residues are readily available to aquatic organisms.

This study has shown that HCB has considerable potential to bioaccumulate in the aquatic environment, and is very persistent when soil incorporated. The combination of these two characteristics makes HCB a potentially hazardous compound to the environment. A soil contaminated with a large amount of HCB would presumably retain HCB for many years. If this soil is removed by erosion, HCB can be introduced into a nearby aquatic environment and become available to aquatic organisms. Thus, known HCB sources should be prevented from directly or indirectly being introduced into waterways.

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Received for review April 12, 1976. Accepted August 5, 1976. Mention of a proprietary product does not constitute a warranty of the product by the U.S. Department of Agriculture nor imply its approval to the exclusion of other suitable products.

Translocation and Metabolic Fate of Monosodium Methanearsonic Acid in Wheat (*Triticum aestivum* L.)

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The distribution and metabolic fate of the arsenical herbicide monosodium [^{14}C]methanearsonate (MSMA) in wheat (*Triticum aestivum* L. var. Waldon) was investigated 2, 4, and 13 weeks following foliar applications. Atomic absorption was used to analyze arsenic and liquid scintillation spectrometry was used to analyze ^{14}C residues in roots, leaves, and seeds harvested from wheat. Results suggested primarily symplastic, but some apoplastic movement of [^{14}C]MSMA. A total of only 0.2% of the leaf applied 89 μg of [^{14}C]MSMA was detected in the seeds. ^{14}C (20% of that applied) exuded out of the roots into the soil. Using unlabeled MSMA at 3.36 kg/ha resulted in increased residues in wheat seed grown in greenhouse conditions. Thin-layer and ion-exchange chromatographic analyses of extracts from root, shoot, treated leaves, and seeds indicated that the carbon–arsenic bond remained intact during the 3-month study.

The use of organic arsenical herbicides has increased over the last decade. MSMA/DSMA (monosodium and disodium methanearsonate) is registered for selective weed control in cotton and citrus crops (*Fed. Regist.*, 1972). Application is postemergence because the organic arsenicals have little preemergence activity at rates used for weed control (Hiltbold, 1975).

Application is by directed spray before bloom to minimize residues in the cottonseed (Baker et al., 1969). Their primary entrance into plants is through leaves and stems. The degree of absorption and translocation of methanearsonates depends upon the rate of application, temperature, and plant species (Arle and Hamilton, 1971; Keeley and Thullen, 1971). Several workers have reported that methanearsonates are translocated by acropetal (apoplastic), as well as basipetal (symplastic), processes (Rumburg et al., 1960; Sckerl and Frans, 1969; Duble et al., 1969; Sachs and Michael, 1971; Keeley and Thullen, 1971).

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There is little evidence that the C–As bond of MSMA is severed in plants. Very little $^{14}CO_2$, which indicates bond rupture, was evolved from [^{14}C]MSMA treated purple nutsedge (Duble et al., 1968) or coastal Bermuda grass (Duble et al., 1969). MSMA is usually found unchanged or in a complexed form in plants. Sachs and Michael (1971) showed that in bean plants (*Phaseolus vulgaris* L.), MSMA formed a complex with some plant component. Similar complexes were reported by Sckerl and Frans (1969) in Johnsongrass treated with MAA (methanearsonic acid), and by Duble et al. (1969) in coastal Bermuda grass (*Cynodon dactylon*) treated with DSMA. Braman (1975) suggested that grass sprayed with arsenicals may metabolize them to gaseous methylarsines, but Sachs and Michael (1971) did not detect arsine gas evolution from bean plants treated with cacodylic acid.

Wild oat (*Avena fatua* L.) is considered a major weed problem on approximately 100 million acres of land in the United States and Canada. Estimated yearly crop losses from wild oat are $1.5 billion in the Northern United States and adjoining Canadian provinces. MSMA has shown considerable promise as a foliar spray to control wild oats in wheat (Moore, 1975). However, basic information concerning uptake, translocation, metabolic fate, and persistence of MSMA in wheat plant or seeds is not known. This study was undertaken to determine the movement,

distributional pattern, uptake, and metabolic fate of MSMA in wheat plant and seed.

