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EVALUATION OF ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR HERBICIDE ANALYSIS OF WISCONSIN SOILS IN COMPARISON TO GAS CHROMATOGRAPHY

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ABSTRACT

Herbicides leaching from contaminated soil are a significant source of groundwater contamination. The remediation process for contaminated sites usually includes extensive laboratory testing which tends to slow the process and add costs. Enzyme Linked Immunosorbent Assay technology (ELISA) has the potential to significantly reduce the cost and time needed for remediation of herbicide-contaminated sites.

Soil samples collected from various herbicide-contaminated sites, such as mixing and loading facilities, were analyzed to evaluate ELISA in comparison with gas chromatography to determine the suitability of this technology for site assessment and remediation planning.

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INTRODUCTION

Contamination of groundwater by herbicides such as Atrazine and Alachlor has been shown to be a significant problem in Wisconsin. In 1991 the Wisconsin Department of Agriculture Trade and Consumer Protection (DATCP) performed a survey in which 50% of wells tested showed the presence of triazine chemical contamination. Most of the contamination was attributed to the leaching of herbicides into the groundwater from fields that had been subjected to repeated, heavy applications of the herbicides. Additionally, sites used for storage or mixing of the herbicides and areas where spills have occurred have also been implicated as groundwater contamination sources.

Since 1991, Enzyme Linked Immunosorbent Assay technology, commonly known as ELISA or immunoassay (IA), has played a key role in Wisconsin in the screening of water samples for herbicides. This innovative technology may also have possible applications in the testing of soil samples for herbicides, particularly at remediation sites. The advantages of immunoassay are its low cost and simplicity of testing which allows for rapid turnaround times. Another possible advantage of IA technology is the ability to screen soil samples in the field. To evaluate the ability of ELISA to test soil samples, a study was conducted in which soil samples collected from herbicide contaminated sites were analyzed by ELISA for alachlor, atrazine, cyanazine, and metolachlor. Results were compared to conventional procedures involving gas chromatographic analysis.

In the last 3 years the Environmental Immunochemistry Laboratory at the Wisconsin State Laboratory of Hygiene (SLH) has analyzed approximately 8,000 groundwater samples for atrazine by immunoassay to determine the extent of atrazine contamination in Wisconsin's groundwater. As a rule, when used for Wisconsin's groundwaters, the ELISA technology compares favorably with gas chromatography. In 1990 the SLH investigated the potential effect of NO_3 in groundwater as a positive interferant of the triazine ELISA. The study demonstrated that nitrate did not bias the ELISA results (American Waterworks Association, 1992).

This study of herbicide-contaminated soil samples could indicate whether or not interferants may exist in various soil matrices. If good correlation exists between the methodologies it could lead to increased acceptance and usage of ELISA technology as a time and money saving device that remediators can use to protect Wisconsin's groundwater.

SOIL MOISTURE DETERMINATION

A separate portion of each soil sample was weighed, oven-dried to zero moisture, and re-weighed to determine the mass and volume of soil and water, respectively. These values were converted to percentages to make mathematical corrections for the soil moisture in the calculations for standardization purposes.

SAMPLE ANALYSIS

Samples were analyzed using the Ohmicron® Rapid Assays® proprietary procedures, following the instructions provided in the test kits (Ohmicron undated). Separate assays were performed for atrazine alachlor, cyanazine, and metolachlor.

INTERPRETATION

Four different methods of analysis and calculation were used to determine results. Moisture and Non-specific Binding Corrections (NSB) were used in calculations and photometric analysis. Specifically, soil moisture was added to the volume of the extracting solution, and the mass of soil was mathematically corrected to equal the dry mass. The NSB is a photometric reagent blank that compensates for indirect binding of the conjugate to the sample tube, and solid support, which can negatively bias sample concentrations. The calculations for this would be as follows. (Dilutions can be > 50:1 to analyze in a higher concentration range. Calculation numbers 1 through 4 correspond with column numbers in Table 2 through 5).

1. NSB and Moisture Correction

$$\text{NSB assay result} \times \frac{\text{vol. MeOH (mL)} + \text{vol. of soil moisture}}{\text{mass of dry soil (g)}} \times \frac{\text{vol. extract (mL)} + \text{vol. diluent (mL)}}{\text{vol. extract (mL)}} =$$

concentration of analyte in soil;

2. Nonspecific Binding Correction Only

$$\text{N.S.B. assay result} \times \frac{\text{vol. MeOH (mL)}}{\text{mass of soil (g)}} \times \frac{\text{vol. extract (ml)} + \text{vol. diluent (mL)}}{\text{vol. extract (mL)}} =$$

concentration of analyte in soil;

3. Moisture Correction Only

$$\text{assay result} \times \frac{\text{vol. MeOH (mL)} + \text{vol. of soil moisture (mL)}}{\text{mass of dry soil (g)}} \times \frac{\text{vol. extract (mL)} + \text{vol. diluent (mL)}}{\text{vol. extract (mL)}} =$$

concentration of analyte in soil;

4. Not Corrected

$$\text{assay result} \times \frac{\text{vol. MeOH (mL)}}{\text{mass of soil (g)}} \times \frac{\text{vol. extract (mL)} + \text{vol. diluent (mL)}}{\text{vol extract (mL)}} =$$

concentration of analyte in soil.

RESULTS AND DISCUSSION

The original premise for this study was that samples previously tested for herbicides using gas chromatography methods would be plentiful. However, soil samples were not as available as had been originally anticipated. The majority of the soil samples were to come from the Wisconsin Department of Agriculture Laboratory; however, when it became apparent that the DATCP Laboratory was not receiving their usual number of pesticide contaminated soil samples, we broadened our horizons and were able to obtain fourteen samples from the University of Iowa Hygienic Laboratory. Unfortunately, chromatography results were not available for all four analytes for every soil sample. Because of the limited pool of soil samples to choose from, the samples in this study generally have either very low or very high levels of contamination. Although it would have been desirable to have a broader range of concentrations, the results are very interesting and still allow conclusions to be drawn.

Because soil samples can vary from very dry to mud, a portion of each sample was analyzed for moisture content. Visually most of the soil samples could be described as moist. No samples were muddy and no samples appeared exceptionally dry. Percent moisture concentrations ranged from 5.4%-30.5%, with most samples in the 10%-20% moisture range. Samples that are not corrected for moisture will show a negative bias (Table 1). This would be important to consider if an investigator were doing field immunoassays and did not analyze for moisture content. Moisture corrected results are sometimes reported on a "dry weight basis."

Table 1. Comparison of results with and without soil moisture corrections.

Sample Number	Percent Moisture	Analyte Result not Moisture Corrected (ppm)	Analyte Result Moisture Corrected (ppm)
A	30.5	1.73	2.75
G	6.4	0.84	0.92

ELISA testing antibodies are prepared by injecting a host animal such as a mouse or rabbit with the target chemical and collecting the antibodies produced by the animal. Sometimes this results in cross-reactivity of antibodies to analytes that are closely related to the target chemical the antibodies were originally designed for. This phenomenon has made it difficult to compare this technology to traditional, very specific analytic methods such as gas chromatography. Antibodies generally show increased cross-reactivity at low concentrations. For example, using a sample with 0.062 ppb of the atrazine metabolite Desethyl Atrazine (and no parent atrazine) would be measured by the Ohmicron® atrazine Rapid® assay, (antibodies from different manufacturers have different reactivities), as 0.046 ppb. If the sample had 3.21 ppb Desethyl Atrazine, the assay result would be 0.72 ppb. This demonstrates that as concentrations increase,

Table 2 (continued).

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected Only (ppm)	3 H_2O Corrected Only (ppm)	4 Uncorrected (ppm)
103082	<0.1	0.02	0.02	0.02	0.02
103083	<0.1	0.14	0.10	0.15	0.12
103084	<0.1	0.09	0.06	0.09	0.07
103085	<0.1	0.06	0.05	0.07	0.05
103086	<0.1	0.24	0.19	0.28	0.22
103266	<0.1	<0.02	<0.02	<0.02	<0.02
103267	<0.1	<0.02	<0.02	<0.02	<0.02
103268	<0.1	<0.02	<0.02	<0.02	<0.02
103300	5.71	12.9	11.5	11.6	10.2
103305	<0.1	<0.02	<0.02	<0.02	<0.015
103306	2.73	5.63	4.89	5.98	5.20
103307	<0.1	0.02	0.02	<0.02	<0.02
103919	<0.1	0.03	0.02	<0.02	0.02
103920	<0.1	<0.02	<0.02	<0.02	<0.02
105864	<0.1	0.04	0.03	<0.04	0.03
105865	1.04	4.87	3.85	4.76	3.76
105866	<0.1	0.16	0.13	0.19	0.15
106190	<0.1	<0.02	<0.02	<0.02	<0.02
106191	<0.1	2.75	2.22	3.06	2.47
106192	<0.1	0.13	0.11	0.15	0.12
106455	<0.1	<0.02	<0.02	<0.02	<0.02
106456	<0.1	0.08	0.07	0.09	0.08

†NSB is non-specific binding corrections

Table 3. Atrazine soil concentration.

