

different in these two molecules, whereas the charge on C(7) remains essentially invariant. Since the toxicity of the phosphinate analogue to mammals is significantly lower than that of azinphos-methyl and since the structural parameters of the two compounds would appear to be similar, it seems logical to suspect a charge density dependence in the toxic mechanism.

These results, consequently, provide impetus for future calculations of charge densities in other series of organophosphorus insecticides. In addition to the possibility of charge density effects in AChE inhibition, intramolecular restrictions or freedoms may also contribute to the inhibition, and x-ray diffraction analyses of organophosphorus insecticides can provide valuable information to elucidate the mechanisms of acetylcholinesterase inhibition in both mammals and insects.

**Supplementary Material Available:** A listing of the observed and calculated structure factor amplitudes (4 pages). Ordering information is given on any current masthead page.

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## Acid Dissociation Constants of Arsenic Acid, Methylarsonic Acid (MAA), Dimethylarsinic Acid (Cacodylic Acid), and *N*-(Phosphonomethyl)glycine (Glyphosate)

D. Wauchope

Acid dissociation constants have been measured for arsenic acid ( $\text{H}_3\text{AsO}_4$ ), methylarsonic acid ( $\text{CH}_3\text{AsO}_3\text{H}_2$ ; MAA), dimethylarsinic acid [ $(\text{CH}_3)_2\text{AsO}_2\text{H}$ ; cacodylic acid], and *N*-(phosphonomethyl)glycine ( $\text{HO}_2\text{CCH}_2\text{NHCH}_2\text{PO}_3\text{H}_2$ ; glyphosate). A recently devised mathematical technique for  $\text{p}K$  and end point determinations which corrects for strong acid or base impurities was modified and used to analyze automatic titrator titration curves and correct  $\text{p}K$  values to zero ionic strength. Thermodynamic  $\text{p}K$  values =  $-\log (a_{\text{HA}}/a_{\text{AH}})$  at 25 °C are:  $\text{H}_3\text{AsO}_4$ ,  $\text{p}K_2 = 7.089 \pm 0.01$ ; MAA,  $\text{p}K_1 = 4.114 \pm 0.01$ ,  $\text{p}K_2 = 9.148 \pm 0.01$ ; cacodylic acid,  $\text{p}K_1 = 6.288 \pm 0.01$ ; glyphosate,  $\text{p}K_1 = 2.32 \pm 0.03$ ,  $\text{p}K_2 = 5.86 \pm 0.03$ ,  $\text{p}K_3 = 10.86 \pm 0.03$ .

Dimethylarsinic acid (cacodylic acid; hydroxydimethylarsine oxide) and methylarsonic acid (methane-

arsonic acid; MAA) and its sodium salts (MSMA and DSMA) are extensively used as postemergence herbicides applied to the foliage of weeds. Lead and calcium salts of arsenic acid were formerly used in large quantities as insecticides and are still used to a limited extent, and *N*-(phosphonomethyl)glycine (glyphosate) is a new broad-spectrum, translocatable herbicide with potential uses in agronomic crops. These materials differ from many

Southern Weed Science Laboratory, U.S. Department of Agriculture, Agriculture Research Service, in cooperation with the Mississippi Agricultural and Forestry Experiment Station, Stoneville, Mississippi 38776.

pesticides in that they are ionic and water soluble. The arsenic and phosphorus acid moieties in the compounds, as in inorganic phosphate, affect their movement and inactivation by soils (Dickens and Hiltbold, 1967; Hiltbold, 1974; Johnson and Hiltbold, 1969; Kardos, 1964; Sprankle et al., 1975; Von Endt et al., 1968; Woolson et al., 1973; Woolson and Kearney, 1973; Wauchope, 1975), and there is some evidence that the phytotoxicity of the arsenical herbicides may be a function of the state of dissociation of the acid (Shepherd et al., 1966; Keeley and Thullen, 1971; Arle and Hamilton, 1971). Accurate acid dissociation constants for these acids are needed if these and other aspects of the solution chemistry of these materials are to be interpreted in terms of the actual ion species present. A new mathematical technique for treating titration data has been used (Briggs and Stuehr, 1974) which allows simultaneous calculation of pK values and end points. The technique was slightly modified for our conditions and is particularly suitable for materials of uncertain purity such as herbicide preparations.

