CASE REPORT

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A Paraquat Fatality—The Dilemma of Multiple Analyses

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ABSTRACT: Presented is a case of suicidal paraquat poisoning. Postmortem analytical measurement of paraquat content in autopsy specimens was accomplished at two different analytical laboratories using different methodological approaches. Despite some disparate results, all findings indicated acute paraquat poisoning.

KEYWORDS: toxicology, paraquat, comparative analyses

This report concerns itself with the comparative analytical findings developed from a well documented case of suicidal paraquat poisoning. Paraquat poisoning and the analytical findings associated with these episodes are well documented in the literature [*1-5*] and thus not the focus of this report. This case is presented to highlight an occurrence that is becoming a practice in forensic toxicology and forensic pathology; that is, the multicenter evaluation of a single forensic science case. This becomes even more important when one considers the strong impetus from practitioners for a Forensic Toxicology Proficiency Testing Program. We wish to relay in this report that, in some cases, different investigators using well documented but analytically distinct methods may generate data that may appear disparate. The data developed however may not be misleading, at least in a case of this type.

Case Report

A 27-year-old married father of three spent an active day on his farm, had dinner, and sat down to watch television with his wife. Several hours later, he got up and said that he was going to get some tools from his tool box in the basement. After 10 to 15 min, his wife asked

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him what he was doing. He indicated that he would be right up and returned to watch television. Shortly thereafter, he went to bed.

A few minutes later, he came downstairs and asked his wife to call an ambulance because he had drunk some poison and was coughing up blood. His wife did as he requested and was subsequently told that her husband had ingested Paraquat C/L (29% paraquat dichloride). The husband was transported to a local hospital 35 min post ingestion, admitted, and immediately lavaged. His condition deteriorated and he was transferred to a tertiary center. Despite medical intervention, he expired some 15 h following ingestion.

Toxicological Methods

Simple verification of the paraquat ingestion was obtained easily by using the classical dithionite reaction (yielding a blue color) on filtrates from the gastric lavage, urine, and blood. The findings reasonably established the cause of death as paraquat poisoning—suicidal. Other tissues and organs were subsequently obtained at autopsy.

Pure paraquat dichloride was obtained as a reference standard from Chevron Corp. Two of the authors (J.B. and B.D.) were involved in establishing gas chromatography/mass spectrometry (GC/MS) reference assays for quaternary amine substances at this time and therefore received a set of postmortem specimens from this case.

The specimens processed in Erie County were subjected to sodium dithionite either directly or following the preparation of a protein free filtrate. The color that was produced was measured spectrophotometrically (Fig. 1). The absorbance reading correlated to the concentration of paraquat using a simultaneously run calibration curve.

In New York, the specimens were processed according to the schema in Fig. 2. Following a

SIMPLE PROCEDURE FOR COLORIMETRIC DETERMINATION OF PARAQUAT

Standards Processed: 0 - 20 mcg/ml

Serum and Urine Were Processed as Aqueous Standards

Tissues -- 25% Aqueous Homogenate
-- Coagulation of Proteins in Boiling Water -- 15 minutes
-- Filter; Process as an Aqueous Solution

PROCEDURE:

1. Scan (Specimens and Standards) From 625-380 nm.
2. Add 0.4 ml 1% $Na_2S_2O_4$ in 1M Na OH.
3. Scan Specimen and Standards From 625-380 nm.

CALCULATIONS:

$$(ABS\ 396_2 - ABS\ 396_1)\ \frac{1.0}{1.4} = O.D.$$

$$\frac{O.D.\ SPECIMEN}{O.D.\ STANDARD} \times CONCENTRATION\ OF\ STANDARD = CONCENTRATION\ OF\ UNKNOWN$$

FIG. 1—*Assay of paraquat, procedure used by Erie County Laboratories, Division of Forensic Pathology and Toxicology.*