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected (ppm)	3 H_2O Corrected (ppm)	4 Uncorrected (ppm)
A	2.70	2.44	1.54	2.75	1.73
B	N.A.	<0.18	<0.16	<0.18	<0.16
C	N.A.	0.47	0.42	0.36	0.32
D	N.A.	<0.18	<0.16	<0.18	<0.16
E	N.A.	<0.18	<0.16	<0.18	<0.16
F	N.A.	0.89	0.80	0.92	0.84
G	N.A.	0.91	0.84	0.91	0.84
H	N.A.	<0.20	<0.16	<0.20	<0.16
I	N.A.	<0.19	<0.16	<0.18	<0.16
J	N.A.	<0.2	<0.16	<0.20	<0.16
K	0.10	<0.19	<0.16	<0.19	<0.16
L	0.22	0.29	0.26	0.21	0.19
M	0.32	0.67	0.61	0.64	0.58
N	0.16	<0.19	<.16	<0.19	<0.16
112484	1.52	1.86	1.67	2.04	1.83
112485	0.32	0.33	0.30	0.33	0.30
112486	1.36	0.71	0.64	0.71	0.64
80575	N.D.	<0.19	<0.16	<0.19	<0.16
80576	N.D.	<0.20	<0.16	<0.20	<0.16
80577	N.D.	<0.20	<0.16	<0.20	<0.16
80578	N.D.	<0.18	<0.16	<0.18	<0.16
80579	N.D.	<0.20	<0.16	<0.20	<0.16
80580	N.D.	<0.20	<0.16	<0.20	<0.16
80581	N.D.	<0.20	<0.16	<0.20	<0.16
80582	N.D.	<0.19	<0.16	<0.19	<0.16
80583	N.D.	<0.20	<0.16	<0.20	<0.16
80584	N.D.	<0.21	<0.16	<0.21	<0.16
80585	N.D.	<0.21	<0.16	<0.21	<0.16

Table 3 (continued).

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected (ppm)	3 H_2O Corrected (ppm)	4 Uncorrected (ppm)
103082	<0.05	<0.01	<0.01	-	-
103083	<0.05	0.02	0.02	-	-
103084	<0.05	0.02	0.02	-	-
103085	<0.05	0.03	0.02	-	-
103086	<0.05	0.04	0.03	-	-
103266	<0.05	<0.01	<0.01	-	-
103267	<0.05	<0.01	<0.01	-	-
103268	<0.05	<0.01	<0.01	-	-
103300	<0.05	0.07	0.06	-	-
103305	<0.05	<0.01	<0.01	-	-
103306	<0.05	0.09	0.08	-	-
103307	<0.05	<0.01	<0.01	-	-
103919	0.08	0.15	0.12	-	-
103920	<0.05	0.03	0.03	-	-
105864	<0.05	0.02	0.01	-	-
105865	0.94	2.04	1.61	-	-
105866	<0.05	0.14	0.11	-	-
106190	<0.05	0.10	0.08	-	-
106191	42.9	66.0	53.0	-	-
106192	38.2	19.0	15.3	-	-
106455	<0.05	<0.01	<0.01	-	-
106456	<0.05	0.09	0.08	-	-

†NSB is non-specific binding correction.

Table 4. Cyanazine soil concentration.

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected (ppm)	3 H_2O Corrected (ppm)	4 Uncorrected (ppm)
A	<0.10	0.31	0.19	0.31	0.19
B	N.A.	0.01	0.01	0.01	0.01
C	N.A.	<0.14	<0.13	<0.14	<0.13
D	N.A.	<0.14	<0.13	<0.14	<0.13
E	N.A.	<0.14	<0.13	<0.14	<0.13
F	N.A.	<0.14	<0.13	<0.14	<0.13
G	N.A.	<0.14	<0.13	<0.14	<0.13
H	N.A.	<0.16	<0.13	<0.16	<0.13
I	N.A.	<0.14	<0.13	<0.14	<0.13
J	N.A.	<0.01	<0.01	<0.01	<0.01
K	<0.10	<0.15	<0.13	<0.15	<0.13
L	<0.10	<0.14	<0.13	<0.14	<0.13
M	<0.10	<0.14	<0.13	<0.14	<0.13
N	<0.10	0.02	0.02	0.02	0.02
112484	0.41	0.68	0.61	0.72	0.64
112485	0.19	0.25	0.23	0.19	0.18
112486	0.15	0.25	0.23	0.25	0.23
80575	N.A.	<0.15	<0.13	<0.15	<0.13
80576	N.A.	<0.16	<0.13	<0.16	<0.13
80577	N.A.	<0.16	<0.13	<0.16	<0.13
80578	N.A.	<0.15	<0.13	<0.15	<0.13
80579	N.A.	<0.16	<0.13	<0.16	<0.13
80580	N.A.	<0.15	<0.13	<0.15	<0.13
80581	N.A.	<0.16	<0.13	<0.16	<0.13
80582	N.A.	<0.15	<0.13	<0.15	<0.13
80583	N.A.	<0.16	<0.13	<0.16	<0.13
80584	N.A.	<0.17	<0.13	<0.17	<0.13
80585	N.A.	<0.17	<0.13	<0.17	<0.13

Table 4 (continued).

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected Only (ppm)	3 H_2O Corrected Only (ppm)	4 Uncorrected (ppm)
103082	<0.1	<0.1	<0.01	<0.01	<0.1
103083	<0.1	<0.01	<0.01	<0.01	<0.01
103084	<0.1	<0.01	<0.01	<0.01	<0.01
103085	<0.1	<0.01	<0.01	<0.01	<0.01
103086	<0.1	<0.01	<0.01	<0.01	<0.01
103266	<0.1	<0.01	<0.01	<0.01	<.01
103267	<0.1	<0.01	<0.01	<0.01	<0.01
103268	<0.1	<0.01	<0.01	<0.01	<0.01
103300	<0.1	0.01	0.01	0.01	0.01
103305	<0.1	<0.01	<0.01	<0.01	<0.01
103306	<0.1	0.01	0.01	0.01	0.01
103307	<0.1	<0.01	<0.01	<0.01	<0.01
103919	<0.1	0.01	0.01	0.01	0.01
103920	<0.1	<0.01	<0.01	<0.01	<0.01
105864	<0.1	<0.01	<0.01	<0.01	<0.01
105865	<0.1	0.04	0.04	0.04	0.04
105866	<0.1	0.01	0.01	0.01	0.01
106190	7.93	4.97	4.50	5.62	4.51
106191	<0.1	0.26	0.22	0.27	0.22
106192	<0.1	0.21	0.19	0.24	0.19
106455	<0.1	<0.01	<0.01	<0.01	<0.01
106456	<0.1	0.03	0.03	0.03	0.03