#### MATERIALS AND METHODS

Disodium methylarsonate and sodium cacodylate, specified 99% pure, were obtained from K&K Laboratories (Plainview, N.Y.) and used without further purification. Disodium arsenate and potassium dihydrogen phosphate were reagent grade. Methylarsonic acid was prepared by acidification of a concentrated solution of the technical disodium salt followed by recrystallization twice from water, and dried at 110 °C. Glyphosate acid was 97% assay. Potassium hydroxide (0.1002 M) was prepared, purified by ion exchange, and checked for carbonate contamination by titration of histidine, using the methods of Albert and Serjeant (1971).

Samples (2.4 mmol) of the compounds were weighed into the inner cell of a Radiometer water-jacketed titration vessel and dissolved in 35.0 or 40.0 ml of 0.05 M KCl. One or two equivalent amounts of 0.1 M HCl were added in the case of the mono- or disodium salts, and the titrations were done under nitrogen at 25.0 °C using a Radiometer titrator recorder with 2.5-ml syringe autoburette. The recorded curves were readable to 0.005 pH unit and 0.002 ml. Recorder chart calibration was monitored throughout the course of each titration. The glass-calomel electrode pair was calibrated with 4.01 and 9.18 commercial NBS-formula buffers and the calibration was checked before and after each titration. Stability and reproducibility were 0.01 pH unit or less in every case. At least three samples of each material were titrated.

The method of Briggs and Stuehr (1974) was used to calculate pK values. Nine to 16 points from each titration curve were fit by the method of least squares to a modification of the linear equation (slope  $K_r$ ; intercept  $V_e$ ):

$$V + R - \Delta V = -a_H(V + R + \Delta V)K_r + V_e \quad (1)$$

$$R = (C_H - C_{OH})V_t/C_b \quad (2)$$

where  $V$  is the volume of base added,  $\Delta V$  is the volume of base neutralized by any strong acid impurities present in the initial solution (negative if any strong base is present),  $a_H$  is the hydrogen ion activity as measured by the glass electrode,  $K_r$  is the reciprocal of the "mixed" acid dissociation constant, i.e.,  $K_r = C_{HA}/a_H C_A$ ,  $V_e$  is the "end-point" volume of base required to neutralize the weak acid only,  $V_t$  is the total volume =  $V + V_0$ ,  $C_b$  is the base concentration, and  $C_H$ ,  $C_{OH}$ ,  $C_A$ , and  $C_{HA}$  are the equilibrium concentrations of hydrogen ion, hydroxide ion, conjugate base, and conjugate acid, respectively.

Briggs and Stuehr wrote a computer program in which an initial estimate of  $\Delta V$  is used, along with titration curve

data, to calculate the quantities in eq 1 and the goodness-of-fit to eq 1 and 2. Incorrect values of  $\Delta V$  produce a poor fit (curvature) and the computer program varies  $\Delta V$ , with recalculation of the deviation from linearity, until two successive iterations produce a negligible change in fit.

Because  $K_r$  is the reciprocal of a mixed ionization constant, it will be constant throughout a titration only if the ionic strength remains constant. Briggs and Stuehr used their method on data for monoprotic acids titrated with 0.1 M 1:1 base in 0.1 M supporting electrolyte. Under these conditions, the ionic strength remains very nearly constant throughout the titration and only small corrections to  $K_r$  due to dilution effects need be made. This was done using the activity coefficient expression of Davies (1962), correcting  $K_r$  at each point to 0.1 ionic strength, a procedure that also minimizes the effects of uncertainties in activity coefficients. The resulting average  $K_r$  for all points is therefore the reciprocal of the mixed ionization constant for the acid at 0.1 ionic strength.

In this work, second ionization constants were also obtained. In each case, these were sufficiently separated from first ionization constants [ $\Delta pK > 2.7$ ; Albert and Serjeant, 1971] so that eq 1 could be applied, but ionic strength changes will be large during such titrations unless very high concentrations of supporting electrolyte are used. High ionic strengths, however, introduce uncertainties in liquid junction potentials, because the calibrating buffers have an ionic strength of only 0.05 (Bates, 1964). This problem can be solved rather straightforwardly for the compounds in this study by titrating at low initial ionic strength and correcting  $K_r$  at each point to zero ionic strength, because accurate activity coefficients can be calculated for the individual ions.

