

## **Rapid Qualitative Procedure for the Identification of Petroleum Products in Animal Tissue by Gas-Liquid Chromatography**

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Following the long history of the oil business, which involves exploration, drilling, production, transportation, refining and petrochemical operations, no one will deny that the industry has been a significant polluter of land, waterways, oceans and the atmosphere. A potential hazard exists to livestock and wildlife in all phases of the oil industry.

Clinical reports have been published on petroleum intoxication in ruminants. The circumstances as to why these animals ingest crude oil and other petroleum products are as varied as the clinical signs.

The Oklahoma Animal Disease Diagnostic Laboratory has investigated many cases of suspected petroleum hydrocarbon intoxication. Many of the cases were valid and many were not. The potential for litigation required that the laboratory develop analytical procedures for the identification and quantitation of petroleum hydrocarbons in intestinal contents and tissues.

This analytical procedure is a reasonably rapid, extremely accurate, qualitative test that lends itself to modification as a quantitative procedure. Petroleum compounds from water, stomach contents, lung and feces may be positively matched with implicated petroleum products by a method of chromatographic "fingerprinting" (Tanacredi 1977, Ramsdale & Wilkinson 1968, Gruenfeld and Frank 1977).

Each petroleum mixture, when separated by gas-chromatography, will be represented by a specific reproduceable recorder tracing or "fingerprint". When recovered from biological samples, the petroleum mixture will retain this fingerprint and can be identified by direct comparison.

### **MATERIALS AND METHODS**

A 20 g sample is extracted with 20 mL of Freon 113 (E. I. DuPont de Nemours, Inc.) (U.S.E.P.A. 1974) by shaking for 2 min in a 125-mL separatory funnel. The freon is then placed on a 25 x 500 mm cleanup column (with stopcock) prepared by placing 15 to 20 cm of Florisil in the glass column and adding 5 mL of distilled water. The Freon 113 is collected but not concentrated and dried over 10 g of sodium sulfate. Five  $\mu$ L of sample freon is injected into a gas chromatograph equipped with a flame ionization detector, temperature programmer and a 1.83 m x 2 mm id glass column packed with 10% Carbowax 20 m on 80/100 mesh Supelcoport. The inlet temperature was 200 C and the column was programmed from 100 C to 200 C over 8 min at 32 $^{\circ}$ /min.

Standards are prepared by extracting 1 mL of suspect compound with 10 mL Freon 113.

#### DISCUSSION

Each crude oil or petroleum product has an unique combination of hydrocarbons and relative concentrations. Identification of a petroleum product from biological samples is made by matching peak retention times of the suspect petroleum product and extracts from biological samples. Relative concentrations may be altered in biological samples by differential absorption and should, therefore, not be used as a criteria for identification.

This rapid qualitative procedure has been successfully used to identify petroleum products in ppm concentrations in rumen content, lung tissue and feces.

#### REFERENCES

GRUENFELD, M. and U. FRANK: Proceedings 1977 Oil Spill Conference March 8-10 (1977). New Orleans, A. P. I., Washington, D. C.

RAMSDALE, S. J. and R. E. WILKINSON: J. Institute Petroleum, 54, 539 (1968).

TANACREDI, J. F.: J. Water Pollut. Contr. Fed. pp 216-226 (1977).

U. S. ENVIRONMENTAL PROTECTION AGENCY: Manual of Methods for Chemical Analysis of Water and Wastes, p. 227 (1974).