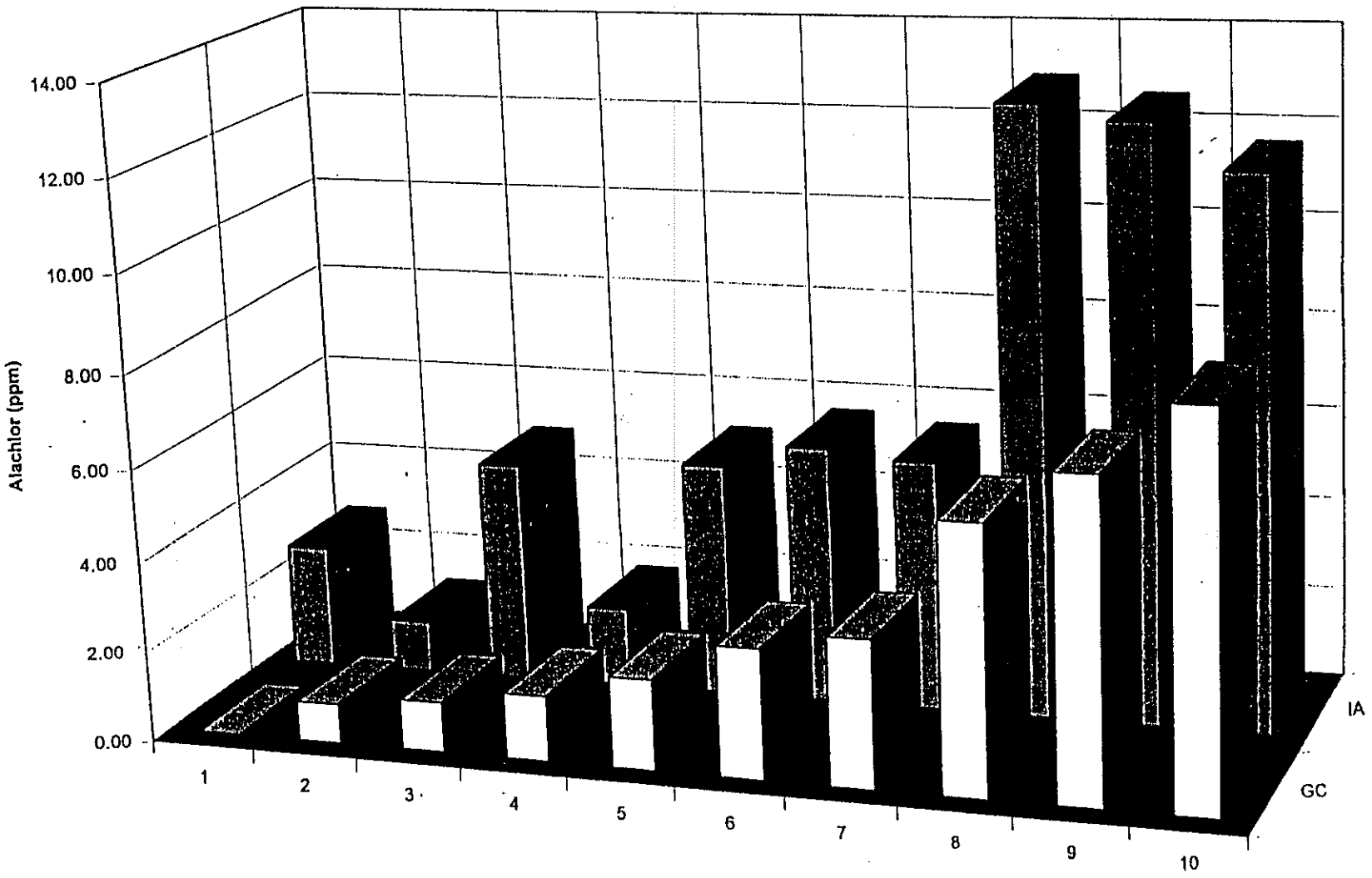

Figure 1. Low range alachlor (NSB + H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

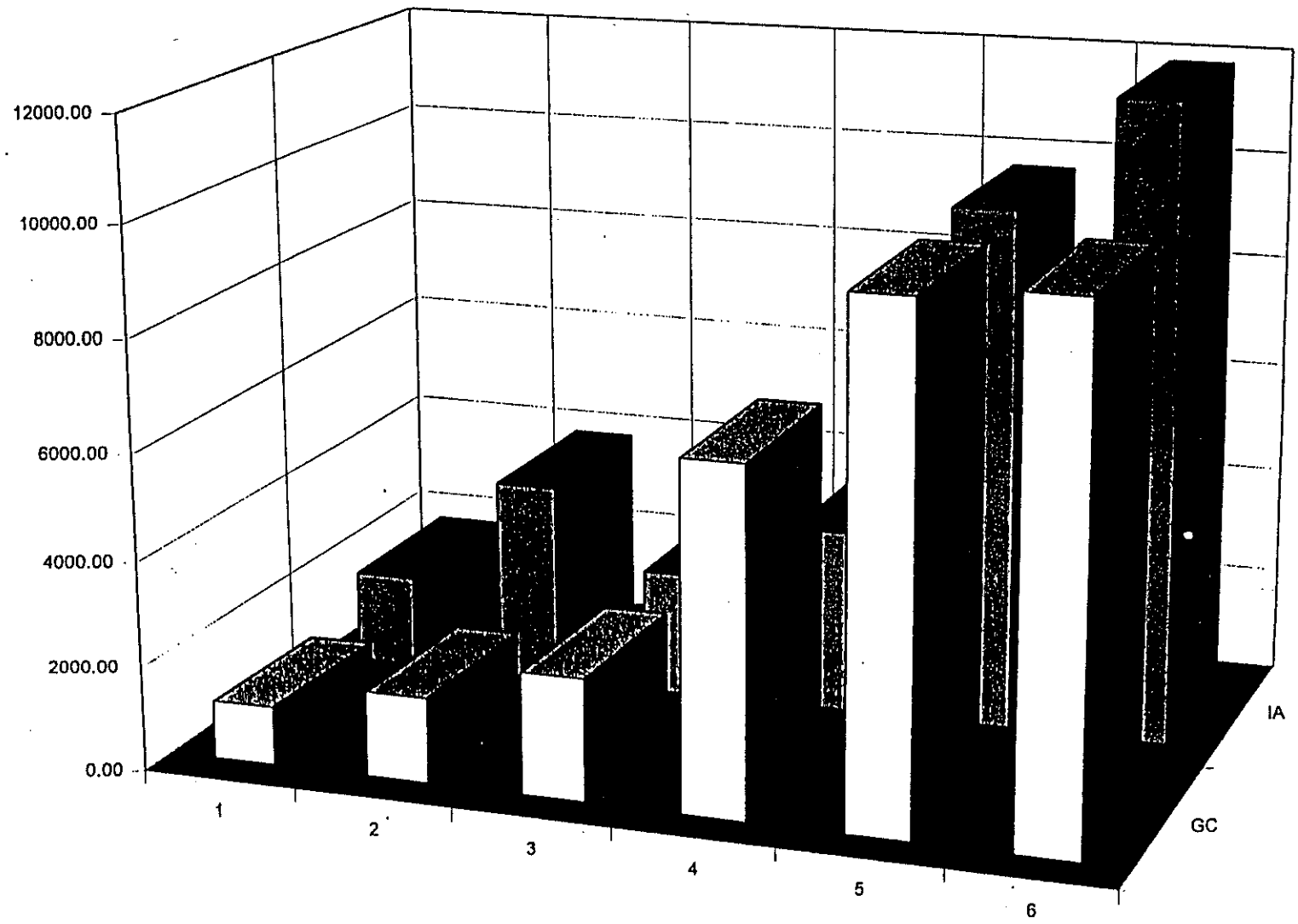

Figure 2. High range alachlor (NSB + H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