Initially, titrations were run without supporting electrolyte, following the procedures of Albert and Serjeant (1971). The precision of the data was high enough, however, to justify using 0.05 M supporting electrolyte to remove liquid junction potential differences with respect to the buffers used for calibration (Stuehr, 1975). This refinement raised the final calculated pK values by about 0.02–0.03 unit.

The data were fit to the equation:

$$V + R + \Delta V = [-a_H(V + R + \Delta V)\gamma^A/\gamma^{AH}]K_r^0 + V_e \quad (3)$$

where the slope,  $K_r^0$ , is the reciprocal of the thermodynamic ionization constant at infinite dilution and zero ionic strength, and  $\gamma^A$  and  $\gamma^{AH}$  are the activity coefficients of the ionized and un-ionized acid species, respectively. Accurate values of the activity coefficients for the anions of the arsenic compounds and phosphate may be calculated using the Debye-Huckel relation, e.g., for the ionized species (Cookson, 1974; Benet and Goyan, 1967; Klotz, 1964):

$$\log \gamma^A(25.0 \text{ }^\circ\text{C}) = -0.5091(z_A)^2\sqrt{I}/(1 + 0.3286 \times 10^8 r_A \sqrt{I}) \quad (4)$$

where  $I$  is the ionic strength at each point and  $z_A$  and  $r_A$  are the charge and effective ionic radius of the ion, respectively. Values for  $r_A$ , which is essentially an adjustable parameter (Klotz, 1964), are available for  $\text{H}_2\text{PO}_4^-$  ( $4.3 \times 10^{-8}$  cm),  $\text{HPO}_4^{2-}$  ( $4.0 \times 10^{-8}$  cm), and  $\text{H}_2\text{AsO}_4^-$  ( $4.5 \times 10^{-8}$  cm) (Kielland, 1937) and reproduce experimental (mean) activity coefficients within 2% at  $I \geq 0.1$  when used in eq 4. Similar values should reasonably hold for sodium methylarsonate and dimethylarsinate ions and the dianion of arsenic acid, viz.,  $\text{AsO}_4\text{H}^{2-}$  and  $\text{CH}_3\text{AsO}_3^{2-}$  ( $4.2 \times 10^{-8}$

Table I. Typical Calculation Results: Sequential Titration of 64.64 mg of Disodium Methylarsonate Dissolved in 35.00 ml of 0.05 M KCl + 2 Equiv of HCl:  $V_0 = 38.80$  ml;  $C_b = 0.1002$  M;  $T = 25.0$  °C

pH	$V$ , ml	$\gamma_{AH}$	$\gamma_A$	pK	$\Delta pK$	$I$ , mol $l^{-1}$
3.50	0.380	1.000	0.813	4.1176	-0.0016	0.057
3.60	0.503	1.000	0.813	4.1180	-0.0012	0.057
3.70	0.628	1.000	0.813	4.1205	0.0013	0.057
3.80	0.759	1.000	0.813	4.1211	0.0019	0.057
3.90	0.894	1.000	0.813	4.1209	0.0017	0.057
4.00	1.030	1.000	0.813	4.1201	0.0009	0.058
4.10	1.167	1.000	0.812	4.1189	-0.0003	0.058
4.20	1.298	1.000	0.812	4.1183	-0.0010	0.058
4.30	1.420	1.000	0.812	4.1194	0.0002	0.058
4.40	1.535	1.000	0.812	4.1188	-0.0004	0.058
4.50	1.641	1.000	0.812	4.1162	-0.0030	0.058
4.60	1.731	1.000	0.812	4.1184	-0.0084	0.058
4.70	1.810	1.000	0.812	4.1202	0.0010	0.058
4.80	1.879	1.000	0.812	4.1207	0.0584	0.058
$\overline{pK} = 4.119 \pm 0.002^a$			$V_e = 2.174$		$\Delta V = 0.028$	
8.19	0.413	0.810	0.424	9.1432	-0.0002	0.060
8.29	0.491	0.810	0.423	9.1439	0.0003	0.060
8.39	0.582	0.809	0.423	9.1423	-0.0011	0.061
8.49	0.680	0.809	0.422	9.1448	0.0014	0.061
8.59	0.793	0.809	0.421	9.1425	-0.0009	0.061
8.69	0.910	0.808	0.420	9.1438	0.0004	0.062
8.79	1.033	0.808	0.419	9.1446	0.0012	0.062
8.89	1.162	0.807	0.418	9.1425	-0.0009	0.063
8.99	1.286	0.807	0.417	9.1437	0.0002	0.063
9.09	1.408	0.807	0.417	9.1431	-0.0004	0.063
9.19	1.520	0.806	0.416	9.1452	0.0018	0.064
9.29	1.629	0.806	0.415	9.1418	-0.0017	0.064
9.39	1.723	0.806	0.414	9.1429	-0.0005	0.064
9.49	1.808	0.805	0.414	9.1423	-0.0013	0.065
9.59	1.880	0.805	0.413	9.1449	0.0015	0.065
$\overline{pK} = 9.143 \pm 0.001^a$			$V_e = 2.174$		$\Delta V = 0.031$	