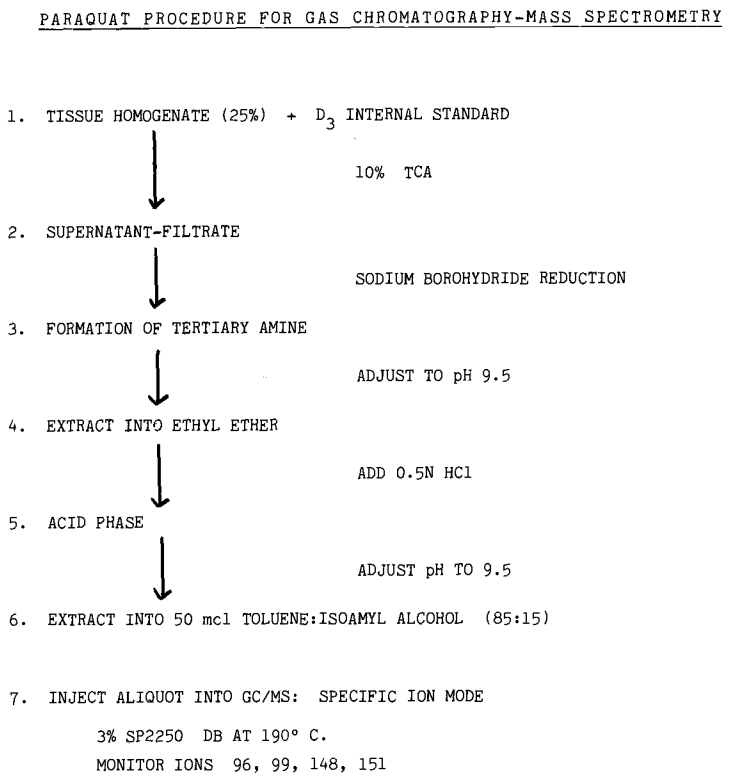

FIG. 2—*Procedure for detection and measurement of paraquat by GC/MS.*

trichloroacetic (TCA) filtrate, the quaternary amine was reduced to a tertiary amine with sodium borohydride. This amine was isolated by extraction and aliquots of the extract were subjected to GC/MS in a specific ion monitoring mode. A deuterated paraquat derivative was used as an internal standard that was added to each specimen and standard before the preparation of the TCA filtrate. The ion chromatograms obtained are presented in Fig. 3. Calibration data are presented in Fig. 4. This analytical method was a modification of that described by Draffen et al [*3*]. Absolute recovery of an aqueous paraquat standard (5 mg/L) was 67%. Relative recovery (to the internal standard) was better than 98%. A within-run precision study using the 5-mg/L standard resulted in a coefficient of variation of 11.5%.

Results and Discussion

Table 1 presents the data that were obtained from the two laboratories on equivalent specimens. Although the ratios on one sample to others in the sample set have some measure of consistency when compared on an interlaboratory basis, the concentrations themselves vary markedly, in some instances, as high as 650%. In all samples processed by the colorimetric approach, those prepared by acid hydrolysis consistently yielded lower recovered paraquat concentrations (from 33 to 78%) than those processed with simple heat coagulation of proteins. This would indicate potential degradation of the paraquat molecule in the digestion process or that the simple coagulation approach is less specific.

The concentrations obtained by the GC/MS approach using a deuterated internal stan-

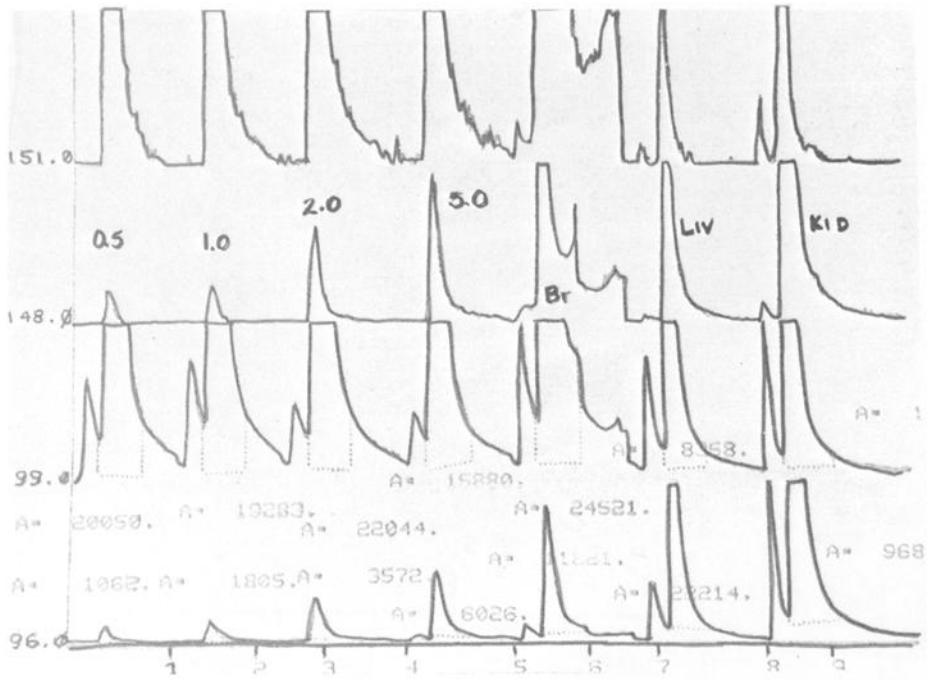

FIG. 3—*Mass spectrometric specific ion chromatograms for paraquat content in postmortem tissues. (0.5, 1.0, 2.0, and 5.0 refer to standards processed in the sample run with postmortem brain* [BR], *liver* [LIV], *and kidney* [KID]. *Ion currents at* m/e *96 and 148 are indicative for* D_0*-paraquat and at 99 and 151 for* D_3*-paraquat, respectively, internal standard).*