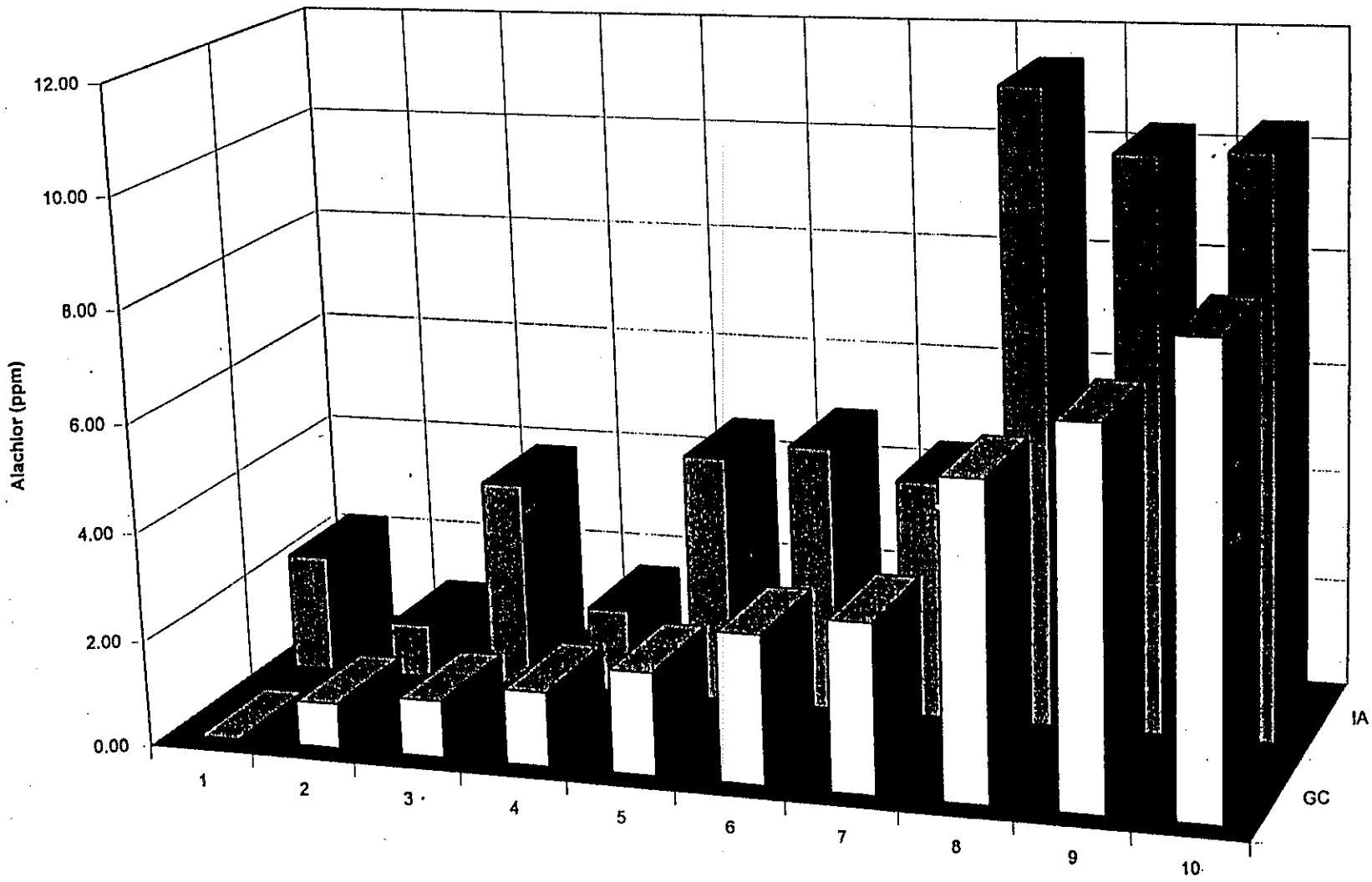

Figure 3. Low range alachlor (NSB corrected) ELISA (IA) vs. gas chromatography (GC).

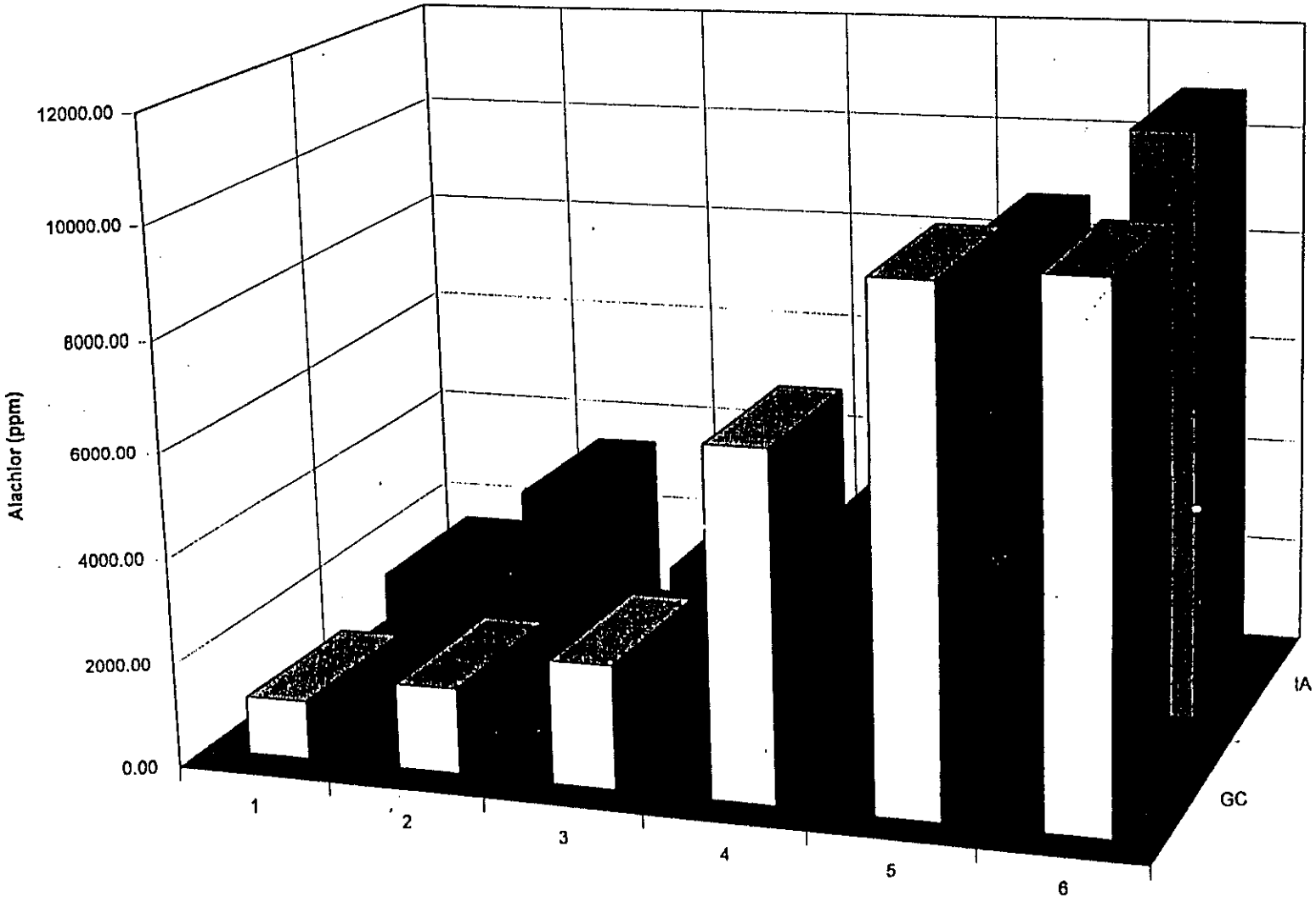

Figure 4. High range alachlor (NSB corrected) ELISA (IA) vs. gas chromatography (GC).

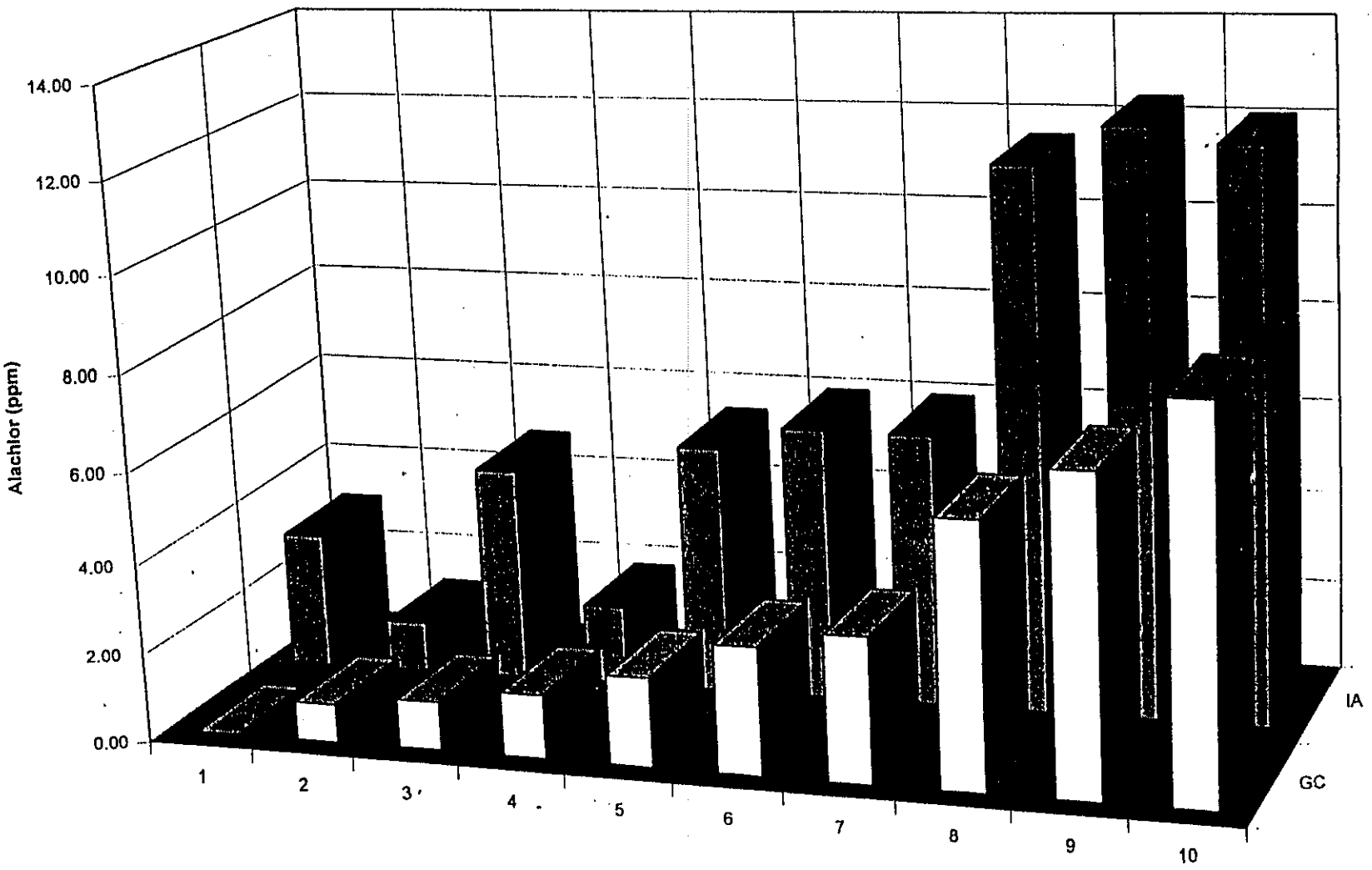

Figure 5. Low range alachlor (H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