<sup>a</sup> One standard deviation.

cm);  $CH_3AsO_3H^-$  and  $(CH_3)_2AsO_2^-$  ( $4.5 \times 10^{-8}$  cm). Organic anions and dianions similar in size to glyphosate have an  $r_A$  of about  $6 \times 10^{-8}$  cm, and, assuming the effective ionic radius increases by about  $0.5 \times 10^{-8}$  cm with ionization (based on the values given by Kielland (1937) for citrate), radii of 5.5, 6.0, and  $6.5 \times 10^{-8}$  cm were assigned to glyphosate anion, dianion, and trianion, respectively.

## RESULTS AND DISCUSSION

In the present work, the limits of precision were set by the reproducibility and readability of the electrode standardization, about  $\pm 0.005$  pH unit from pH 4 to 9. Points from individual curves fit eq 3 with a standard deviation in pK of 0.004 or better (see the typical results of Table I), providing confidence in the precision of the

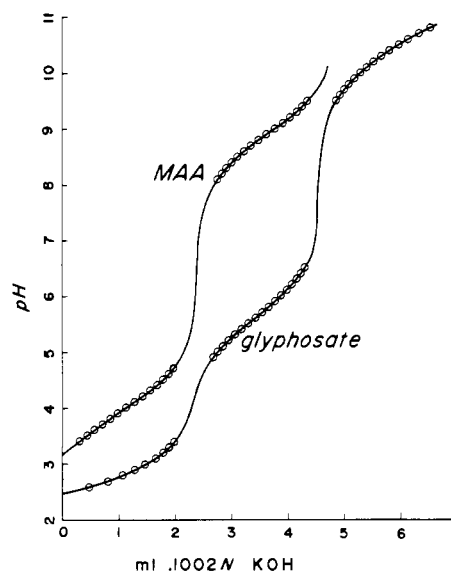


Figure 1. Titration curves for 33.99 mg of methylarsonic acid and 39.44 mg of glyphosate acid; initial solution volume 35.00 ml, supporting electrolyte 0.05 M KCl. Circles are data points used for pK calculation.

titration data, sample purity, and the linearity of the electrode response to  $\log a_H$ . The absolute accuracy attainable from a grand average pK obtained from three or more titrations is suggested by our results for the second pK of phosphate, for which quite accurate values are available; our agreement within 0.01 pK unit of the best values from Sillen and Martell (1964, 1971) indicates the 90% confidence levels of the pK values reported in Table II are about  $\pm 0.01$  for the pK values between 4 and 9 and  $\pm 0.03$  for the pK values outside that range, where electrode standardization and activity corrections are more uncertain (Bates, 1964; Albert and Serjeant, 1972). Glyphosate gave the poorest fit to eq 3 (although still within calibration uncertainty) and the widest range of average pK values from different titrations, especially in the high and low pH range, as expected. Sprankle et al. (1975) showed that glyphosate may be back-titrated with no change in titration curve, ruling out hydrolysis reactions from pH 2 to 12. Titration curves for glyphosate and MAA are given in Figure 1, and the species suggested by Sprankle et al. (1975) for glyphosate are shown in Figure 2. These results show that the use of a recording titrator need not entail a sacrifice of precision as compared to manual methods (Albert and Serjeant, 1972).