METHODS AND MATERIALS

Experiment I. (1) *Treatment Solution.* [^{14}C]MSMA was prepared from [^{14}C]MAA as follows. A [^{14}C]MAA stock solution was prepared containing 5.0 μCi dissolved in 100 ml of water (sp act. 28 μCi/mg). A 10-ml aliquot of the stock solution was reduced to 1-ml volume. Ansurf 286 (a product of The Ansul Co.) surfactant solution was added to it. The pH of the solution was adjusted to 6.0 (to obtain [^{14}C]MSMA with NaOH and diluted to 2.0 ml with distilled water.

(2) *Plant Material and Treatment.* Wheat seeds were sown in 15-cm plastic pots containing Matapeake silt loam (pH 5.3; organic matter 1.5%; sand, silt, and clay, 38.4, 49.4, and 12.2%). Soil in each pot was supplemented with about 10 g of 10-10-10-2 (N-P-K-Mg) fertilizer. The pots were kept in the greenhouse at about 25 °C and were watered daily. One week after germination, at the two–three leaf developmental stage, each seedling was treated with 2.5 μCi of [^{14}C]MSMA (89 μg/plant). The treatment was such that each of the first two leaves was spotted with five 1-μl drops. The treated plants were harvested 2, 4, and 13 weeks after treatment. The roots were thoroughly washed in running tap water, and the plants separated into treated leaves, roots, shoot, and seed heads, the latter from the 90-day harvest. The chaff removed from the seeds was discarded.

(3) *Extraction and Cleanup.* To follow the distribution of radioactivity in the plant and to prepare the samples for further characterization, the treated leaves, roots, shoots, and seed tissues were extracted with 80% methanol in Soxhlet extractors for 12 h. Aliquots of the methanol extracts were placed in 10 ml of liquid scintillation solution (prepared by mixing 750 ml of Triton X-100, 2250 ml of *p*-xylene, 16.5 g of 2,5-diphenyloxazole (PPO), 1.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP)) and assayed by liquid scintillation spectrometry (LSS).

The procedures of Still and Mansager (1973) were modified for cleanup of the extracts prior to thin-layer chromatography (TLC). Equal amounts of chloroform and water mixture (2:1, v/v) were added to the MeOH extract. The resulting mixture was shaken and allowed to separate into two phases. Both were collected in separate flasks. This step was repeated and the lower and upper phases were saved separately. The nonpolar, chloroform phases were combined, extracted with an equal amount of methanol and water mixture (2:1, v/v), and assayed for ^{14}C. No ^{14}C activity was detected and, therefore, the mixture was discarded. The two upper polar phases were combined, flash evaporated to 10 ml at 40 °C, and analyzed by ion-exchange chromatography.

Wheat seeds containing nonmethanol extractable (or bound) ^{14}C residues (residues after Soxhlet extracting) were subdivided and assayed both by combustion to $^{14}CO_2$ and further vigorous extraction (Schuth et al., 1974). Methanol extracted seed samples and new or nonextracted seed samples were extracted sequentially with cold trichloroacetic acid (Cl_3CCOOH), 75% EtOH, and hot Cl_3CCOOH (Woolson et al., 1976). All fractions including combustion of the extracted residual material were assayed for radioactivity by use of LSS.

(4) *Thin-Layer Chromatography.* Extracted ^{14}C was compared with standard [^{14}C]MSMA by concentrating the aqueous phase to 2.0 ml under a gentle stream of N_2. Aliquots varying from 5 to 15 μl were chromatographed on silica gel and cellulose coated plates. The eluting solvents were methanol–10^{-3} M ammonium hydroxide–10% Cl_3CCOOH–water (50:15:5:30) (Von Endt et al., 1968) for silica gel plates, and pyridine–ethanol–water (1:1:1) for cellulose plates (Sckerl and Frans, 1969). Radioactive areas were detected by autoradiography using no-screen x-ray film.

(5) *Ion-Exchange Chromatography.* To further verify the identity of the recovered radioactivity, a cation-exchange column, 23 cm × 1.9 cm (i.d.), filled with Dowex 50W-X8 resin (50–100 mesh), was used to separate the ^{14}C-labeled compounds in the aqueous phase. The detailed procedures for ion-exchange chromatography have been described elsewhere (Yamamoto, 1975).