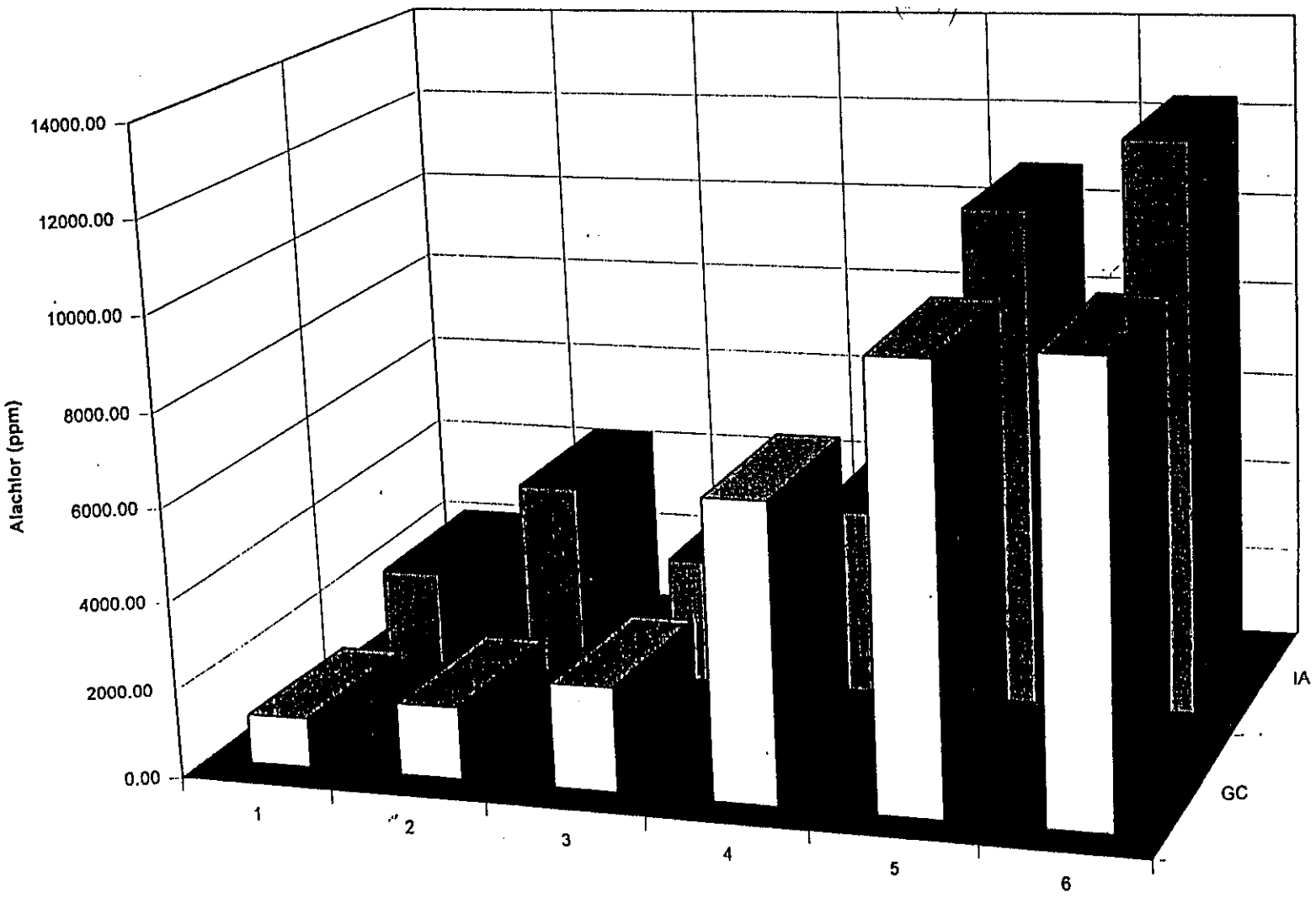

Figure 6. High range alachlor (H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

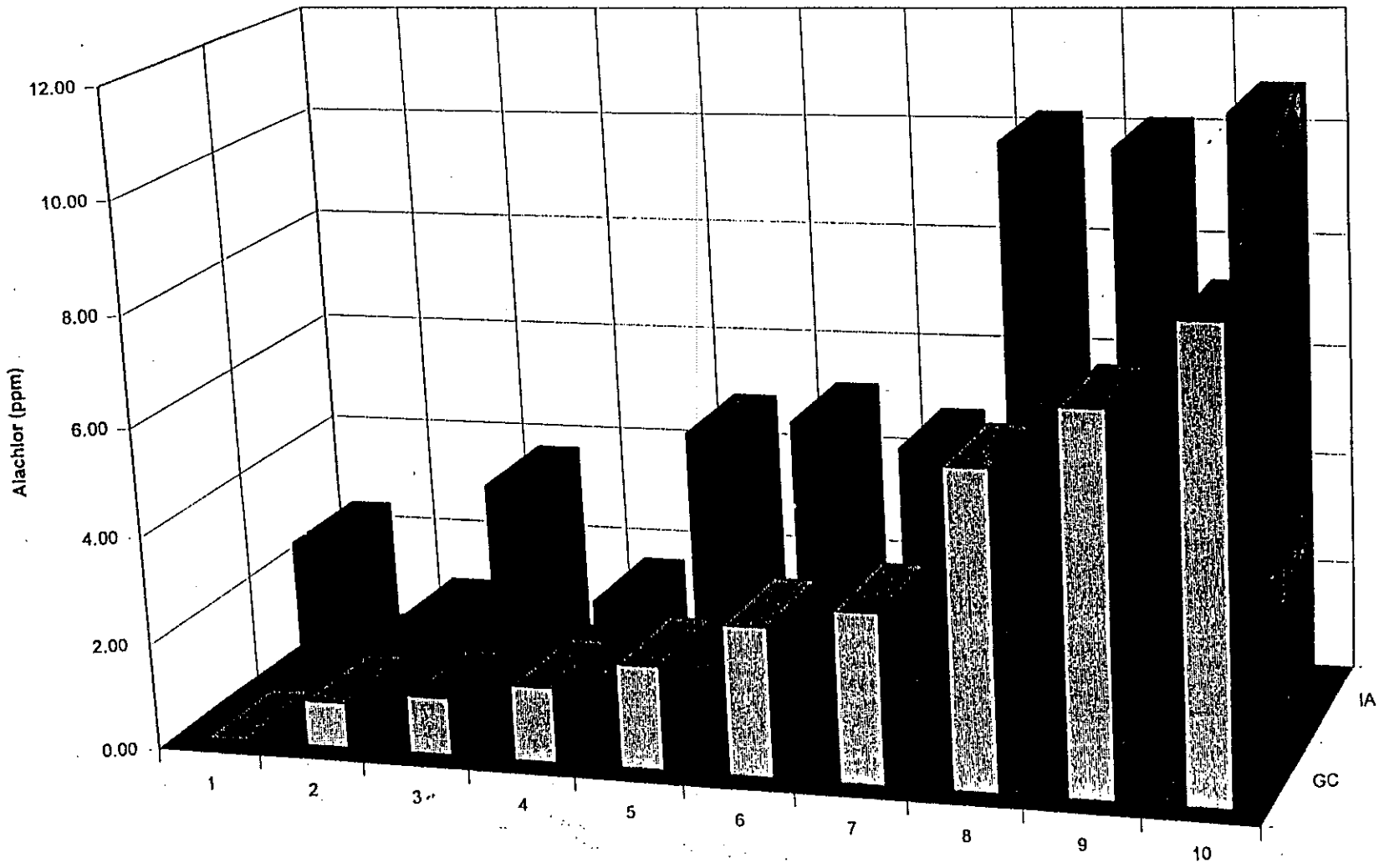

Figure 7. Low range alachlor (uncorrected) ELISA (IA) vs. gas chromatography (GC).

