentration from the aerosol produced at steady rates by the generator; the chamber was rapidly flushed by the carrier aerosol and the initial concentration build-up occurred within the first 5 to 10 min of each test. Rather, concentrations increased throughout the duration of each test as increasing MES deposits volatilized from test subject and exposure chamber surfaces. Aerosol concentration regression equations presented in the figure are representative of the period between 5 and 60 min, the period during which bubbler samples were obtained. The equations do not represent the initial rapid build-up of aerosol concentration within the exposure chamber.

TABLE 3.1. Methyl Salicylate Aerosol Concentration Results Determined from Bubbler Samples

Test	Sample	Sample			Solvent Volume (mL)	MES		Aerosol Conc. (mg/m ³)
		Time (min)	Period (min)	Volume (L)		Conc. (mg/L)	Total (mg)	
MES-00	1a	9.8	10.0	5.88	49.1	71.8	3.52	600
	1b					≤0.1		
	2a	26.7	7.0	3.69	49.0	63.6	3.12	850
	2b					≤0.1		
	3a	40.5	9.0	4.77	48.7	86.9	4.23	890
	3b					≤0.1		
MES-01	1	10.0	10.0	5.34	49.4	14.1	0.70	130
	2	25.0	10.0	5.25	49.4	20.2	1.00	190
	3	40.0	10.0	5.21	49.1	20.3	1.00	190
	4	55.0	10.0	5.16	49.4	24.1	1.19	230
MES-02	1	10.0	10.0	4.98	48.9	72.6	3.55	710
	2	25.2	10.0	4.94	49.7	89.0	4.42	890
	3	40.0	10.0	4.82	49.2	91.7	4.51	940
	4	55.2	10.0	4.95	49.3	101.7	5.01	1010
MES-03	1	10.0	10.0	5.25	48.6	66.6	3.24	620
	2	25.6	11.0	5.70	48.9	87.2	4.27	750
	3	40.6	11.0	5.52	49.0	89.9	4.40	800
	4	55.0	10.0	4.92	49.1	83.8	4.11	830

The aerosol concentration during MES-01 was 1.9 times the target value and that during MES-02 was 0.89 times the target value. The differences were due in part to the difficulty of predicting the depositional losses of vapor to chamber and test subject surfaces, and in part to the opposite effect of revolatilization of deposited material during the later stages of the tests. The concentrations in the low-dose test were greater than the respective target values, and the concentrations in the high-dose test were less than the respective target values. This difference may be related to the concentration of MES within the chamber. Specifically, the partition between vapor and particulate phases of MES within the chamber may have differed in the two tests; relative vapor concentration may have been greater during the low-dose test because the greater actual vapor concentration during the high-dose test limited the volatilization rate from MES particles. Two results support this conclusion: 1) deposition velocity measurements (Section 3.1.2) were greater at greater aerosol concentrations, and

FIGURE 3.1. Methyl Salicylate Aerosol Concentration Versus Time. Concentration history regressions for the exposure tests are valid for periods between 5 and 60 min; they do not consider the initial aerosol concentration build-up between 0 and 5 min.

2) a slight fog (indicating the presence of high concentrations of particles) was observed within the exposure chamber during the high-dose test but not during the low-dose test. The target and actual concentration levels during MES-03 were nearly identical; this was attributed to experience gained during the high-dose test.

3.1.2 Deposition Velocity to Surrogate Surfaces

Measurement of the deposition velocities of MES aerosols to the surrogate-surface coupons was difficult because of the rapid depuration of MES associated with the coupons (Section 3.1.3). For samples contacted with solvent at 2, 3, and 1 min after the end of MES-01, MES-02, and MES-03, respectively, the corresponding deposition velocities were 0.0011 ± 0.0002 , 0.0068 ± 0.0013 , and 0.0122 ± 0.0018 cm/sec. The deposition velocity for MES-01 (low dose) was 16% of that for MES-02 (high dose) and 9% of that for MES-03 (also high dose). This large difference supports the conclusion

that increasing aerosol concentration influenced the vapor/particle partition of the aerosol. It is likely that the vapor phase of the MES aerosols would have deposited to plant, soil, and chamber surfaces at slower rates than would the particulate phase of the aerosol. This is because particulate MES would deposit under the influence of gravitational and diffusional forces that do not affect the diffusion of the vapor phase. If a greater relative concentration of vapor to particle mass was present during the low-dose test, more of the aerosol would be likely to remain suspended in the exposure chamber and be exhausted with the carrier flow, and less would be deposited to surfaces. On the other hand, if the greater vapor concentrations present during the high-dose tests caused a relative increase in the fractional portion of the aerosol mass consisting of particles, then deposition rates would increase.

The deposition velocity for MES-03 was about twice that for MES-02, possibly because of the difference in time until contact with solvent. That difference, between 3 and 1 min, was roughly equal to half of the half-life of MES on the surfaces (Section 3.1.3).

The uncertainty limits for MES deposition velocity results equal the differences between the masses of MES measured on each one of the pairs of deposition coupons analyzed from each test. As a percentage of the averages, the uncertainty limits are 18, 19, and 15% for tests MES-01, MES-02, and MES-03, respectively. Additional data for the 11 pairs of coupons, representing both low- and high-dose tests and having MES masses above the detection limit, yielded an average uncertainty limit of $11 \pm 7\%$ of the average of the pairs. This indicates that the use of randomly selected pairs of deposition coupons provided similar results, that the deposition of MES to glass fiber coupons was uniform, regardless of position within the plant canopy, and that the aerosol within the chamber was well-mixed.