(6) *Sample Preparation for Gas–Liquid Chromatography.* As a further check on identity of MSMA in the cold Cl_3CCOOH extract, methylarsine was generated by reduction and detected by GLC. The reducing reaction vessel was a three-necked separatory funnel containing 100 ml of 4% $NaBH_4$ and 1 pellet of KOH, in a N_2 atmosphere. The openings on the flask were covered with a balloon, a septum, and a stopper. One milliliter of Cl_3CCOOH extract (20% in HCl) was injected through the septum into the reduction mixture. An alkylarsine–H_2 gas sample was taken immediately from the gaseous phase above the reaction mixture and injected into the GLC. Retention times for authentic samples of methylarsine, dimethylarsine, and trimethylarsine were 2.0, 4.3, and 6.2 min, respectively, for our conditions. The evolved gas was removed with N_2 flushing between samples.

GLC parameters were: flame ionization detector; glass column (temperature, 150 °C; size 1.82 m × 0.64 cm o.d.) packed with 80–100 mesh Chromosorb 101; and N_2 carrier gas (40 ml/min).

Experiment II. *$^{14}CO_2$ and Alkylarsine Evolution.* Because of incomplete recovery of ^{14}C in experiment I, the formation of $^{14}CO_2$ and [^{14}C]alkylarsines (which may be generated from the plant and/or soil) was investigated. Matapeake silt loam soil was layered on the bottom of all glass tanks and wheat seeds planted. One week after germination, the seedlings were treated with [^{14}C]MSMA as described in experiment I. After treatment, each of the chambers was sealed with glass plates containing two openings. An ascarite- and drierite-filled drying tube passed through a stopper in one of the openings. The other opening was connected in series to a CO_2 trap (0.5 N KOH), an arsine trap (I_2 crystals in 0.001 M KI), and a vacuum outlet. Periodically, a 1-ml sample was taken from each trap and assayed for radioactivity. This experiment was terminated 2 weeks after treatment.

Experiment III. Wheat seeds were planted in two 1.52 m × 1.52 m metallic flats in the greenhouse. The flats contained Matapeake silt loam fertilized as before at a rate of 1120 kg/ha. The seeded rows were spaced at 15-cm intervals and the flats watered daily. At the third leaf development stage, the seedlings were sprayed with commercial MSMA (Ansar 529 H.C.) at a rate equivalent to 3.36 kg/ha. A few plants grown on Matapeake silt loam soil in 15-cm plastic pots were used as controls. Approximately 3 months after treatment, the wheat crop was harvested and the chaff separated from the seeds. The seeds were ground in a Wiley mill to 20 mesh.

Analysis of Total As. One-gram samples of ground material were used for total arsenic determination. These samples were digested with a 20-ml mixture of HNO_3–$HClO_4$–H_2SO_4 (20:4:1) until SO_3 fumes appeared. After cooling the solution, 10 ml of water was added twice and brought to a boil. Digestion continued for 2 more h and the solution was diluted to 25 ml after cooling.

Table I. Recovery of Applied Radioactivity from [^{14}C]MSMA Treated Wheat Plants[a]

Plant part	Weeks after treatment: 2 Extracted,[b] %	2 Nonextracted,[c] %	2 Total, %	4 Extracted, %	4 Nonextracted, %	4 Total, %	13 Extracted, %	13 Nonextracted, %	13 Total, %
Root	15.1	1.2	16.3	4.9	1.4	6.3	0.4	1.2	1.6
Shoot	9.1	2.2	11.3	9.9	2.4	12.3	9.5	2.2	11.7
Treated leaf	24.6	2.2	26.8	20.1	2.3	22.4	19.9	3.1	23.0
Seeds							0.1	0.1	0.2
	48.8	5.6	54.4	34.9	6.1	41.0	29.9	6.6	36.5

[a] The values represent mean of three replications. [b] Extracted with 80% MeOH in soxhlet apparatus. [c] Determined by combustion of the extracted seed and liquid-scintillation counting of the trapped $^{14}CO_2$.

A 1.0-ml aliquot of the digest was added to 3.5 ml of 1% $NaBH_4$ stabilized with 0.2% KOH to generate arsine. The generated arsine–H_2 gas was analyzed on an atomic absorption spectrometer (AA) by sweeping into a heated tube (900 °C) with argon gas (Chu et al., 1972; Wauchope, 1976). The AA was equipped with an As electrodeless discharge lamp. A strip chart presented 10-s integrated response.