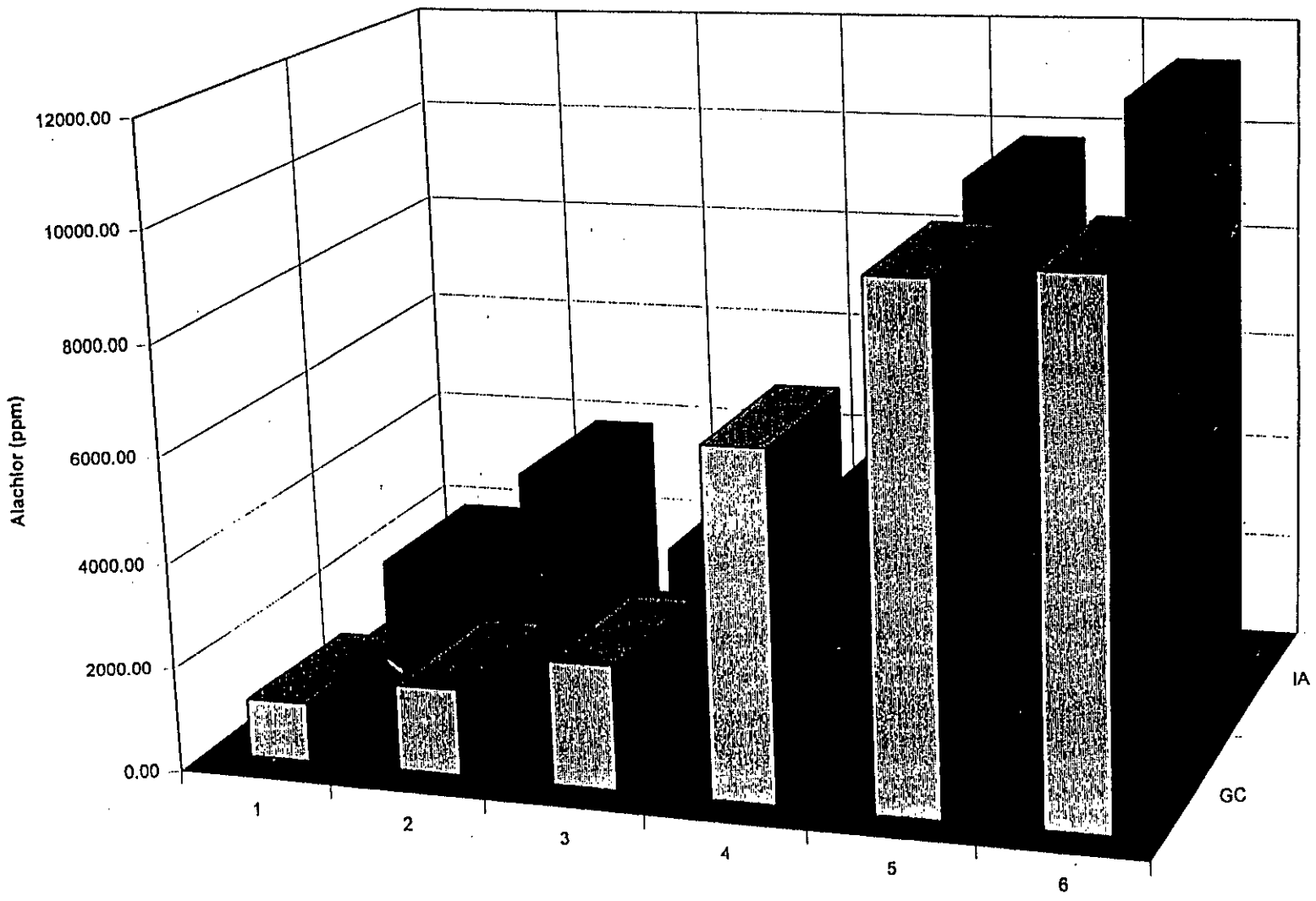

Figure 8. High range alachlor (uncorrected) ELISA (IA) vs. gas chromatography (GC).

