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Ecological Research Series

ETHYLMERCURY: FORMATION IN PLANT TISSUES AND RELATION TO METHYLMERCURY FORMATION

Environmental Monitoring and Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
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ETHYLMERCURY: FORMATION IN PLANT TISSUES AND RELATION TO METHYLMERCURY FORMATION

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FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound integrated monitoring data base through multidisciplinary, multimedia programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report presents data demonstrating the biotransformation of elemental mercury in plants. The mercury, taken up as a vapor in its elemental state, is converted by the common garden pea to the toxic organomercurials, methylmercury and ethylmercury. The significance of this biotransformation will be considered by EPA and others in making decisions concerning regulations of activities known to generate mercury emissions. For additional information, please contact the Pollutant Pathways Branch, Environmental Monitoring and Support Laboratory-LV, P. O. Box 15027, Las Vegas, Nevada 89114.

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INTRODUCTION

Increased utilization of coal for energy production and continued industrial use of mercury may be expected to increase the levels of mercury pollutants in the environment in the future. Data are needed to define the effects of mercury pollutants on plants, the uptake and distribution of mercury within plants, and the species of mercury present and their biotransformations within plants. Such data are essential to fully describe the impact of mercury on plants.

The objective of this study was to examine the fate of one possible mercury pollutant, elemental mercury, in the tissues of Pisum sativum.

CONCLUSION

Both ethylmercury and methylmercury are formed in the pea following exposure to elemental mercury vapor. The pattern of change in concentration suggests both are metabolites of a single pathway. Methylmercury is identified as an intermediate product of the pathway. The exact role of ethylmercury in this pathway cannot be defined at this time.

MATERIALS AND METHODS

Plant Material: Seeds of Pisum sativum, cv. Little Marvel were grown hydroponically in a greenhouse for 15 days before exposure.

Exposure Conditions: Seventy seedlings were placed in a Plexiglas® chamber of 58,000 cm^3 volume, with 10 ml of elemental mercury in a tray placed on the floor of the chamber. The chambers were placed into a hood with constant air flow in order to maintain a temperature of $25^{\circ} \pm 1^{\circ}$ C, regardless of lighting conditions. Incandescent lighting at 110 $\mu E\ m^{-2}\ sec^{-1}$ was used for a 12-hour daily light period. The concentration of elemental mercury vapor (Hg^{0}) in the chamber was determined by gas chromatography and calculated to be 1.71 ppb (Long, Scott and Thompson, 1973) within 30 minutes. A saturation level of 7.35 ppb was reached at 3 hours.

Tissue Extraction: Tissue was ground by mortar and pestle with 2.2N HCl (2 ml/g). The resultant brei was centrifuged at 10,000 rpm for 30 minutes. The supernatant fluid was then decanted and extracted with an equal volume of benzene (re-distilled nanograde) by vigorous shaking for 1 minute followed

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by centrifugation at 10,000 rpm for 10 minutes. This benzene fraction was passed through Whatman Phase Separating paper and then analyzed by gas chromatography.

The efficiency of the benzene extraction, as determined in our laboratory, agreed with the average of 76% reported by Talmi (1975). The efficiency of the extraction procedure for plant material was determined by extraction of 5-g plant samples to which known quantities of methylmercury (CH_3HgCl) and ethylmercury (C_2H_5HgCl) had been added. Efficiency of the extraction was determined to be 74% ± 2% for CH_3HgCl and 69% ± 2% for C_2H_5HgCl. When a second benzene extraction of the aqueous phase was performed, an additional 21% of the original CH_3HgCl and 20% of the added C_2H_5HgCl was obtained.

For convenience and reduced handling of large numbers of samples during time-course experiments, the second benzene extraction was dropped. Having determined the benzene extracted to be 76% efficient, standards were prepared by a method similar to the plant extractions, i.e., known amounts of CH_3HgCl and C_2H_5HgCl were added to 2.2N HCl, which was extracted with an equal volume of benzene. When this is used as the standard, reported values can be considered to be 95% ± 3% of actual concentration, without the necessity of using a correction factor.