3.1.3. Depuration from Surrogate Surfaces

The evaporation of three MES droplets from a glass slide is shown in Figure 3.2. The surface was maintained in the dust cover of a semi-micro mass balance with one sliding door positioned to allow gas exchange with the laboratory. The analysis was gravimetric, and thus the initial and final compositions of the mass present on the surface were not determined, although a strong odor of wintergreen oil was noted both initially and after 30 h. Additionally, a small amount of crystalline residue remained after

~36 h. These observations indicated a linear decrease of mass between 0 and 30 h, with a mass half-life of 15.8 h. This analysis was performed to provide guidance for depuration measurements of MES from glass fiber deposition coupons.

The depuration of MES from glass fiber coupons during the three aerosol exposure tests is shown in Figure 3.3. The mass of MES, measured chemically, on pairs of coupons is plotted versus time of sampling for each test. The uncertainty limits shown on the plot are the range of the MES mass on each pair of coupons. Sampling extended to 4 h; however, no detectable MES was present on any sample aged longer than 57 min after the end of the 1-h test period (the detection limit of the analytical procedure was $\pm 1 \mu\text{g}$ of MES per coupon contacted with 10.0 mL of acetonitrile). The two high-dose tests (MES-02 and MES-03) provided similar results both in terms of the mass of MES per coupon per sampling period and in terms of the initially rapid and then less rapid depuration rate. Although the mass per coupon for the low-dose test (MES-01) was less than that for the high-dose tests, the rate of MES depuration was similar. In all three cases, the half-life of MES on the glass fiber coupons was estimated to be ≤ 5 min.

FIGURE 3.2. Evaporation of Three Droplets of Methyl Salicylate from a Glass Cover Slip under Standard Conditions (Gravimetric Analysis)

FIGURE 3.3. Depuration of Methyl Salicylate Deposited to Glass Fiber Coupons During Aerosol Exposure Tests (Chemical Analysis)

3.2 MASS LOADING ON AND DEPOSITION VELOCITIES TO FOLIAR SURFACES AND SOILS

A variety of physical and environmental factors combine to determine the rate at which atmospheric pollutants transfer from the air column through the near surface boundary layer to a receptor surface. These factors, which include such atmospheric conditions as wind speed and humidity, aerosol diameter, and vapor pressure, as well as the physical and chemical structure of the receptor surface, combine to determine the net deposition to a surface. Two removal mechanisms operate on atmospheric pollutants, one for gas-phase compounds and one for compounds in the aerosol phase. Although the two deposition mechanisms depend on different parameters, the net deposition to a surface can be calculated when an average gas/aerosol deposition velocity is known. A deposition velocity, which is normally expressed as V_d in units of cm/sec, is analogous to a mass-transfer coefficient and describes the rate at which an atmospheric pollutant is deposited on a given surface. Without the deposition velocity, the dosing levels to a receptor surface cannot be predicted.

The relationship between chemical dose and damage or effect may be quantified by estimating the total surface deposition, or mass loading, if the aerosol concentration, deposition velocity, and surface exposure time are known. Because of the complexity of deposition processes, specific deposition velocities are rarely known and must be either measured directly or calculated. In this study, we have directly measured surface mass loading by subsampling a known area of an exposed surface and then extracting and quantifying any deposited chemical species. Subsequently, these data were combined with the measured aerosol concentrations and exposure times to compute the deposition velocity. The formula for calculating V_d is

$$V_d \text{ (cm/sec)} = \frac{\text{mass load (ng compound/cm}^2 \text{ leaf)}}{\text{aerosol conc (ng compound/m}^3 \text{)}} \times \frac{1 \times 10^6 \text{ cm}^3}{\text{m}^3} \times \frac{1}{\text{exposure time}}$$

Quantifying the deposition process for specific receptor surfaces permits a comparison of the relative importance of atmospheric variables and of the characteristics of the canopy and receptor surfaces (plant and soil) to the net deposition efficiency. The following subsections discuss the mass loading of MES to vegetative and soil surfaces and the calculated deposition velocities. All plant results were obtained from exposure tests MES-01 (890 mg/m³) and MES-03 (750 mg/m³); soil results are based on MES-03 only.

3.2.1 Vegetative Surfaces

Average air concentrations of MES for the low-dose exposure were 190 mg/m³, for the high-dose exposure 890 mg/m³, and for the depuration experiment 750 mg/m³. Exposure duration was 60 min. Foliar mass loading ranged from 0.78 to 1.85 µg/cm² in MES-01 and from 4.19 to 46.1 µg/cm² in MES-02 (Table 3.2). Short-needle pine and tall fescue exhibited lower mass loadings than sagebrush, particularly under high-dose conditions. Differences in mass loadings for all three species in the low-concentration exposure are not significant ($P \geq 0.1$). The extent of mass loading to sagebrush was significantly greater than that to tall fescue and short-needle pine in both the high-dose test and the depuration test ($P \leq 0.1$). Mass-loading levels between the high- and low-dose exposures were 4 to 23 times higher than the factor of four difference in air concentration. This would indicate that the volatility of MES prohibits accumulation on foliar surfaces at lower air concentrations.

