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ANALYSIS OF ORTHOCHLORONITROBENZENE IN OTTO FUEL II BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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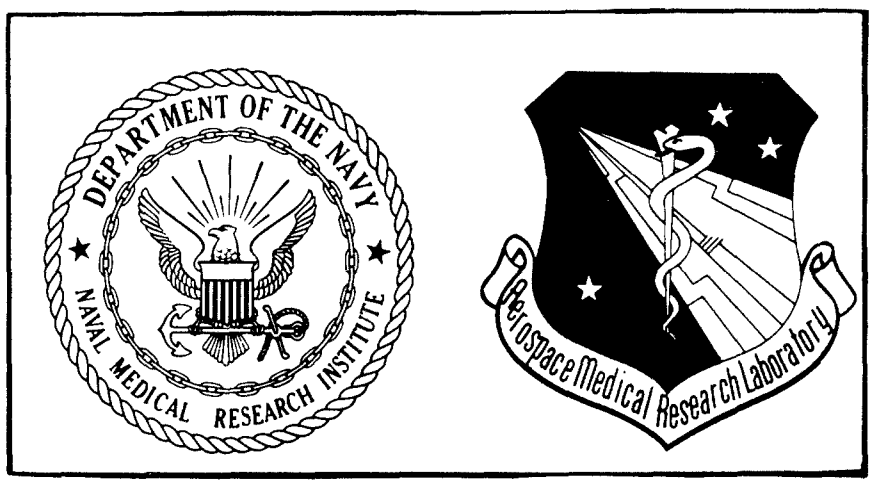

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FOR THE COMMANDER

Roger C. Inman

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Orthochloronitrobenzene (OCNB) is a trace contaminant in Otto Fuel II (OFII), a nitrated ester used as a torpedo propellant by the U. S. Navy. In a published study, OCNB has been reported to produce multiple life tumors in male and female rats. The quantification of OCNB in OFII was performed by high pressure liquid chromatography. The OFII was found to contain 0.00165% OCNB and a distillate of OFII was found to contain 0.0009% OCNB. It was estimated that over a 12 month inhalation exposure to 240 mg/m^3 of OFII an animal would receive a total dose of

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0.05 mg OCNB per kilogram of body weight. This is insignificant compared to the total dose required for the previously published results. OCNB is not considered to be a factor in the outcome of the current studies.

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes an Interim Report on the Analysis of Orthochloronitrobenzene in Otto Fuel II by HPLC. The research covered in this report began in October 1981, was completed in December 1981, and was performed under Air Force Contract No. F33615-80-C-0512, Work Unit 63020115 with funding provided by the Naval Medical Research Institute under Work Unit No. MF58524001.0001. M. K. Pinkerton served as the Contract Technical Monitor for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., served as the Laboratory Director for the THRU of the University of California, Irvine and a co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. LCDR R. L. Hilderbrand served as the Study Coordinator for the Toxicology Detachment of the Naval Medical Research Institute. Acknowledgement is made to H. F. Leahy, W. J. Bashe, J. L. Scheerschmidt and J. A. Sizemore for their significant contributions and assistance in the preparation of this report.

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INTRODUCTION

The Toxic Hazards Research Unit (THRU) of the Department of Community and Environmental Medicine, University of California, Irvine, was requested by the Naval Medical Research Institute/ Toxicology Detachment to determine the concentration of ortho-chloronitrobenzene (OCNB) in Otto Fuel II and in animal chamber atmospheres for one year exposures to 1.4 and 240 mg/m^3 Otto Fuel II, respectively.

The carcinogenic potential of OCNB was reported by Weisburger et al. (1978) in a feeding study using rats and mice. A sustained 12-month OCNB dose of 500 milligrams per kilogram of body weight produced multiple late-life tumors in male rats and hepatocellular tumors in female rats.

In this report the OCNB concentration in both Otto Fuel II liquid and an estimate of the concentration in exposure chamber atmosphere were obtained by analyzing samples using high pressure liquid chromatography (HPLC). Since the Naval Ordnance Station, Indian Head located in Maryland had already measured the concentration of OCNB in liquid Otto Fuel II, this value will provide additional confirmation on the analytical data.

In order to estimate the chamber concentrations, samples of Otto Fuel II distillate were taken for HPLC analysis. It was assumed that the composition of this distillate approximated the composition of Otto Fuel II vapor in the exposure chambers. This technique of sample simulation was adopted because of difficulties in sampling and concentrating Otto Fuel II directly from the exposure atmosphere.

MATERIALS AND METHODS

OTTO FUEL II AND ORTHOCHLORONITROBENZENE

Otto Fuel II (NMRI/TD Sample #0171-1 through 10) and a sample of orthochloronitrobenzene were supplied by the Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Dayton, Ohio.

OTTO FUEL II DISTILLATE

Otto Fuel II was distilled using a micro distilling apparatus (Corning/Pyrex, 14/20 T ground glass joint) at a temperature of 85°C. Air was passed through the still at a rate of 2 liters per minute to speed the volatilization of Otto Fuel II which thermally degrades at 121°C. This procedure has been routinely employed for the preparation of standard bags of propylene glycol dinitrate (PGDN) during exposure of animals to Otto Fuel II vapor.