Sample Analysis: A Gas Chromatograph-Microwave Emission Spectrometer (GC-MES) system (Talmi, 1975) with a Gay-Frank optical system modification (Gay and Frank, 1977) was used for analysis of both air and benzene samples with the following operating conditions: Column: 3-foot by ¼-inch glass, packed with 4% FFAP (80/100 mesh) on Supelcoport® (Supelco Inc., Bellefonte, PA); Column temperature: 150° C for benzene samples, 30° C for air samples; Carrier gas: ultrapure argon at a flow rate of 11 ml/min; Temperature of injection port and detector: 225° C; Microwave generator output: 50 W incident, 3 W reflected power; Photomultiplier voltage: 500 V; Monochromator slit width: 70 μm; and Spectral wavelength: 253.7 nm. The concentration of mercury compounds, expressed as nanograms per gram (ng/g) in this report, represents nanograms Hg^+ per gram fresh weight of identical tissues of control plants. This convention was necessary since the effect of Hg^0 toxicity is dessication of the leaf.

RESULTS

Initially, plants were exposed to elemental mercury for 24 hours to determine whether any organomercury compounds would be formed. As indicated in Table 1, $C_2H_5Hg^+$ and CH_3Hg^+ were detected in all parts of the plant. There was no definite pattern of distribution of either metabolite within the plant with the exception of consistently high levels of both mercury species in the oldest lateral branch (stem and leaf). This lateral was the first to show visible signs of mercury toxicity. The pea, having a single growing point, was an ideal plant for study of a gas known to effect plant senescence (Speitel and Siegel, 1975). Damage to the plant occurred in a gradient from the first lateral to the top of the plant. The effects of mercury vapor

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toxicity consisted of wilting, followed by severe dessication of the leaf. An exposure duration was lengthened, each successive lateral up the stem wilted, then became severely dessicated, although no abscission occurred during the 48-hour period.

TABLE 1. THE FORMATION OF ETHYLMERCURY AND METHYLMERCURY IN THE PEA AFTER EXPOSURE TO ELEMENTAL MERCURY VAPOR FOR 24 HOURS (Values reported are the average of triplicate samples and represent ng Hg^+/gram fresh weight of identical tissue of control plants.)

Plant Part	$C_2H_5Hg^+$ (ng/g)	CH_3Hg^+ (ng/g)
1st Lateral*	12.82 ± 0.60	19.21 ± 12.82
2nd Lateral*	9.93 ± 0.35	8.07 ± 1.77
3rd Lateral*	5.40 ± 0.05	4.21 ± 0.79
4th Lateral*	8.05 ± 1.81	9.95 ± 7.05
5th Lateral*	6.99 ± 3.59	6.49 ± 0.47
Apex	11.00 ± 1.67	17.26 ± 5.66
Stem	5.22 ± 3.33	10.77 ± 7.59
Root	4.85 ± 1.34	11.55 ± 6.35
Control	N.D.**	N.D.**

*Includes both leaf and stem, the 1st lateral branch being the lowest and oldest branch off the central axis.

**None detectable in any plant part.

In an attempt to define the relationship between the two mercury compounds, a time-course of exposure was performed. Concentrations of both CH_3Hg^+ and $C_2H_5Hg^+$ varied considerably over the 48 hours of exposure (Figure 1). In the aerial portion of the plant $C_2H_5Hg^+$ concentration rose during the 48-hour period from 2.20, 4.05, and 4.25 ng/g to 25.92, 19.48, and 24.07 ng/g for the stem, apex, and laterals, respectively. A similar rise in concentration was not observed for CH_3Hg^+. Concentrations ranged from 1.79 ng/g to 17.28 ng/g in the aerial portion of the plant, with an average concentration of 4.66 ng/g. It is significant that in all aerial parts the CH_3Hg^+ concentration after 48 hours of exposure was nearly the same as that recorded at the first extraction (4 hours).