Table 3.2 also provides calculated deposition velocities for plant canopies, based on the average air concentration and mass-loading levels. For the low-dose treatment, deposition velocity values averaged 0.002 cm/sec for the three plant canopies and were not significantly different ($P \geq 0.1$). Again, this may indicate that the volatility of MES is such that it either prevents effective deposition or is rapidly revolatilized from surfaces once it has been deposited. At the higher exposure concentrations, deposition velocity values for the sagebrush were 2 to 7 times higher than for the other two plant canopies. Collection efficiency remained lower for the pine and the tall fescue than for the sagebrush. This difference is likely to be related to the canopy structure and to the surface texture of the rougher sagebrush leaves. Differences in deposition velocities for sagebrush and the other two species are significant ($P \geq 0.05$). The differences in mass loading and deposition velocity for the low-dose compared with the high-dose exposures are probably related to rates of deposition compared to revolatilization from foliar surfaces.

TABLE 3.2. Average Foliar Mass Loading and Deposition Velocities for Methyl Salicylate on Vegetative Surfaces During Low- and High-Dose and Depuration Experiments (n=4).

Treatment	Mass Loading ($\mu\text{g}/\text{cm}^2 \pm \text{SD}$)	Deposition Velocity ($\text{cm}/\text{sec} \times 10^3 \pm \text{SD}$)
<u>Low Dose</u>		
Sagebrush	1.85 ± 0.72	2.71 ± 1.06
Short-Needle Pine	0.78 ± 0.35	1.14 ± 0.52
Tall Fescue	1.30 ± 1.15	2.09 ± 1.68
<u>High Dose</u>		
Sagebrush	46.06 ± 6.58	14.40 ± 2.05
Short-Needle Pine	6.12 ± 1.51	1.91 ± 0.47
Tall Fescue	4.19 ± 1.61	1.31 ± 0.50
<u>Depuration Exposure</u>		
Sagebrush	35.44 ± 10.36	13.10 ± 3.84
Short-Needle Pine	17.44 ± 15.70	6.46 ± 5.82
Tall Fescue	3.27 ± 0.23	1.21 ± 0.08

3.2.2 Soil Surfaces

Soil mass-loading levels, following 60 min of exposure at the dose level of 750 mg/m³, were 378 and 370 µg/cm² for the Burbank and Palouse soils, respectively (Table 3.3). There was no significant difference in mass loading between the two soils ($P \geq 0.05$). This would indicate that deposition and fixation/sorption in the two soils are similar and that revolatilization rates are comparable.

Deposition velocities calculated for the soils were 0.140 and 0.137 cm/sec for the Burbank and Palouse soils, respectively. These values are not statistically different ($P > 0.1$).

3.3 PHYTOTOXICITY

3.3.1 Gross Phytotoxicity

The determination of foliar toxicity effects can present several problems. Although the plants used for this study were of natural genetic stock, individual differences in physiological variability and toxicity response within each test species were expected. Further, both under field conditions and in the stirred exposure system employed for these studies, where air moves along a given vector (i.e., wind direction), substantial amounts of deposition to canopies can occur irregularly, depending on canopy structure and density and the presence of back eddies. Finally, the most cost-effective and consistent manner in which to quantify damage to vegetation must be considered. Taking these issues into account, a nonparametric grading system, a Modified Daubenmire Rating Scale (MDRS; Daubenmire 1959), was used as a damage index for the evaluation of contact toxicity.

The criteria used to compile the MDRS are described in Table 3.4. These criteria were used to describe the extent of visual damage to the plants caused by exposure over a given period at each concentration of MES. Damage can be subjectively quantified using any one or more of the listed symptoms. The intensity of foliar damage can be further quantified by determining the physical length of the needle or leaf damage. The data generated are nonparametric estimates of foliar damage.

TABLE 3.3. Average Mass Loading and Deposition Velocities of Methyl Salicylate on Soil Surfaces During the Depuration Exposures (n=6).

Soil Type	Mass Loading ($\mu\text{g}/\text{cm}^2 \pm \text{SD}$)	Deposition Velocity ($\text{cm}/\text{sec} \times 10^3 \pm \text{SD}$)
Burbank	378.38 ± 71.66	140.0 ± 26.5
Palouse	369.76 ± 78.12	137.0 ± 28.9

Table 3.5 provides the gross toxicity data for the three plant species exposed to MES (foliar exposure only). At the low dose, $190 \text{ mg}/\text{m}^3$ for 60 min, only the sagebrush exhibited any phytotoxicity following foliar contamination. This consisted primarily of necrotic spotting, leaf tip burn, and leaf drop. In the short-needle pine, only a slight amount of tip burn was observed, and in the grass, neither old nor new growth was affected. In the low-dose treatment, mass loadings were relatively low and comparable for the three plant species. Thus it appears that sagebrush may have a higher sensitivity to MES. At the higher dose, $890 \text{ mg}/\text{m}^3$ for 60 min, a rapid and severe onset of toxicity occurred for both the sagebrush and the tall fescue. Two days after exposure, leaf curl (LC) and tip burn/necrosis (TB) were evident. The extent or intensity of damage was most severe in the sagebrush, which had received a much higher dosage (46 versus $4 \mu\text{g}/\text{cm}^2$), but the fact that the grass also exhibited significant damage at the higher concentration indicates that grass is also sensitive to MES at mass loadings of $4 \mu\text{g}/\text{cm}^2$.

In view of the severity of damage from contact toxicity with MES at the high dosage levels, residual effects were assessed using tall fescue. The objective of the residual plant effects study was to determine whether foliar toxicants had any lasting effect on root systems that were not exposed directly. Control and treated plant canopies were harvested 30 days post-exposure and dry-matter production was noted. Cropped plants were then allowed to regrow for a second harvest. The results in Table 3.6 indicate a significant ($P \leq 0.05$) reduction in biomass following foliar exposure in the high-dose treatment, both after the first 30 days and following the second harvest. At low dose levels, no statistically significant ($P > 0.1$) residual effects on dry-matter production are observed for the first or the second harvests.