Otto Fuel II distillate prepared in this manner consisted predominantly of PGDN, the most volatile component of Otto Fuel II, about 0.2% of dibutylsebacate (DBS), and a trace of OCNB.

OTTO FUEL II CONDENSATE

Otto Fuel II condensate was obtained from a vapor trap located in the contaminant introduction line of the low level exposure chamber.

LIQUID CHROMATOGRAPHY (HPLC)

HPLC was the method of choice for OCNB analysis since it had been previously used effectively in this laboratory to separate and measure major Otto Fuel II components as part of a quality control analysis procedure. Analytical conditions for this method were as follows:

ANALYTICAL CONDITIONS FOR HPLC - OTTO FUEL II QUALITY CONTROL

Instrument:	Waters Associates Liquid Chromatograph M 6000
Recorder:	Hewlett Packard 3388A Integrator
Detector:	Waters Differential Refractive Index R-401
Stationary Phase:	Spherisorb (5μ) C^{18} Column - 4.5 mm - I.D., 25 cm - length
Mobile Phase:	Methanol-Water, 90% - 10% (v/v)
Flow Rate:	0.6 milliliter/minute
Injection Volume:	1.0 μliter

Preliminary chromatograms of Otto Fuel II employing this reversed-phase method revealed separation problems which prevented adequate measurement of the OCNB concentration in the fuel. A normal-phase procedure was then tried producing a chromatographic separation much more acceptable for quantitation of OCNB. Therefore, a normal phase HPLC method with conditions detailed below was selected for the determination of OCNB.

ANALYTICAL CONDITIONS FOR HPLC - OCNB IN OTTO FUEL II ANALYSIS

Instrument:	Waters Associates Liquid Chromatograph M 6000
Recorder:	Varian G2000, Span - 50 millivolts
Detector:	Differential U.V., 0.02 Absorbance Range
Stationary Phase:	Spherisorb (5μ) Silica Column - 4.5 mm - I.D., 25 cm - length
Mobile Phase:	Hexane, 0.15% Isopropanol (v/v)
Flow Rate:	0.6 milliliter/minute
Injection Volume:	3.0 μliter
Chart Speed:	1 inch/min

RESULTS AND DISCUSSION

An initial attempt to identify a minor peak in reversed-phase liquid chromatogram of Otto Fuel II as being OCNB by sample spiking was successful. A UV detector was substituted for the refractometer because of the preferential absorptivity of aromatics such as OCNB in the UV range. It became apparent that quantification would be a problem in the reversed-phase LC method due to the poor resolution between the small OCNB peak and the large and tailing PGDN peak. Figure 1 demonstrates this problem.

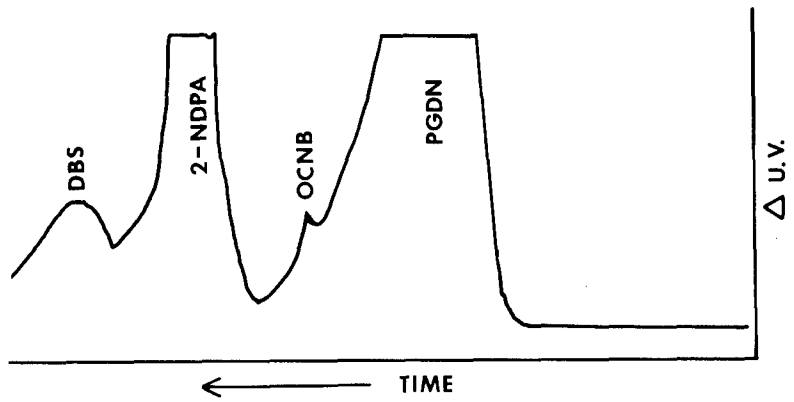

Figure 1. Reversed phase liquid chromatogram of Otto Fuel II. Column - C^{18}; Mobile Phase - 90% MeOH, 10% H_2O (v/v)

Consequently, normal phase LC method was chosen since inverting the elution order allowed the small OCNB peak to avoid being masked by tailing of the PGDN peak.

Figure 2 is a normal phase liquid chromatogram of Otto Fuel II. Absorbance peaks were identified by comparison with retention times of Otto Fuel II components on separate chromatograms. It was observed that the PGDN peak now followed OCNB. While the OCNB was now on the tail of the absorbance peak of the 2-nitrodiphenylamine (2-NDPA) and dibutylsebacate (DBS), its resolution was greatly enhanced.