Given the pK values and the functional dependence of activities on ionic strength in eq 4, the distribution of ionic

Table II. Thermodynamic pK° Values [ $-\log(a_H a_A/a_{AH})$ ] for Acids at 25.0 °C; Comparison with Reported Values

Acid species	No. samples	pK°		
		Range	Av <sup>a</sup>	Lit. <sup>b</sup>
$H_2PO_4^-$	4	7.201-7.217	7.210 $\pm$ 0.03	7.206, 7.22, 7.198, 7.211, 7.22, 7.199, 7.200, 7.18
$H_2AsO_4^-$	3	7.080-7.096	7.089 $\pm$ 0.01	7.08, 6.98, 6.94, 6.94 <sup>c</sup>
$(CH_3)_2AsO_2H$	6	6.286-6.292	6.288 $\pm$ 0.01	6.23, <sup>d</sup> 6.27 <sup>e</sup>
$CH_3AsO_3H_2$	4 <sup>f</sup>	4.110-4.119	4.114 $\pm$ 0.01	3.61 (18 °C), 4.46, <sup>g</sup> 4.58 <sup>h</sup>
$CH_3AsO_3H^-$	4 <sup>c</sup>	9.141-9.154	9.148 $\pm$ 0.01	8.24 (18 °C), 8.38, <sup>g</sup> 7.82 <sup>h</sup>
Gly $H_3$	3	2.312-2.329	2.32 $\pm$ 0.03	2.6 <sup>i</sup>
Gly $H_2^-$	3	5.844-5.869	5.86 $\pm$ 0.02	5.6 <sup>i</sup>
Gly $H^-$	3	10.830-10.882	10.86 $\pm$ 0.03	10.6 <sup>i</sup>

<sup>a</sup> Estimated 90% confidence limit. <sup>b</sup> Unless otherwise noted, values from Sillen and Martell (1964, 1971). <sup>c</sup> Flis et al. (1959). <sup>d</sup> Morton (1928). <sup>e</sup> Kirkpatrick (1949). <sup>f</sup> Includes two samples of MAA and two samples of DSMA; see Materials and Methods. <sup>g</sup> Gillet et al. (1971). <sup>h</sup> B. D. Chernokal'skii et al. (1966). <sup>i</sup> P. Sprankle et al. (1975).

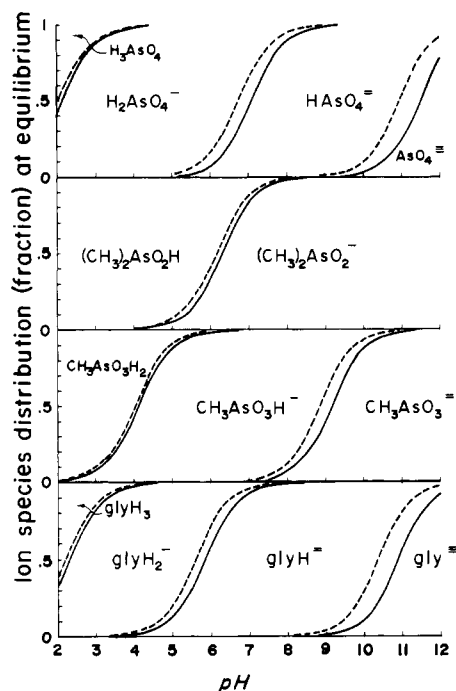


Figure 2. Ion species distributions at zero ionic strength (solid curves) and at 0.1 ionic strength (broken curves).

species for a given pH and ionic strength may be calculated by eq 5:

$$C_A/C_{HA} = \exp_{10} \left[ \text{pH} - \text{pK}^0 + 0.5091\sqrt{I} \left( \frac{z^2(A)}{1 + 0.3286r_A\sqrt{I}} - \frac{z^2(\text{HA})}{1 + 0.3286r_{\text{AH}}\sqrt{I}} \right) \right] \quad (5)$$

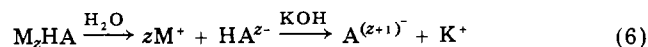
where  $\text{pK}^0$  is the negative log of the thermodynamic dissociation constant  $a_{\text{HA}}a_{\text{A}}/a_{\text{AH}}$ , and  $a_{\text{H}}$  is defined as  $\exp_{10}(-\text{pH})$ . This equation, of course, applies only to systems with  $\text{pK}$  values sufficiently separated so that only one dissociation equilibrium is significant at any given pH. The results for the compounds of this study are given in Figure 1. Accuracies of the calculated ratios are about  $\pm 2$  and  $\pm 7\%$  for  $\text{pK}$  values with 0.01 and 0.03 90% confidence limits, respectively.