TABLE 3.4. Coding for Modified Daubenmire Rating Scale and Associated Phytotoxicity Symptoms

Symptom/Intensity	Description
Modified Daubenmire Rating Scale	
0	no obvious effects over controls
1	5% of plant foliage affected
2	5% to 25% of foliage affected
3	25% to 50% of foliage affected
4	50% to 75% of foliage affected
5	75% to 95% of foliage affected
6	95% to 100% of foliage affected
Phenotypic Responses	
OGA	old growth affected
NGA	new growth affected
O&NGA	old and new growth affected
TB	tip or leaf edge burn
LBD	leaf burn and leaf drop
NS	necrotic spotting
LD	leaf abscission or needle drop
Chl	chlorosis
BD	blade dieback
LC	leaf curl
W	wilting
GD	growing tip dieback
D	plant dead
F/SA	floral or seed/fruit abortion
(value)	length (cm) that needles or leaves exhibit dieback or burn

TABLE 3.5. Plant Symptomology Following Atmospheric Exposure to Methyl Salicylate at Low and High Concentrations

Treatment/ Plant Species	Days Post- Exposure	Foliar Mass Loading (µg/cm ²)	Toxicity Response ^(a)	
			Damage Index (DR Scale)	Symptomology
<u>Low Dose</u>				
Sagebrush	3	1.85 ± 0.72	1.0	O&NGDH ^(b)
	14		2.5	NS,TB, LD
Short-Needle Pine	3	0.77 ± 0.35	1.0	O&NGDH
	14		1.5	TB (0.5)
Tall Fescue	3	1.30 ± 1.15	1.0	O&NGDH
	14		1.0	NS, O&NGDH
<u>High Dose</u>				
Sagebrush	3	46.0 ± 6.58	4.5	NS,LD
	14		6.0	D ^(c)
Short-Needle Pine	3	6.11 ± 1.51	1.0	O&NGDH
	14		2.0	TB (1.0)
Tall Fescue	3	4.19 ± 1.61	5.0	LC,NS,TB
	14		4.0	LC, NS,TB

(a) Daubenmire scale and symptomology definitions (see Table 3.4).

(b) New growth developing healthy.

(c) One plant dead.

TABLE 3.6. Effects of Methyl Salicylate on the Regrowth (Dry-Matter Production) of Exposed^(a) Tall Fescue 30 and 60 Days Post-Exposure

Treatment	Dry-Matter Production	
	30 Days Post-Exposure (Avg. g dry wt. $\pm$ variance, n=2)	60 Days Post-Exposure
Control	4.64 ± 0.62	5.44 ± 0.09
Low Dose	$4.89 \pm 0.53^{(b)}$	$4.98 \pm 0.09^{(b)}$
High Dose	2.81 ± 0.27	3.50 ± 0.06

(a) See Table 3.2 for rates of foliar mass loading.

(b) Not significant.

3.3.2 Metabolic Effects on Plants

Whole-Leaf Photosynthesis/Respiration

Tissue samples were removed from the exposed plants in both the low-dose and the high-dose experiments before and immediately after exposure and at intervals over the following two weeks. The samples were assayed for intact leaf photosynthesis and dark respiration capability using polarographic techniques (O_2 electrode) on a $\mu\text{Mol } O_2$ (uptake or evolution)/g dry weight basis. The results from these samples were then compared to those of samples taken from non-exposed control plants maintained in the same growth chamber and the data were expressed as percent control rate and plotted for the sagebrush, short-needle pine, and tall fescue (Figures 3.4, 3.5, and 3.6).

Immediately following exposure, a reduction in both the photosynthetic and dark respiratory rates proportional to the exposure concentration was observed in all plants. In the case of the sagebrush (Figure 3.4), at the high concentrations the photosynthetic rate decreased to zero while the respiratory rate declined to <10% of the controls. However, within two days recovery was apparent, and within two weeks both rates had returned to the control level in the surviving plants (a plant that had died in the high-dose experiments was not sampled). The recovery was aided by the production of new leaves during this time.

The short-needle pine also exhibited a decline following exposure (Figure 3.5) and a general recovery over the following two weeks; however, this recovery was not as rapid as with the other species. This may in part be accounted for by the slower growth rate of the plant during this period. Although new needles were beginning to emerge, statistically they were within the range of the control tissues by the end of the sampling period.

The tall fescue (Figure 3.6) also exhibited a rapid recovery, apparently by virtue of regrowth over the two-week sampling period. Note that the low-dose plants had returned to the control rates within 28 h after exposure, but the high-dose plants, which received only twice as much of the simulant, took a full 14 days to recover.

FIGURE 3.4. Oxygen Electrode Data Expressed as Percent Control Activity for (A) Net Photosynthesis and (B) Dark Respiration of Sagebrush Leaves Following Exposure to Low and High Atmospheric Concentrations of Methyl Salicylate. Data are average $\pm$ SD (n=4).

FIGURE 3.5. Oxygen Electrode Data Expressed as Percent Control Activity for (A) Net Photosynthesis and (B) Dark Respiration of Short-Needle Pine Leaves Following Exposure to Low and High Atmospheric Concentrations of Methyl Salicylate. Data are average $\pm$ SD (n=4).