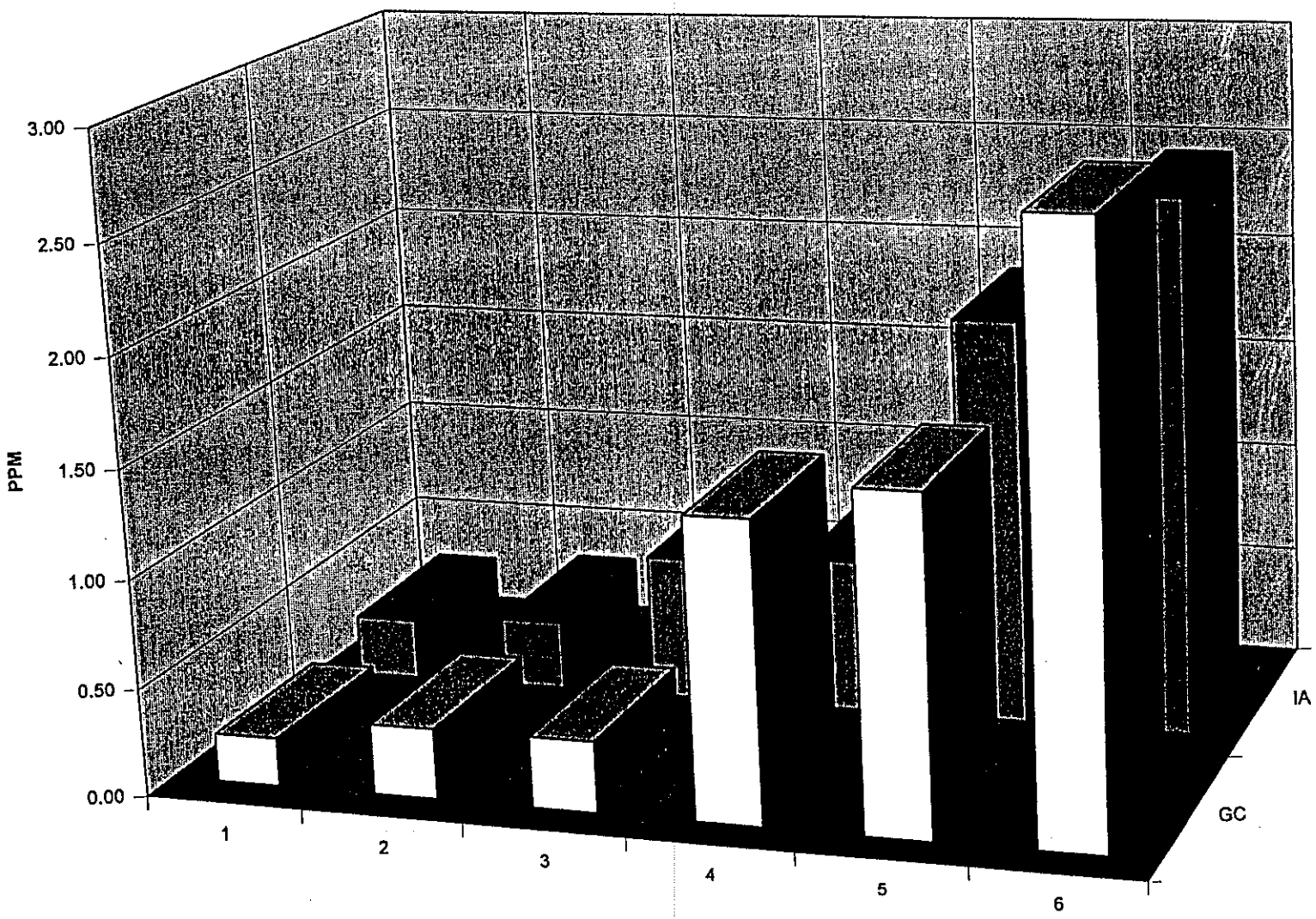

Figure 9. Atrazine (NSB + H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

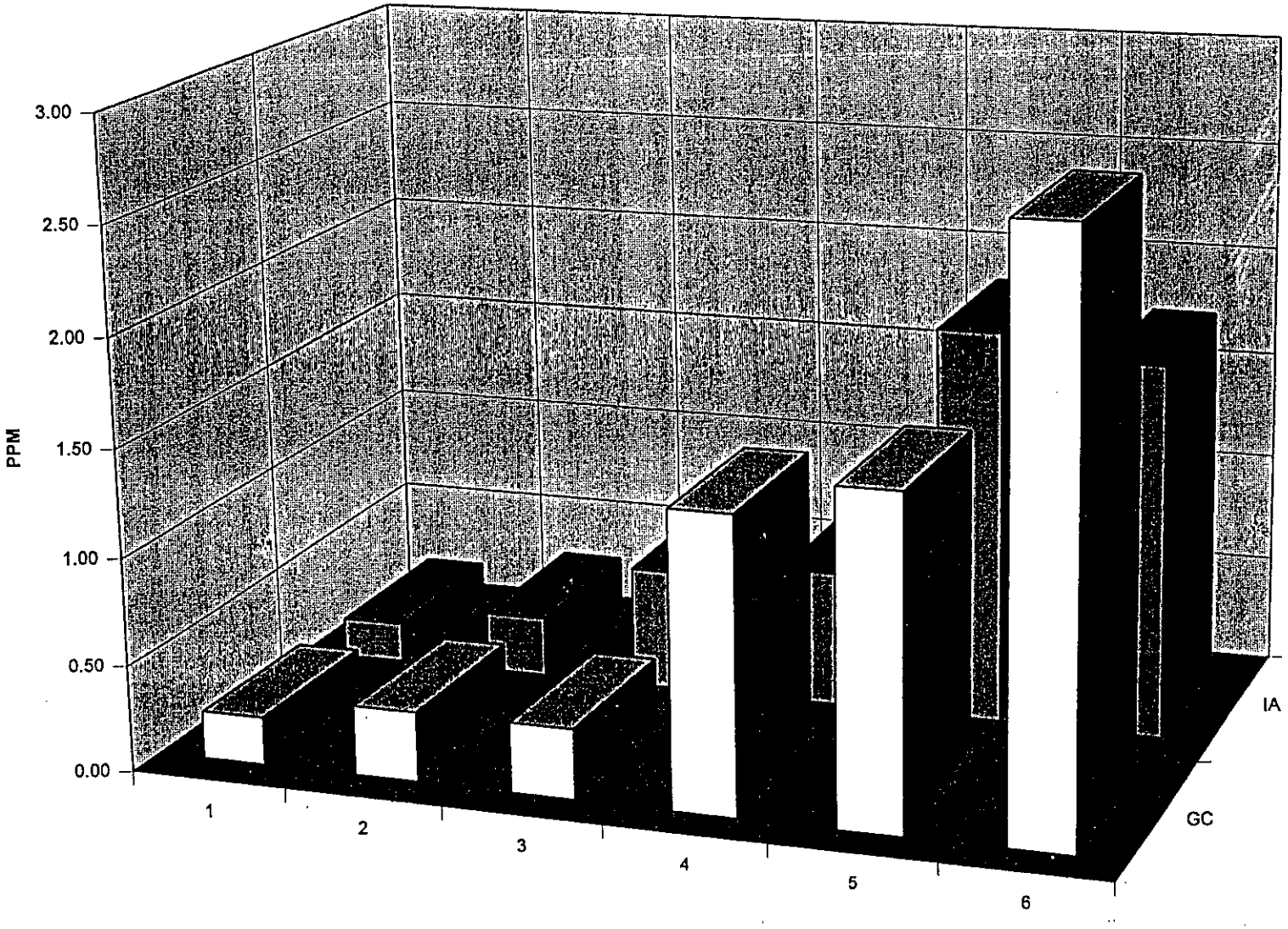

Figure 12. Atrazine (uncorrected) ELISA (IA) vs. gas chromatography (GC).

is not enough data for statistical analysis, Figure 13 compares result of the two methods for samples where Cyanazine was detected. Note that sample number 106190 was not included in Figure 13 since the value was substantially higher.

Twenty-nine samples were analyzed by GC for Metolachlor. Comparative data is presented in Table 5. Non-detections occurred with both methods on 15 of the samples. The immunoassay method detected the compound in seven cases where the GC method did not. The five samples that had detections with both methods are compared in Figure 14. The R^2 for this data is 0.58 which is somewhat low due to the large differences between methods for the first and last samples. Sample number 106191 was not included in Figure 14 because the metolachlor concentration was very high and out of scale with the other samples.

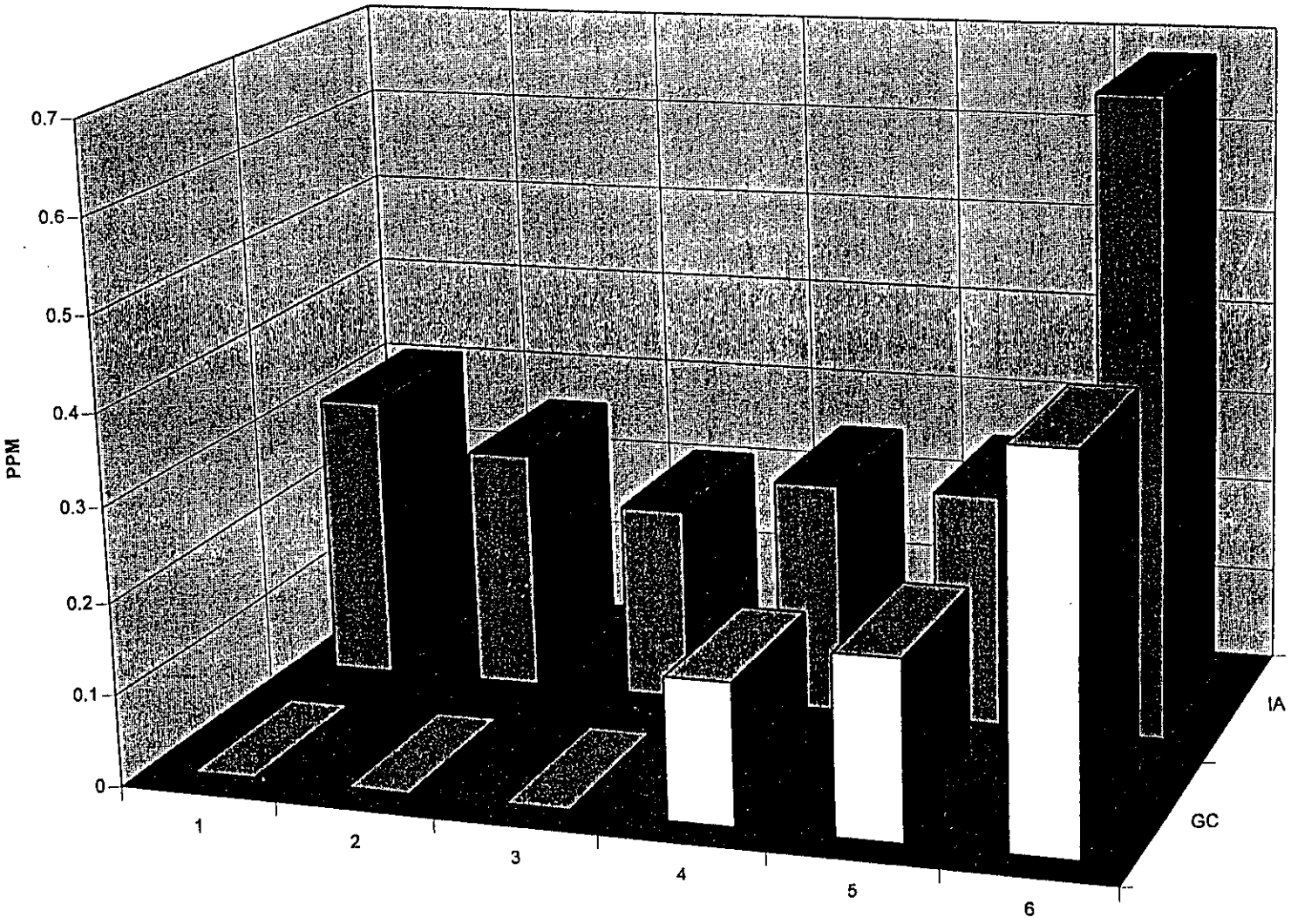

Figure 13. Cyanazine (NSB + H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

Table 5. Metolachlor soil concentration.