RESULTS AND DISCUSSION

Experiment I. Radioactivity from MSMA was translocated from the point of application throughout the plant within 2 weeks after treatment. Of the total ^{14}C applied to wheat, 25% was extracted from the treated leaves, 9% from shoot, and 15% from root (Table I). Four weeks after treatment, only 5% of the applied ^{14}C was extracted from the root. Overall, extractable ^{14}C had substantially decreased in roots between the two harvests while it remained the same in the shoots. At the 13-week harvest, a further loss of extractable ^{14}C from roots (0.4%) was observed. This loss in extractable radioactivity might be due to exudation of [^{14}C]MSMA through roots into the soil, degradation of [^{14}C]MSMA resulting in the release of $^{14}CO_2$, or reduction to [^{14}C]alkylarsines. A small amount of ^{14}C (0.2% of the 89 μg of MSMA applied) was in the seeds. Over 3 months, only 36% of the total applied ^{14}C was recovered in the plant.

Experiment II. Thin-layer chromatographic analysis of the 80% methanol extract from root, shoot, treated leaf, and seeds indicated that all of the extracted radioactivity was present in the form of [^{14}C]MSMA by cochromatography with standard [^{14}C]MSMA. This was further supported by ion-exchange chromatography since the elution patterns of both standard [^{14}C]MSMA and methanol extracts of root, shoot, treated leaf, and seeds were similar (Table II). In contrast, Johnsongrass (Sckerl and Frans, 1969) and beans (Sachs et al., 1971) formed a conjugated complex with MSMA.

All parts of the plant contained radioactivity in a nonextracted bound form (Table I). Fifty percent of the total ^{14}C in the seeds was present in the bound nonextractable form. To characterize this bound ^{14}C in wheat seed, extraction procedures described by Woolson et al. (1976) were employed (Table III). ^{14}C was found in all four fractions.

Methanol and cold Cl_3CCOOH appeared to extract the same material since the Soxhlet and non-Soxhlet-extracted samples were similar. To identify the forms of ^{14}C in all the extracts, the three extracted fractions were passed through the ion-exchange column. The elution pattern of all fractions corresponded with that of [^{14}C]MSMA. Differences of 5 to 10 ml in elution behavior are not significant. In addition, methylarsine, from MSMA, was detected by GLC analysis in the cold 5% Cl_3CCOOH extract. These results suggest that [^{14}C]MSMA was present in an unchanged form or was conjugated to non-alcohol-extractable lipid, nucleic acid, and protein materials.

Table II. Elution[a] of ^{14}C from Extracts of [^{14}C]MSMA-Treated Plants on a Cation-Exchange Column

Eluent vol, ml	% act. added to the column for indicated sample[b]: [^{14}C]-MSMA	Root	Shoot	Tr. leaf	Seeds
0-5	0	0	0	0	0
5-10	0	0	0	0	0
10-15	0	0	0	0	0
15-20	0	0	0	0	0
20-25	0.1	0.2	0.9	0.4	0.2
25-30	0.6	2.2	3.7	3.3	0.2
30-35	2.7	6.8	7.7	8.8	1.6
35-40	7.8	11.3	11.3	14.0	1.8
40-45	11.2	13.8	12.6	16.1	4.8
45-50	17.1	12.5	11.9	14.8	9.5
50-55	15.4	10.8	10.1	11.2	12.3
55-60	11.3	9.0	8.8	8.8	15.2
60-65	8.1	7.8	7.7	7.0	12.0
65-70	6.9	6.2	6.3	5.2	11.5
70-75	5.1	5.3	5.4	4.0	8.1
75-80	4.2	4.6	4.6	2.9	6.3
80-85	2.7	3.8	3.1	1.6	4.1
85-90	1.8	2.4	2.3	1.0	3.5
90-95	1.0	1.4	1.5	0.6	2.7
95-100	0.5	1.0	0.9	0.3	1.4
100-150	0.2	0.4	0.6	0.4	1.0

[a] Successive eluents were 25 ml of 0.2 M trichloroacetic acid (pH 1.7), 30 ml of 1.8 N sodium acetate (pH 6.5), 50 ml of 1.0 N sodium acetate (pH 11.8), 50 ml of 1.0 N NaOH. Arsenate elutes from 0 to 25 ml and cacodylic acid from 75 to 175 ml. [b] The samples are from methanol extracts of wheat 90 days after treatment. Similar results were obtained from extracts prepared 14 to 28 days after treatment.