FIGURE 3.6. Oxygen Electrode Data Expressed as Percent Control Activity for (A) Net Photosynthesis and (B) Dark Respiration of Tall Fescue Leaves Following Exposure to Low and High Atmospheric Concentrations of Methyl Salicylate. Data are average $\pm$ SD (n=4).

For all three plant species, metabolic damage results from the deposition of MES to foliage. This damage is evident at low mass loadings, 1 to 4 $\mu\text{g}/\text{cm}^2$, and affects both respiration and photosynthesis. However, the rapid recovery that did occur would indicate that the damage is related more to loss of foliar surface area as a result of contact phytotoxicity than to long-term metabolic damage to the plant. The residual effects and regrowth studies indicate, however, that MES is transported to the root (systemic) and does have a negative effect on near-term growth.

Chloroplast Electron Transport

In an attempt to determine the basis of the metabolic disruption by MES, subsequent experiments were conducted with isolated plant organelles. Isolated spinach chloroplasts were exposed to MES at a final concentration of 100 ppm to determine whether the simulant would induce any direct effect on a primary plant metabolic process. As seen in Table 3.7, even after incubation within the cuvette for as long as 15 min, there was no apparent interaction of the MES with the electron transport systems of the spinach chloroplasts. This observation supports the suggestion that contact phytotoxicity, perhaps through direct damage of the outer cell membranes of the leaf cells in contact with the simulant, rather than a systemic intercellular action, is the principal mechanism of action for MES.

TABLE 3.7. Interaction of 100 ppm of Methyl Salicylate with Isolated Spinach Chloroplasts

<u>Whole Chain</u>	<u>Electron Transport System (% Control)</u>	
	<u>Photosystem I</u>	<u>Photosystem II</u>
101.3 $\pm$ 8.9 ^(b)	100.5 $\pm$ 3.5 ^(b)	97.38 $\pm$ 4.5 ^(b)

(a) In vitro amendment of simulant, exposure duration approximately 5 min. Data are avg. $\pm$ SD (n=3).

(b) Not significant based on two-tailed t-test, $P > 0.1$.

3.4 TERRESTRIAL PERSISTENCE

3.4.1 Persistence of Methyl Salicylate in Soil

Soils contaminated in the high-dose test were sampled at intervals over a five-day period. At predetermined post-exposure times, MES was extracted from the soils and the extracts were analyzed to determine the amount of MES. The quantity of MES in each sample was divided by the soil or leaf area, resulting in values expressed in terms of $\mu\text{g MES/cm}^2$. The semilogarithmic plot of these values as a function of post-exposure time allowed calculation of the environmental half-life of MES on the different surfaces. The depuration plots of MES in both Burbank (Figure 3.7A) and Palouse (Figure 3.7B) soils were biphasic. Given that MES has a significant vapor pressure, volatilization probably accounts for the observed losses. Of the $370 \mu\text{g MES/cm}^2$ initially deposited to the Palouse soil, only $51 \mu\text{g/cm}^2$ remained after 2 days. In Burbank soil, $<5 \mu\text{g MES/cm}^2$ remained after 2 days.

The initial rapid loss of MES represents volatilization of the surface-deposited material; the slower loss rate seen in the second phase may represent losses due to volatilization and to abiotic and biotic decomposition.

3.4.2 Persistence of Methyl Salicylate on Foliar Surfaces

Depuration rates for the plant canopies were based on the mass loadings, as determined from chromatographic analysis of deposition coupons for experiment MES-03, a high-dose treatment (Figure 3.8). As in the case of the soils, depuration of MES from the foliar surfaces is biphasic. The initial rapid-loss phase had a half-life of 0.9 to 2.3 h for sagebrush, tall fescue, and short-needle pine. The longer depuration component had half-lives of 31, 10, and 6 h for sagebrush, short-needle pine, and tall fescue, respectively. The mass loading for the tall fescue was significantly lower ($3.28 \mu\text{g/cm}^2$) than in experiments with either sagebrush ($35.4 \mu\text{g/cm}^2$) or short-needle pine ($17.4 \mu\text{g/cm}^2$). At most, only $3 \mu\text{g/cm}^2$ (sagebrush) remained associated with the foliar surfaces after 2 days of depuration. It is likely that the cutin and wax surface layers of the foliage affect sorption and thus the subsequent volatilization of MES.

3.5 EFFECT OF METHYL SALICYLATE ON EARTHWORM SURVIVAL

The earthworm bioassay was performed using artificial soil having contamination doses of 56 and 331 $\mu\text{g MES}/\text{cm}^2$. Earthworms were evaluated for survival and activity 1, 10, and 21 days after exposure (Table 3.8). In the low-dose treatment, the survival was 96% after 21 days, with a slight overall loss in activity. At the high-dose level,

FIGURE 3.7. Persistence of Methyl Salicylate on Surfaces of (A) Burbank and (B) Palouse Soils Following Atmospheric Exposure for 60 min at a Concentration of 750 mg/m^3

FIGURE 3.8. Persistence of Methyl Salicylate on Foliar Surfaces of (A) Sagebrush, (B) Short-Needle Pine, and (C) Tall Fescue Plants Following Atmospheric Exposure for 60 min at a Concentration of $750 \text{ mg}/\text{m}^3$

survival decreased to 86%, but activity was judged to be affected in only 20% of the individuals. Given the rapid loss through volatilization on other soil surfaces, we concluded that the worms received only a small exposure even in the high-dose experiments.