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected (ppm)	3 H_2O Corrected (ppm)	4 Uncorrected (ppm)
A	N.A.	<0.25	<0.16	<0.25	<0.16
B	N.A.	<0.25	<0.16	<0.26	<0.16
C	N.A.	>17.9	>16.1	>17.9	>16.1
D	N.A.	>17.8	>16.1	>17.8	>16.1
E	N.A.	>18.0	>16.1	>18.0	>16.1
F	N.A.	>17.7	>16.1	>17.7	>16.1
G	N.A.	>17.5	>16.1	>17.5	>16.1
H	N.A.	<0.20	<0.16	<0.20	<0.16
I	N.A.	<0.18	<0.16	<0.18	<0.16
J	N.A.	<0.20	<0.16	<0.20	<0.16
K	<0.1	<0.19	<0.16	<0.19	<0.16
L	<0.1	<0.18	<0.16	<0.18	<0.16
M	<0.1	<0.18	<0.16	<0.17	<0.16
N	<0.1	<0.19	<0.16	<0.19	<0.16
112484	1.69	3.90	3.50	4.65	4.17
112485	0.65	0.44	0.39	0.40	0.35
112486	1.11	0.96	0.87	1.06	0.90
80575	N.A.	<0.19	<0.16	<0.19	<0.16
80576	N.A.	<0.20	<0.16	<0.20	<0.16
80577	N.A.	<0.20	<0.16	<0.20	<0.16
80578	N.A.	<0.18	<0.16	<0.18	<0.16
80579	N.A.	<0.20	<0.16	<0.20	<0.16
80580	N.A.	<0.19	<0.16	<0.19	<0.16
80581	N.A.	<0.20	<0.16	<0.20	<0.16
80582	N.A.	<0.19	<0.16	<0.19	<0.16
80583	N.A.	<0.20	<0.16	<0.20	<0.16
80584	N.A.	<0.21	<0.16	<0.21	<0.16
80585	N.A.	<0.21	<0.16	<0.21	<0.16

Table 5 (continued).

	Type of Analysis				
		Enzyme Linked Immunosorbent Assay			
Sample Number	Gas Chromatography (ppm)	1 Moisture and NSB Corrected (ppm)	2 NSB Corrected Only (ppm)	3 H_2O Corrected Only (ppm)	4 Uncorrected (ppm)
103082	<0.1	<0.01	<0.01	<0.01	<0.01
103083	<0.1	<0.01	<0.01	<0.01	<0.01
103084	<0.1	<0.01	<0.01	<0.01	<0.01
103085	<0.1	<0.01	<0.01	<0.01	<0.01
103086	0.54	1.79	1.41	N.A.	N.A.
103266	<0.1	<0.01	<0.01	<0.01	<.01
103267	<0.1	<0.01	<0.01	<0.01	<0.01
103268	<0.1	<0.01	<0.01	<0.01	<0.01
103300	<0.1	0.10	0.09	0.15	0.13
103305	<0.1	<0.01	<0.01	<0.01	<0.01
103306	<0.1	0.07	0.06	0.09	0.08
103307	<0.1	<0.01	<0.01	<0.01	<0.01
103919	<0.1	0.40	0.34	0.42	0.36
103920	<0.1	0.09	0.08	0.10	0.09
105864	<0.1	<0.01	<0.01	<0.01	<0.01
105865	<0.1	0.05	0.04	0.06	0.04
105866	<0.1	0.02	0.01	0.02	0.02
106190	<0.1	<.01	<0.01	<0.01	<0.01
106191	236.00	>190	>153	>190	>153
106192	<0.1	0.95	0.75	0.77	0.61
106455	<0.1	0.01	0.01	0.01	0.01
106456	0.70	0.99	0.86	0.87	0.75

†Non-specific binding correction.

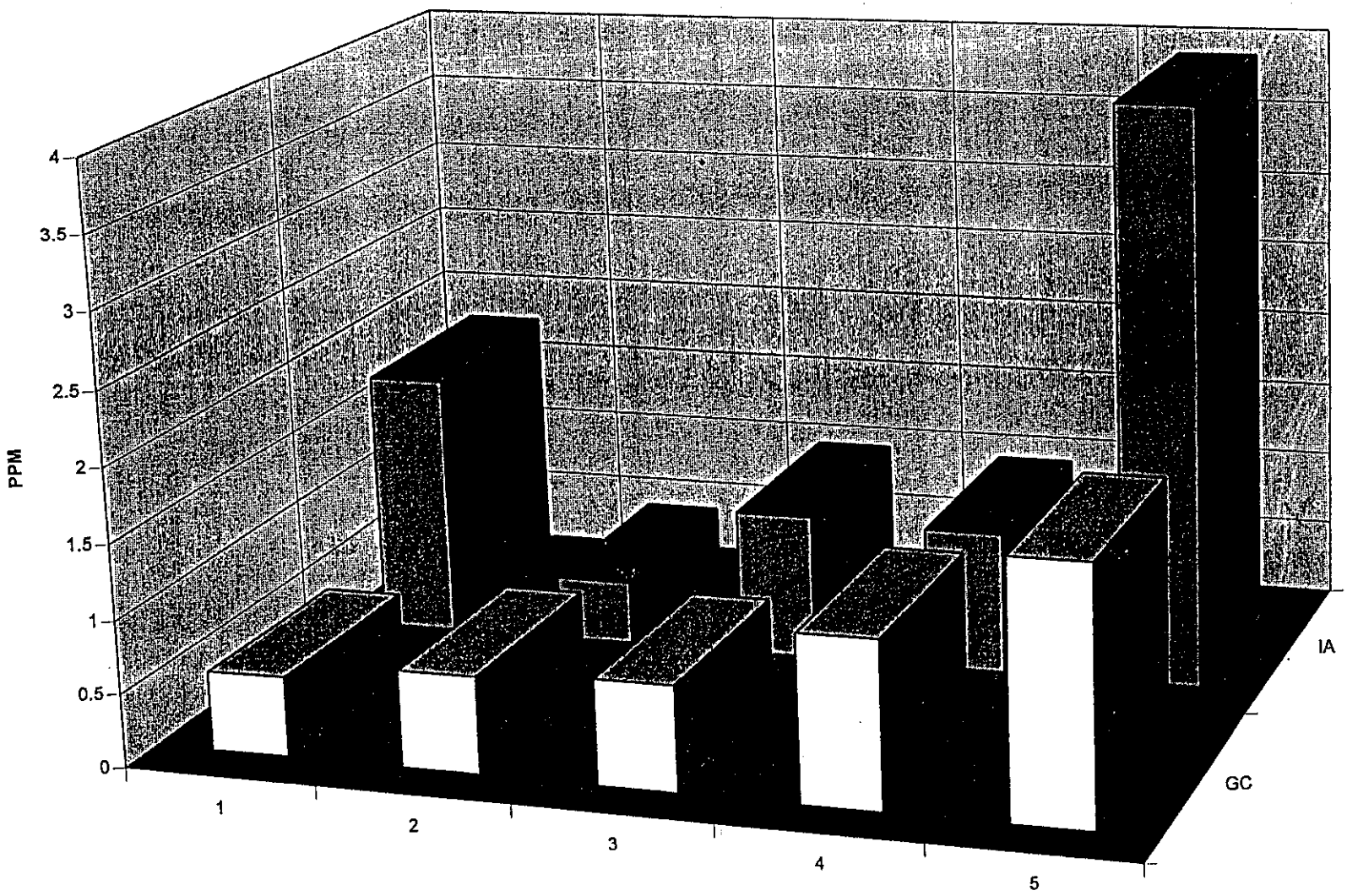

Figure 14. Metolachlor (NSB + H_2O corrected) ELISA (IA) vs. gas chromatography (GC).

CONCLUSIONS

Immunoassays appear to be a dependable analytic method for screening soil samples. Nearly all soil samples that had GC results $\leq$ 0.1 ppm also had immunoassay results $\leq$ 0.2 ppm. Generally speaking, Immunoassay and Gas Chromatography results are very well correlated on samples that have low herbicide concentrations. From a practical standpoint, if you were to choose a soil herbicide concentration of 1.0 ppm as a threshold value, you would nearly always make the proper decision using immunoassay results. Immunoassays usually show a positive bias, most likely due to antibody crossreactivity. In this regard immunoassays are conservative, that is, they tend to overestimate pesticide concentrations. Thus, an immunoassay on the screening result below a limit or standard would provide reasonable assurance that the true concentration is below the standard. Based on the results of this study, immunoassays are recommended for screening soils for the presence of herbicides. They provide a relatively quick, inexpensive alternative to conventional gas chromatographic techniques. The technique is especially cost-effective when investigating only one or two herbicides.

One final point is that analysis of multiple herbicides by immunoassay requires different testing kits for each herbicide. For example, an Atrazine immunoassay requires a different proprietary kit than is required for Alachlor. Consequently, the cost-effectiveness of the immunoassay is reduced when several herbicides must be analyzed on the same sample. In such cases, more conventional analysis (by GC or possibly gas chromatography - mass spectrometry) may be more cost-effective.

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