Comparison of graphs a, b, and c of Figure 1 indicates a distinct pattern of concentration change in the aerial portion of the plant. Periods of

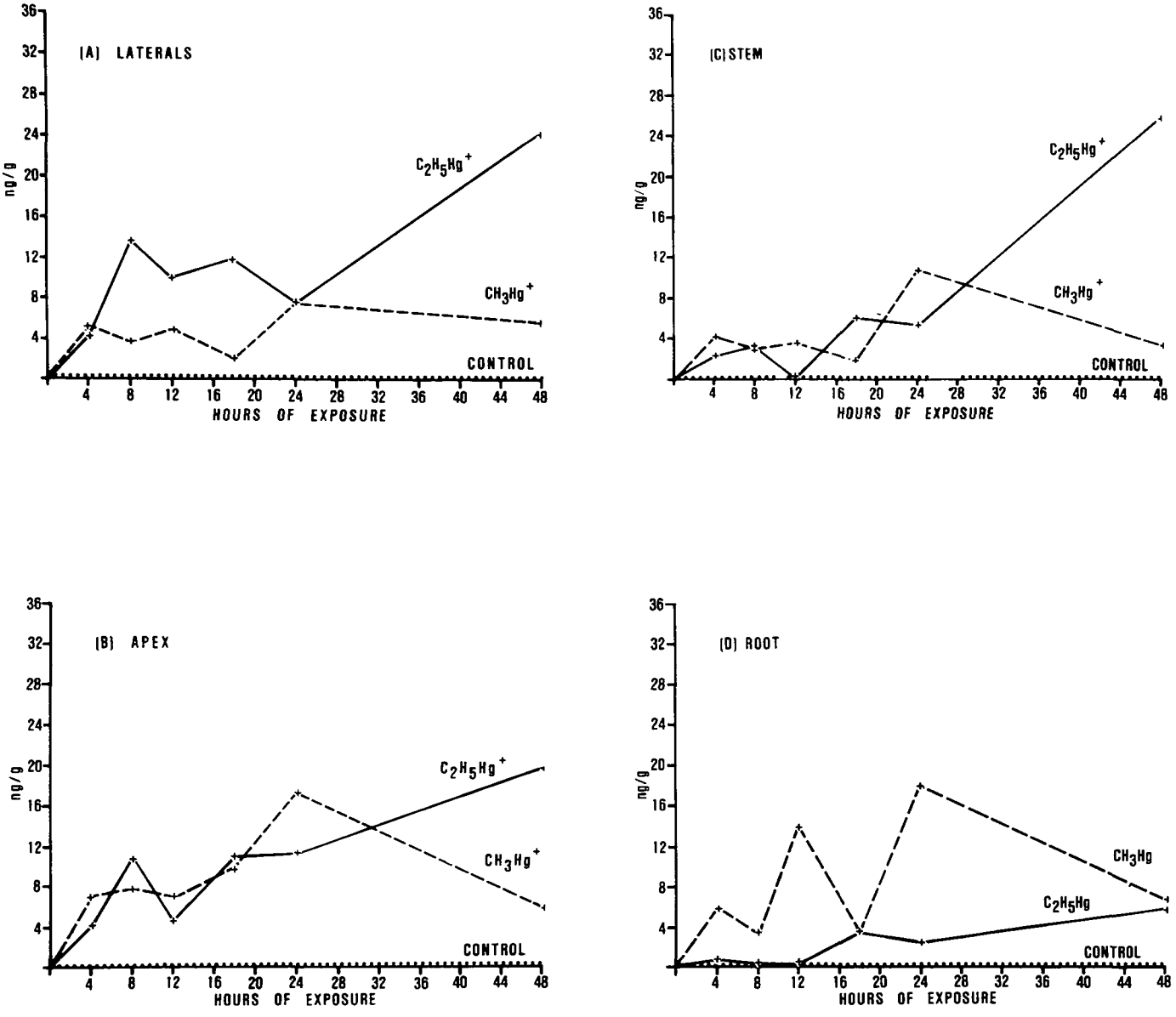

Figure 1. Changes in the concentration of ethylmercury and methylmercury in various plant parts of peas exposed to elemental mercury vapor for 48 hours.

high $C_2H_5Hg^+$ concentration are periods of low CH_3Hg^+ concentration, and vice versa, so that the two curves are similar but are out of phase.