3.6 SOIL MICROBIAL EFFECTS OF METHYL SALICYLATE

The inhibition of enzymes that drive key metabolic reactions in microbial cells is one primary cause of a chemical's toxicity to microorganisms. Microbial dehydrogenase enzyme systems catalyze the oxidation of organic material and fill an important role in the carbon cycle of the soil. The assay of soil dehydrogenase activity is a general indicator of the potential activity of the soil microbial population (Skujins 1976). Phosphatases, which can exist extracellularly, are a broad group of enzymes that cleave esters and anhydrides of phosphates from complex organophosphates, and they are believed to be important in the mineralization of this element from soil organic matter (Ramirez-Martinez 1968). For these reasons, activities of these two enzymes were used in this study to assess MES toxicity to soil microorganisms and soil biochemical processes.

TABLE 3.8. Influence of Soil-Deposited Methyl Salicylate on the Survival of Earthworms (*Eisenia foetida*). Artificial soils (70 g) and worms were exposed to MES and held for 21 days post-exposure.

Treatment	Days Post-Exposure	Soil Mass Loading ($\mu\text{g}/\text{cm}^2$)	Earthworm Survival	Earthworm Condition
(avg $\pm$ SD, n=3)				
Control	1	0	30/30	Active
	10		30/30	Active
	21		30/30	Active
Low Dose	1	56.61 $\pm$ 18.40	30/30	Active
	10		30/30	Active
	21		29/30	Moderately Active
High Dose	1	331.81 $\pm$ 93.57	30/30	Active
	10		28/30	Moderately Active
	21		26/30	Moderately Active

The effect of MES on soil dehydrogenase activity is shown in Table 3.9. One day after the addition of MES, dehydrogenase activity in Burbank soil decreased to 67% of the control with a MES amendment of 500 $\mu\text{g/g}$ dry soil; it further decreased to 5% of the control level at 2500 $\mu\text{g/g}$. After 42 days incubation, MES concentration at 100 $\mu\text{g/g}$ or less stimulated enzyme activity (150-157% of control level). The degree of inhibition by the 500 to 1250 $\mu\text{g/g}$ MES amendments remained approximately the same at 42 days as after 1 day. Inhibition by 2500 $\mu\text{g/g}$ MES recovered from 5% to 35% of the control level. In Palouse soil, soil dehydrogenase activity was initially impacted (86 to 89% of control) by the addition of MES at concentrations of 100 $\mu\text{g/g}$ or higher, but activity was greatly enhanced after 42 days incubation. Values for EcD_{50} , an expression of the ecological dose of MES that would cause 50% inhibition (Babich et al. 1983), were calculated from response curves in Figure 3.9 and are presented in Table 3.10. The EcD_{50} of MES for soil dehydrogenase activity in Burbank soil stayed roughly the same after 42 days, and the EcD_{50} for Palouse soil increased from 1338 $\mu\text{g/g}$ to no inhibition by MES. In fact, soil dehydrogenase activity was greatly stimulated with increased incubation time. These findings suggested that the effect of MES on soil dehydrogenase activity was transient and that soil microorganisms might be able to metabolize this simulant, resulting in an increase in their activity. The results also indicate that Palouse soil was impacted less than Burbank soil.

Soil phosphatase activity was not decreased by MES after 1 day (Table 3.11). In fact, it was slightly enhanced (118% of control level) by the addition of MES at 2500 $\mu\text{g/g}$ in Burbank soil and at 100 $\mu\text{g/g}$ in Palouse soil. With prolonged incubation time, soil phosphatase activity declined moderately to 75% and 92% in the Burbank and Palouse soils, respectively. The EcD_{50} values (Table 3.10) calculated from response curves in Figure 3.10 indicate that the ecological dose of MES that would cause a 50% inhibition of soil phosphatase activity is far greater than the highest MES concentration tested (2500 $\mu\text{g/g}$).

The data show that, in general, the effects of the chemical simulant MES on microbially mediated soil enzyme activities depend on the simulant concentration, the soil enzyme studied, the soil type, and the length of incubation. At 25 to 100 $\mu\text{g/g}$, MES did not significantly impact the activities of soil enzymes studied. Soil dehydrogenase activities were impacted only at concentrations greater than 500 $\mu\text{g/g}$ in the Burbank soil, and Palouse soil dehydrogenase activity was greatly enhanced by MES with increased incubation time. The impact on soil dehydrogenase activity was, in general,

more severe than the impact on soil phosphatase activity, indicating that dehydrogenase activity might serve as a more sensitive indicator of the toxicity of this simulant. The Burbank sandy loam, which has soil with low organic carbon and nitrogen, was more susceptible to MES than the Palouse silt loam, which has higher organic carbon and nitrogen, suggesting that soil organic matter may have an important role in reducing the toxicity of this simulant in the environment. The effects of MES on soil enzyme activities decreased with increased incubation time, indicating that the simulant is not persistent in the soil.

TABLE 3.9. The Effect of Methyl Salicylate on Burbank and Palouse Soil Dehydrogenase Activities

MES Concentration (μg/g)	Dehydrogenase Activities % of Control(a)			
	Burbank Soil		Palouse Soil	
	Incubation Time			
	1 day	42 days	1 day	42 days
0	100 (9)	100 (7)	100 (5)	100 (10)
25	94 (8)	150 (13)*	102 (7)	111 (11)
100	94 (8)	157 (10)*	86 (6)*	120 (9)*
500	67 (6)*(b)	58 (3)*	62 (3)*	135 (10)*
1250	47 (3)*	50 (6)*	66 (9)*	134 (10)*
2500	5 (2)*	35 (7)*	9 (1)*	135 (11)*

(a) Mean ($\pm$ standard deviation), n=3.