The considerable disparity between the present results and previous reports for methylarsonic acid require comment. Gillet et al. (1971) did not describe their method. Chernokal'skii et al. (1966) used the pH at  $1/3$ ,  $1/2$ , and  $2/3$  neutralization, and Backer and Bolt (1935), whose values are quoted in Sillen and Martell (1964), used the pH at  $1/2$  and  $3/2$  neutralization. These techniques are inherently inaccurate (Cookson, 1974), and may be inaccurate by a few tenths of a unit (Benet and Goyan, 1967), as are results of Sprankle et al. (1975) for glyphosate by the same method. The present results for methylarsonic acid were identical for materials from two different sources, and, as stated above, the accuracy is expected to be  $\pm 0.01$  unit.

#### APPENDIX

A note is in order on the computation procedure involved in the modification of Briggs and Stuehr's (1974) eq 2 to eq 3. Fitting the points from a titration curve to eq 2 requires the following input data: paired titration volumes  $V$  and pH values; number of points  $n$ , base concentration  $C_b$ , initial sample volume  $V_0$ , initial 1:1

supporting electrolyte concentration  $C_{\text{SE}}$ , and an initial estimate of the end point error volume  $\Delta V$ . Using eq 3 requires four additional input data: the absolute value for the charge on the acid species  $z$ , the effective ionic radii for the acid and its conjugate base  $r_{\text{HA}}$  and  $r_{\text{A}}$ , and the approximate initial analytical concentration of the acid  $C_t^0$ . These data are used to calculate the ionic strength and activity coefficients  $\gamma^{\text{AH}}$  and  $\gamma^{\text{A}}$  as follows: given the solution and titration of the acid or monovalent metal salt ( $M = \text{Na}^+$  in our studies):



the ionic strength is then calculated by:

$$I = 0.5\sum(z_i^2 C_i) \quad (7)$$

$$= 0.5(C_{\text{H}} + C_{\text{OH}} + C_{\text{K}} + 2C_{\text{SE}} + C_{\text{M}} + z^2 C_{\text{HA}} + (z+1)^2 C_{\text{A}}) \quad (8)$$

Mass balance and electroneutrality considerations lead to the result:

$$I = (z+1)(C_{\text{H}} + z_{\text{HA}}C_t^0(V_0/V_t) + V(C_b/V_t) - z_{\text{HA}}(z+1)C_t^0(V_0/V_t - z_{\text{HA}}C_{\text{OH}} + C_{\text{SE}}(V_0/V_t)) \quad (9)$$

An initial estimate of  $I$  at each point is calculated by estimating  $C_{\text{H}} = \exp_{10}(-\text{pH})$  and  $C_{\text{OH}} = 10^{-14}/C_{\text{H}}$ ;  $\gamma_{\text{H}}$  and  $\gamma_{\text{OH}}$  are then calculated using eq 4 with radii from Kielland (1937), and then  $C_{\text{H}}$  and  $C_{\text{OH}}$  are recalculated from the pH and the ion product of water  $K_w$ , correcting for activities:  $C_{\text{H}} = \exp_{10}(-\text{pH})/\gamma_{\text{H}}$ ;  $C_{\text{OH}} = K_w/[\exp_{10}(-\text{pH})]\gamma_{\text{OH}}$ . These corrected concentrations are then used in eq 5,  $I$  is calculated, and  $\gamma_{\text{AH}}$  and  $\gamma_{\text{A}}$  then calculated as in eq 4.