Table III. Distribution of ^{14}C in Various Fractions Extracted from Seeds of Wheat Treated with [^{14}C]MSMA[a]

Extractant	% of total ^{14}C in seeds for indicated seed sample: Previously extracted with methanol	Unextracted
Cold 5% Cl_3CCOOH	2.1	45.2
Warm 75% EtOH	18.5	17.1
Hot 5% Cl_3CCOOH	16.4	17.7
Nonextracted	18.3	22.2

[a] Values are means of two replications.

Table IV shows the recovery of ^{14}C in various portions of the environment after [^{14}C]MSMA application to wheat. [^{14}C]MSMA was exuded into the soil and degraded to release small amounts of $^{14}CO_2$ and a small amount was reduced to [^{14}C]alkylarsines. However, apparently $^{14}CO_2$ and alkylarsine are released by soil degradation of exuded

Table IV. $^{14}CO_2$ and [^{14}C]Alkylarsine Recovered from Plant and Soil, 2 Weeks after Treatment with [^{14}C]MSMA[a]

Sample	% applied radioact. recovd
Plant	59.4[b]
Soil	19.8[b]
$^{14}CO_2$	0.8[c]
[^{14}C]Alkylarsines	0.1[c]

[a] Values are means of two replications. [b] Determined by combustion and liquid-scintillation counting of the trapped $^{14}CO_2$. [c] Determined by liquid-scintillation counting.

Table V. Arsenic Residue in Wheat Seeds[a]

Experiment	Arsenic residue[b]
Control	0.39
MSMA treated expt I	1.52
MSMA treated expt III	1.16

[a] These values represent mean of duplicates for each of the samples. [b] Analysis by atomic absorption spectrometry.

[^{14}C]MSMA since at the end of 1 week neither $^{14}CO_2$ nor [^{14}C]alkylarsine was detected. Detection occurred only after 2 weeks, sufficient time for root exudation. Von Endt et al. (1968) and Woolson (1976) showed that [^{14}C]MSMA is degraded in soil to release $^{14}CO_2$ or is methylated and reduced to dimethyl- and trimethylarsines.

Experiment III. Application of MSMA at 3.36 kg/ha under greenhouse conditions at the two–three leaf development stage resulted in an increase in the arsenic content of the wheat crop relative to the control (Table V). The shallowness of the soil and the greenhouse environment itself may have enhanced the arsenic residues in the seed. Field studies conducted by Moore (1975) indicated that use of MSMA resulted in a small but insignificant increase in the arsenical content of wheat seeds.

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Received for review April 23, 1976. Accepted July 26, 1976. Mention of proprietary products does not imply endorsement or approval by the U.S. Department of Agriculture to the exclusion of other suitable products.

Pesticide Residue Reduction by the Process of Preparing Whole Orange Puree

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Bearing Marrs orange trees [*Citrus sinensis* (L.) Osbeck] sprayed with a mixture of pesticides (azinphosmethyl, carbophenothion, ethion, parathion, malathion, dioxathion, dimethoate, dicofol, and chlorobenzilate) were harvested at 1, 7, and 21 days after application. Samples from each harvest were analyzed for pesticide residues: (1) unwashed; (2) after washing by a common processing plant method; and (3) after being processed into whole orange puree. Washing eliminated from 8 to 35% of the residue initially present on the unwashed fruit. The whole orange puree had a residue level 71 to 95% less than the unwashed fruit. Individual pesticide residues did not exceed the tolerances established by the Environmental Protection Agency (EPA), even on the unwashed fruit harvested 1 day after the spray application. Residues on fruit harvested 3 months after the last spray application in a normal pesticide program were no higher than 1.4% of the tolerance limit. We concluded that residues in oranges received by processors probably would not exceed EPA tolerances for the pesticides tested, and also that pesticide residues in whole orange puree were substantially less than that of the unwashed fruit and well within the established tolerances for whole fruit.

The fate of pesticide residues on citrus fruits which are processed into whole citrus fruit puree has not been investigated previously. The puree process of Cruse and Lime (1970) incorporates from 85 to 90% of the entire fruit into the final product. In their process whole fruits are water blanched at 100 °C for 10 min before grinding. This blanch and the removal of hard portions of tissue in the finisher would be expected to eliminate a considerable portion of any pesticide residue that might be present in the field-run fruit. Gunther (1969) discussed residues and their removal in the processing of other citrus products.

The purpose of this study was to determine the effects of the puree process on the content of pesticide residues in the puree and to determine whether or not pesticide

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