(b) *Denotes significantly different from control (0 μg MES/g dry soil) based on Student's t-test, $P \leq 0.05$

TABLE 3.10. The Effect of Methyl Salicylate on Enzyme Activities in Burbank and Palouse Soils, Expressed as EcD_{50} Values

Days Post-Exposure	Dehydrogenase		Phosphatase	
	Burbank	Palouse	Burbank	Palouse
	(EcD ₅₀ , $\mu\text{g/g}$ soil) ^(a)			
1	1195	1338	stimulated	>>2500
42	1250	stimulated	>>2500	>>2500

(a) EcD_{50} represents the ecological dose that caused a 50% inhibition of the soil enzyme activity.

FIGURE 3.9. Response Curve for the Effect of Methyl Salicylate on Dehydrogenase Activities in Burbank and Palouse Soils

TABLE 3.11. The Effect of Methyl Salicylate on Burbank and Palouse Soil Phosphatase Activities

MES Concentration (µg/g)	Dehydrogenase Activities % of Control(a)			
	Burbank Soil		Palouse Soil	
	Incubation Time			
	1 day	42 days	1 day	42 days
0	100 (6)	100 (5)	100 (8)	100 (2)
25	83 (13)	100 (5)	107 (6)	99 (5)
100	85 (10)	114 (6)•(b)	118 (7)•	106 (4)
500	89 (16)	104 (5)	94 (7)	105 (3)•
1250	96 (14)	96 (5)	97 (6)	122 (3)•
2500	118 (6)•	75 (6)•	101 (9)	92 (1)•

(a) Average ($\pm$ standard deviation), n=3.

(b) ^{*}Denotes significantly different from control (0 µg MES/g dry soil) based on Student's t-test, $P \leq 0.05$

FIGURE 3.10. Response Curve for the Effect of Methyl Salicylate on Phosphatase Activities in Burbank and Palouse Soils

4.0 CONCLUSIONS

Like many other simulants, MES has a relatively high vapor pressure, which caused both aerosol and vapor phases to be present within the exposure chamber. Analysis of the results from the low- and high-air-concentration generations indicates that air concentrations of MES were a mixture of aerosol and vapor, with vapor dominating in the low-concentration exposures. This particle-vapor partition affected the overall rate of deposition to surfaces. The deposition velocity for MES to soil surfaces was 0.140 cm/sec. For foliar surfaces, deposition velocities ranged from 0.001 to 0.003 cm/sec at low air concentrations and from 0.001 to 0.014 cm/sec for high-dose exposures. Collection efficiency was greatest for sagebrush; efficiencies for short-needle pine and tall fescue were similar but less than that for sagebrush.

The volatility of MES results in its rapid loss from soil and foliar surfaces. The residence times or half-lives of MES deposited to soils was 0.6 to 1 h for the fast depuration phase in Burbank and Palouse soils. The second depuration phase had a half-life of 17 h in Burbank soil and 116 h in Palouse soil. Although the initial release component was similar for both soils, the second component was likely influenced by the sorptivity or porosity of the soils. Methyl salicylate deposited to foliar surfaces also exhibited biphasic depuration curves. The half-life of the short-residence-time component ranged from 1 to 2 h, while the longer-residence-time component had half-lives of 5 to 31 h. Retention on foliar surfaces was greatest for sagebrush and lowest for tall fescue.

Methyl salicylate is phytotoxic at foliar mass-loading levels of <1 to $2 \mu\text{g}/\text{cm}^2$, with sagebrush being more sensitive than grass or pine. At higher mass-loading rates, 4 to $46 \mu\text{g}/\text{cm}^2$, severe damage was evident in both sagebrush and tall fescue. Tall fescue was more affected than was short-needle pine, even though the pine received a higher dose. Regrowth of tall fescue indicated that the effects of MES are residual; growth rates were affected even following the second harvest, but only at the higher exposure concentrations. In vitro studies showed MES to adversely affect both photosynthesis and respiration. However, recovery occurred within 4 to 14 days as new growth was initiated. Evaluation of the effects of MES on photosynthetic electron transport failed to demonstrate any effect on the processes, indicating that MES may simply disrupt membrane function and cell integrity.

Results from in vitro testing with MES indicated that concentrations below 100 µg/g dry soil generally did not negatively impact soil microbial activity. In fact, after 42 days incubation, Palouse soil dehydrogenase activity was enhanced by the addition of MES. The short-term effect of MES (1-day incubation) on soil dehydrogenase activity ($EcD_{50} = 1195 \mu\text{g/g}$) was more profound than it was on soil phosphatase activity (stimulated; EcD_{50} could not be calculated), indicating that soil dehydrogenase is more susceptible to MES than is soil phosphatase. However, soil enzyme activities had increased, recovered, or been enhanced after 42 days incubation, indicating that the effect is transient, with a recovery trend. Although in vitro testing usually provides an indication of a dose response and may be representative of a spill or waste-disposal situation, it is not a true representation of an aerosol deposition. Such factors as mass loading, wind speed, and relative humidity can vary, so that the influence of MES on soil microbial and biochemical activity may be different following an actual depositional event.

Results of the earthworm bioassay indicate survival to be 96% at soil doses of 56 µg MES/cm² soil. At higher dose levels, 331 µg MES/cm² soil, survival decreased to 86% after 21 days exposure. Earthworm mobility or activity was noticeably affected only after 10 days at the higher exposure concentrations.