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## Photolysis of Hexachlorobenzene

Jack R. Plimmer\* and Ute I. Klingebiel

Photodecomposition of hexachlorobenzene exposed to sunlight as a crystalline material or on silica gel was extremely slow and no photodecomposition products were identified. This photodecomposition was sensitized by diphenylamine, but not by benzophenone. Photolysis in methanol or hexane with light of wavelengths greater than 260 or 220 nm, respectively, was rapid and the anticipated products of reductive dechlorination (pentachlorobenzene and tetrachlorobenzene) were obtained in each case. In addition, an unexpected photochemical reaction between hexachlorobenzene and methanol resulted in the formation of small amounts of pentachlorobenzyl alcohol and traces of another photoproduct that was probably a tetrachlorodi(hydroxymethyl)benzene.

Hexachlorobenzene (HCB) is an environmental contaminant that is resistant to biological degradation and demonstrates potential for accumulation in biological organisms (Metcalf et al., 1973). One of the primary sources of the compound appears to be waste streams from the manufacture of chlorinated hydrocarbons. Since HCB is relatively volatile, it may also enter the environment as a vapor in stack gases. Atmospheric transport may be responsible for the movement of HCB from contaminated areas or industrial waste dumps. Industrially, HCB has been used as a plasticizer for polyvinyl chloride.

HCB is registered as a preemergence fungicide to be used as a seed protectant for the control of certain diseases of small grains. However, its use in agriculture is minor. Although the directions for use on the label warn against the use of treated grain as human food or as animal feed, there have been instances outside the U.S.A. of contamination of food or meat when treated grain was improperly used (Schmid, 1960). In Louisiana, beef carcasses brought to slaughter in 1972 were contaminated with HCB, which could have resulted from improper disposal of industrial waste (EPA, 1973).

Decomposition by sunlight is a possible route for the environmental degradation of HCB. It is therefore important to establish the extent to which photodegradation can occur and to identify the photoproducts.

### MATERIALS AND METHODS

**Chemicals and Instrumental Techniques.** Pentachlorobenzene was supplied by the Olin Corporation. Other compounds were synthesized by standard techniques or were obtained commercially and purified.

Low-resolution mass spectra were obtained on either a Perkin-Elmer Model GC 270 or Dupont Model 491 combination gas chromatograph-mass spectrometer using GC or direct inlet systems. Infrared spectra were recorded

in KBr disks on a Perkin-Elmer, Model 621, infrared spectrophotometer. The high-pressure liquid chromatograph (HPLC) was a DuPont Model 830, fitted with an ultraviolet photometric detector and equipped with a 1 m × 2.1 mm i.d. column packed with "Permaphase" ODS using methanol-water mixtures as the mobile phase. A Hewlett Packard Model 7600A gas chromatograph equipped with electron-capture detector and a 6 in. × 0.25 in. o.d. column packed with 3% OV-17 on Gas-Chrom Q (80-100 mesh) was used for quantitative measurements of photoproducts.

Radioactivity was measured using a Nuclear-Chicago Mark IV scintillation counter in a water-miscible counting medium [300 ml of 2-ethoxyethanol, 1500 ml of 1,4-dioxane, 18 g of 2,5-diphenyloxazole, 0.9 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, and 90 g of naphthalene].

Thin-layer chromatography was performed on silica-coated (GS 254-250 μm layer) plates with benzene as solvent.

**Irradiation Experiments.** (1) *Solid HCB.* A layer of crystalline HCB was placed on a glass plate under a quartz cover and exposed for 5 months to a sunlamp or to ambient laboratory illumination. Analysis was by GLC and HPLC.

(2) *On Silica Gel.* A hexane solution of HCB (0.97 μg/μl) was spotted on silica gel coated TLC plates (2 μl/spot). The plates were exposed for 4.5 h to outdoor sunlight, to a 40-W GE sunlamp (maximum output ca. 310 nm), or to laboratory illumination (fluorescent lighting). Extremely small amounts of photoproducts were observed after developing the TLC plate but the major spot was unchanged HCB.

For sensitized photolysis, 2-μl aliquots of the hexane solution of HCB were applied to silica gel coated TLC plates. Solutions of benzophenone (5 μg) or diphenylamine (5 μg) were applied to the HCB spots, and the plates were exposed to sunlight for 4.5 h, with corresponding dark controls. After development of the plates with benzene, radioautographs were obtained. A germicidal lamp was used to detect products absorbing light at 254 nm (benzenoid or aromatic compounds) which were scraped off the

\* Agricultural Environmental Quality Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.