To elaborate this relationship more fully, peas were exposed to elemental mercury vapor for a period of either 24 hours light or 24 hours continual darkness. The results indicate nearly twice as much $C_2H_5Hg^+$ formed in the laterals and apex during the light period than in the dark (Figure 2). No $C_2H_5Hg^+$ could be detected in the stem or root under conditions of continuous darkness, despite concentrations of CH_3Hg^+ comparable to those recorded for the plants exposed in the light. Light had no significant effect on the levels of CH_3Hg^+ in the plant.

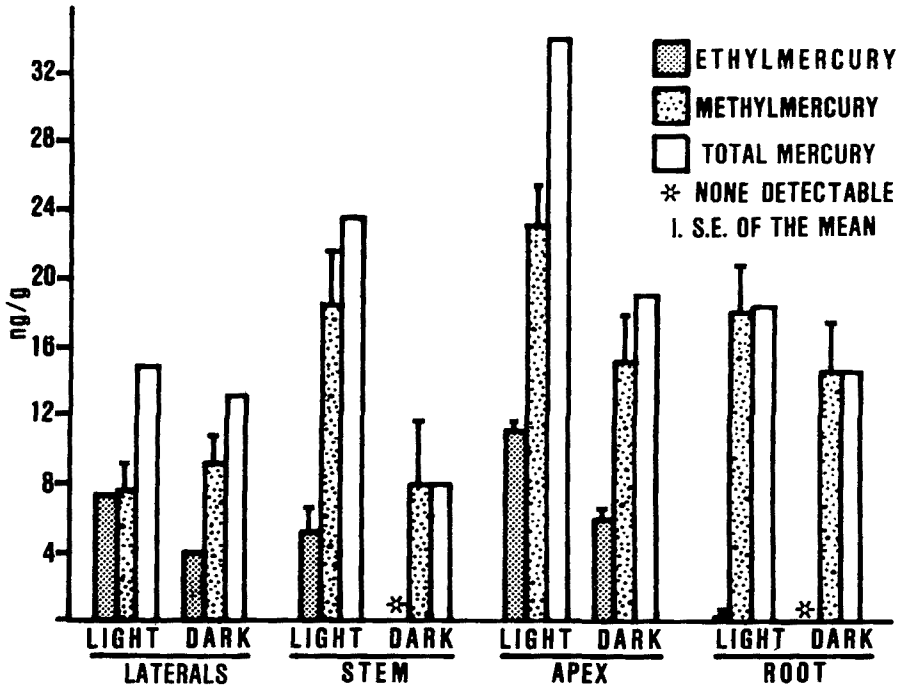

Figure 2. The effect of light on ethylmercury and methylmercury concentration in Pisum sativum after a 24-hour exposure to mercury vapor.

Both $C_2H_5Hg^+$ and CH_3Hg^+ were detected in the root, although appreciable concentrations of $C_2H_5Hg^+$ were not observed until after 12 hours of exposure to Hg^0. The presence of these compounds can be explained by two possible mechanisms. Either they were formed in the root from mercury taken up from the water in which plants were grown, or they were transported from the aerial portion of the plant to the root. A less likely possibility is that all mercury detected in the aerial portion of the plant originated in the root. To determine the source of the organic mercury, the roots were "isolated" from the mercury vapor by placing the roots of seedlings grown in 5-ml microbeakers into a 50-ml test tube, care being taken to form a tight seal between the beaker and test tube. A layer of activated charcoal was placed across the bottom of the microbeaker and was covered by a layer of vermiculite. As is indicated in Table 2, no $C_2H_5Hg^+$ or CH_3Hg^+ was detected in the "isolated"

roots after a 24-hour exposure, despite formation of appreciable concentrations of both organomercury compounds in the aerial portions of the plant.