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APPENDIX A

QUALITY ASSURANCE AND QUALITY CONTROL

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QUALITY ASSURANCE AND QUALITY CONTROL

At the Pacific Northwest Laboratory (PNL), quality control (QC) is the system of activities that ensures a quality product. Quality assurance (QA) is the system of activities that ensures that the quality control system is being performed adequately. The prime responsibility for QA/QC is placed on the Program Manager, Principal Investigator, and Task Leaders; however, the QA audit function is maintained outside of the project and is not directly supported by the project. This audit function was performed by Rob Cuello, the Earth and Environmental Sciences Center's senior Quality Assurance Officer, who was outside the control of the Program Manager or the Principal Investigator.

QUALITY ASSURANCE

The objective of the QA program for this study was to ensure the production of data that are precise and accurate (within the stated acceptance criteria), representative, comparable, traceable, and defensible. To ensure that these conditions were met, PNL's QA program consisted of the following elements: 1) review and approval of standard operating procedures, 2) personnel training, 3) pretest preparation 4) QC of sampling function and analysis function, 5) QA audits, 6) feedback and corrective action (if necessary).

Quality Assurance Training

The activities of the QA training program included 1) introducing personnel to the QA program, 2) identifying training elements, 3) preparing and training supervisory and test personnel, 4) implementing QA audits, and 5) conducting refresher QA training or on-the-job training as needed.

The project team reviewed the procedures that governed the collection and interpretation of field and/or laboratory data and familiarized themselves with what audit procedures were to be used. Supervisory personnel required some training to learn the QA procedures that were employed.

Standard Operating Procedure Review and Approval

Standard operating procedures (SOPs) for this program were developed by the Task Leaders, approved by the Principal Investigator, and reviewed to determine what quality control steps would be incorporated. Each team member used the procedures outlined in the SOPs to 1) prevent contamination, 2) provide the proper sample size, 3) ensure proper taxonomic identification, 4) provide the proper kind and number of blanks, 5) maintain standardization of measuring equipment, 6) guarantee the keeping of usable records.

Quality assurance also ensured that current SOPs were maintained for the program. Whenever it became evident that the data being collected were not sufficiently accurate or appropriate for the intent of the program, the SOP was modified and approved by the Program Manager.

Laboratory Record Books

Work performed on the project was documented in laboratory record books by project personnel. A signature sheet that identified the personnel contributing data to the record book and an assignment form (located inside the front cover of the record book) that indicated the project number and the individual to whom the book was assigned was completed. At the end of the project, the record books and forms were sent to Document Control to be archived.

Sample Identification and Traceability

A system of accountability was used to control the number and variety of samples and the quality of data generated on the research project. The Principal Investigator helped to design the initial system and, because he worked most closely with the experiments, helped monitor the system's effectiveness. Quality assurance, at this stage of the project, was used to ensure that the samples were completed and appropriate. Therefore, the QA Officer

- participated in the development of a system for keeping records of samples and for introducing proper QA steps to ensure dependability of the system

- reviewed sample log system and implementation forms that provide information on samples collected and indicate the work to be done on the samples
- monitored the sample identification system to ensure proper labeling of samples, proper dispersements of samples for analysis, and proper quality control of spikes, blanks, and duplicates.

The Principal Investigator and the Task Leaders were responsible for seeing that the documentation was completed either by themselves or by the team members. A team member was usually assigned the responsibility of handling the samples and storing, retrieving, dispersing, and maintaining records of those samples.

QUALITY CONTROL

The key QC operations emphasized in the program were procurement QC, standardization/calibration, sampling, and analysis.

Procurement Quality Control

Task Leaders were responsible for ensuring that all procured materials (e.g., samples, collection containers) conformed to appropriate specifications. They were also responsible for ensuring that reagents and chemicals with limited shelf-life were identified and used before the specified expiration date.

Sampling Quality Control

A sampling-information document was developed by the Task Leader and used to detail the kind of samples that were taken, the locations where the samples were taken, the time and duration of sampling, the size of samples to be taken, and other pertinent information on the conditions that are useful to the sampling team. From this information, the sampling team selected the labor hours and apparatus necessary to carry out the sampling task and followed the appropriate SOP.

The sampling team then developed a QA plan that included 1) sampling information forms; 2) lists of apparatus, reagents, and supplies; 3) presampling calibrations; 4) onsite checks of apparatus; and 5) post-sampling calibrations.

Analytical Quality Control

Analytical quality was monitored through function checks and control checks. Function checks are performed by the analyst who verified the stability and validity of the sample and the performance of the analytical equipment. Sample validity was assessed in terms of spoilage, container integrity, amount of specimen, sample identification, sample blanks, and general appearance (such as condition of filters or uniformity of sample collection across a filter).

The analytical equipment was checked in terms of calibration and performance of calibrating standards; the latter is part of the permanent record of the analysis. It was recommended that calibration standards that span the working range in factors of two should be run through the entire analysis system at least four times. This develops information on precision and detection limits where appropriate.

Control checks were made by analyzing samples provided by the Task Leader. These samples included blanks, duplicates, spikes, and, if available, standard reference materials in quantities that depended on the total number of samples assigned and on the level of accuracy needed in the analysis. These control samples were introduced into the system in such a manner that the analyst did not give them particular attention.

As a rule, when processing large batches of samples (25 samples or more), a control (spike, blank, or replicate) sample should be run after every five samples. A small batch of samples (up to five samples) may have more control samples than test samples.

For large and continuous amounts of samples, control charts of performance of duplicates and spikes must be maintained. This allows the analyst and Task Leader to know when the system is out of control, which part of the system is the probable cause, and when and what corrective action is to be taken.

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