dard also yielded consistently lower values except in the blood specimen. The brain specimen was remarkably lower by GC/MS than by either colorimetric method. This result is difficult to explain but the GC/MS result was found to be reproducible.

By any of the analytical approaches, the data support an acute episode with paraquat. Considering the different methods used, it was indeed surprising that the data were reasonably close. In light of the current fashion for multiple center analyses in potentially controversial medical examiner cases, disparate data as a result of sample processing differences in each center could be the rule. This phenomena might even reflect the "state of the art" in forensic toxicology and commands the attention of the practicing forensic toxicologist and pathologist alike. The wide range of the quantitative data may be well understood by the forensic toxicologist but it could be a significant problem and, in fact, a reason for the disqualification of the data if the case involves or alleges criminal intent.

The explanations for the general deviation observed in the resultant analytical data are less than obvious. Dithionite methods with spectrophotometric measurement are known to be subject to many interferences. In the forensic science setting, those interferences may be compounded by the type of specimen being assayed, for example, tissue filtrates.

A comparison of our data in this paraquat episode to those of others [*6*], indicates that the values obtained in this case may represent some of the highest paraquat concentrations in blood and urine ever reported. For instance, the severity of this poisoning episode is evidenced by the fact that the blood concentration of paraquat observed was at least twice the highest concentration reported by Houts et al [*6*]. Other tissue concentrations in this case bear a similar profile to those cited in that report.

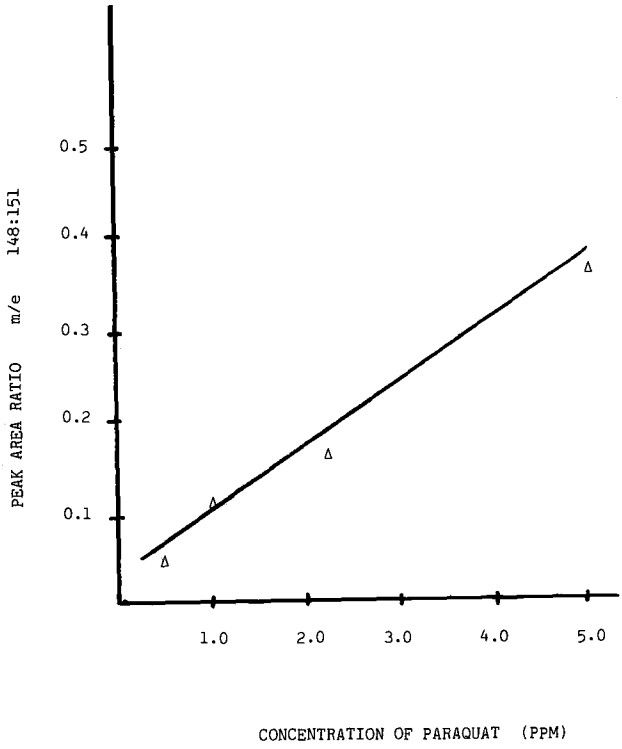

FIG. 4—*Calibration relationship for the GC/MS determination of paraquat.*

TABLE 1.—*Results of paraquat determinations by the two analytical laboratories.*

Specimen	Paraquat Concentrations, ppm	
	Erie County Lab[a]	New York Lab[b]
Liver	157	125
Blood	117 (0.74)[c]	159 (1.3)
Urine	2900 (18.4)	1600 (12.8)
Kidney	414 (2.6)	237 (1.9)
Brain	109 (0.69)	13 (0.69)

[a]Simple colorimetric analysis.
[b]Gas chromatography-mass spectrometry.
[c]Refers to relative concentration ratio to liver concentration found in each instance.

Summary

A case of fatal paraquat poisoning is presented in which two different centers approached the measurement of paraquat content in various tissues. Some disparate results were observed. Multiple center disagreement might be expected in such a situation and as such, may represent the state of the art in forensic toxicology.

Acknowledgment

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