TABLE 2. THE EFFECT OF ISOLATING THE ROOT MEDIUM FROM ELEMENTAL MERCURY VAPOR. (Exposure time was 24 hours.)

	Root Medium Isolated		Root Medium Exposed	
	$C_2H_5Hg^+$ (ng/g)	CH_3Hg^+ (ng/g)	$C_2H_5Hg^+$ (ng/g)	CH_3Hg^+ (ng/g)
Apex	15.99	15.45	12.67	11.62
Lat	15.6	14.35	6.73	4.99
Stem	15.88	N.D.*	8.04	3.18
Root	N.D.*	N.D.*	4.25	6.43

*None Detectable.

DISCUSSION

The formation of both $C_2H_5Hg^+$ and CH_3Hg^+ in peas exposed to Hg^0 offers some fresh insight into the nature of the mercury pathway of the pea. Methylmercury formation in the pea has been reported previously by Gay (1975). In both in vivo and in vitro experiments with inorganic mercury salts, CH_3Hg^+ was identified as an intermediate product. The data reported here suggest that it may also be an intermediate in the biotransformation of Hg^0. This is supported by the following observations.

(a) Although the level of $C_2H_5Hg^+$ increased during the 48-hour exposure period, no corresponding rise in CH_3Hg^+ was observed. A baseline average of 4.66 nanograms CH_3Hg^+ per gram was maintained in the aerial portion of the plant with concentrations at 48 hours being nearly the same as those recorded at 4 hours.

(b) Fluctuations in CH_3Hg^+ concentrations between replicate samples (Table 1 and Figure 2) suggest an intermediary role. The release of volatile mercury products from plants has been reported by Siegel (1974). His work resulted from the observation of analytical inconsistencies in data from replicate leaf samples. In this study, we observed similar inconsistencies in the CH_3Hg^+ data. However, standard error for $C_2H_5Hg^+$ data was small. The efficiency of the extraction and analytical procedures being similar for both CH_3Hg^+ and $C_2H_5Hg^+$, inconsistencies should have been observed for both, but were not. The apparent inconsistencies appear to be artifacts of the time intervals between extractions and the inability to detect other inter-

mediates or final mercury products, possibly because of their volatile nature.

(c) Further verification of this role as intermediate is indicated by the failure of light to have any significant effect on CH_3Hg^+ concentration despite a two-fold increase of $C_2H_5Hg^+$ in the same tissue (Figure 2).

Following the report that Hg^0 vapor induces ethylene formation and abscission in Coleus and Citrus explants (Goren and Siegel, 1976), $C_2H_5Hg^+$ was considered a likely metabolite in the mercury pathway. The accumulation of $C_2H_5Hg^+$ observed in this investigation verifies this initial assumption, but does not enable definition of its role as initial product, intermediary, or final product in this biochemical pathway.

The pattern of concentration changes observed (Figure 1) does indicate that CH_3Hg^+ and $C_2H_5Hg^+$ are both metabolites of a single pathway of mercury in the pea. In the aerial portion of the plant, a high concentration of either metabolite is followed by a high concentration of the other metabolite, with a simultaneous decrease in the concentration (or rate of increase) of the former. The net result of this would be to prevent accumulation of high concentrations of any single mercury metabolite, presumably by conversion to other, presently unidentified, mercury products.

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16. ABSTRACT

Seedlings of the common dwarf garden pea, Pisum sativum, cv. Little Marvel, exposed to elemental mercury vapor formed both methylmercury and ethylmercury in all parts of the plant. Concentrations of both organomercury compounds fluctuated considerably over a 48-hour exposure period, but the total of detectable forms of mercury continued to rise due to increased ethylmercury formation. Ethylmercury formation was greater in the light than in the dark, but methylmercury concentration did not differ significantly. The pattern of change in the concentrations of methylmercury and ethylmercury suggests both are metabolites of a single mercury pathway in peas.

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