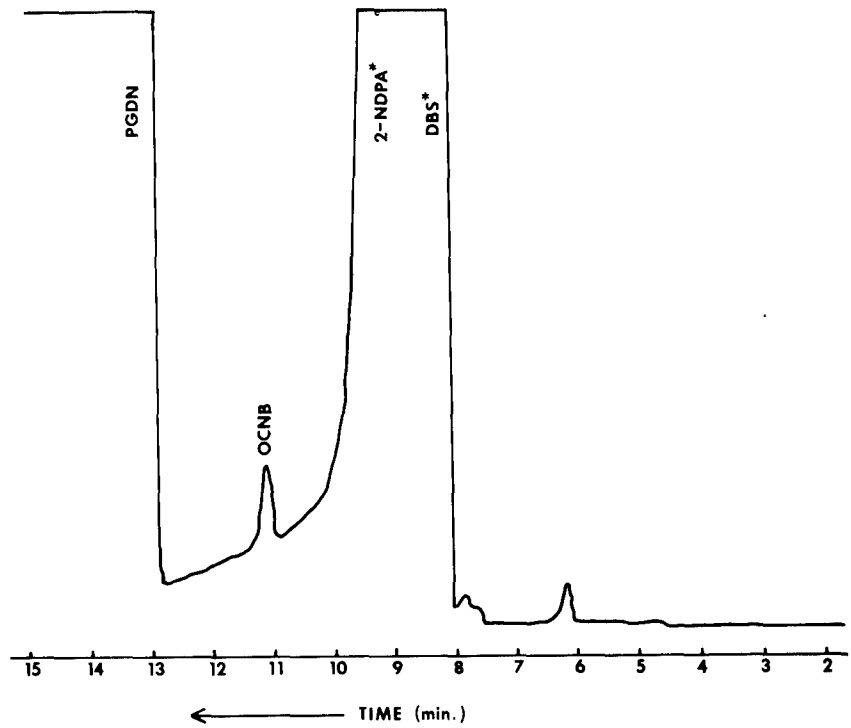

Figure 2. Liquid chromatogram of Otto Fuel II. *DBS and 2-NDPA appear as a composite peak in this chromatogram due to both components being over-range at this U.V. sensitivity setting. At a lower sensitivity, DBS and 2-NDPA yielded poorly resolved peaks at retention times of 8.6 minutes and 9.2 minutes, respectively. The slight shoulder at 9.2 minutes on the DBS peak in the chromatograms in Figure 3 could be 2-NDPA and is so labeled.

In the distillate itself, the relative concentrations of 2-NDPA and DBS were significantly lowered so that they represented no interference to the OCNB peak as shown in Figure 3.

For calibration of the normal phase HPLC methods, a primary standard of 0.25 mg/ml OCNB was made by dissolving 0.025 grams of OCNB in hexane and diluting to 100 ml. Working standards were then generated by dilution of the primary standard with hexane. Table 1 is a summary of calibration data for these standards which were run using the normal phase HPLC method. The calibration curve shown in Figure 4 is the regression line of the means of two or more replicate samples.

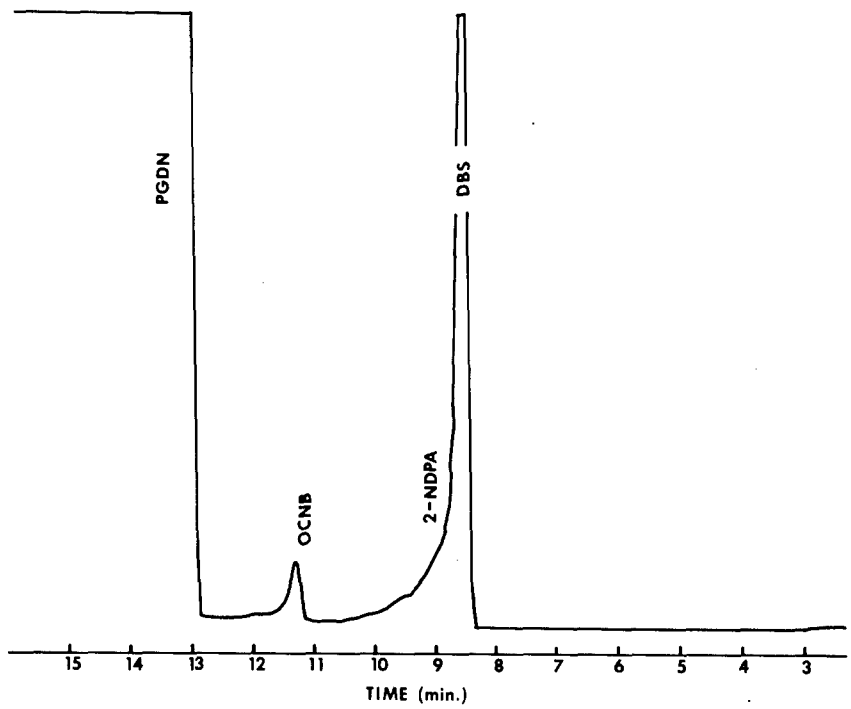

Figure 3. Liquid chromatogram of Otto Fuel II distillate.

TABLE 1. CALIBRATION OF HPLC METHOD FOR OCNB

OCNB Concentration µg/ml	Peak Height in Inches
17	0.8, 1.2
50	3.1, 3.3, 2.8
83	4.5, 4.2
117	5.85, 6.15, 5.95
167	8.0, 8.25, 8.3
Slope:	0.0474
Intercept:	0.38
Correlation Coefficient:	0.9954

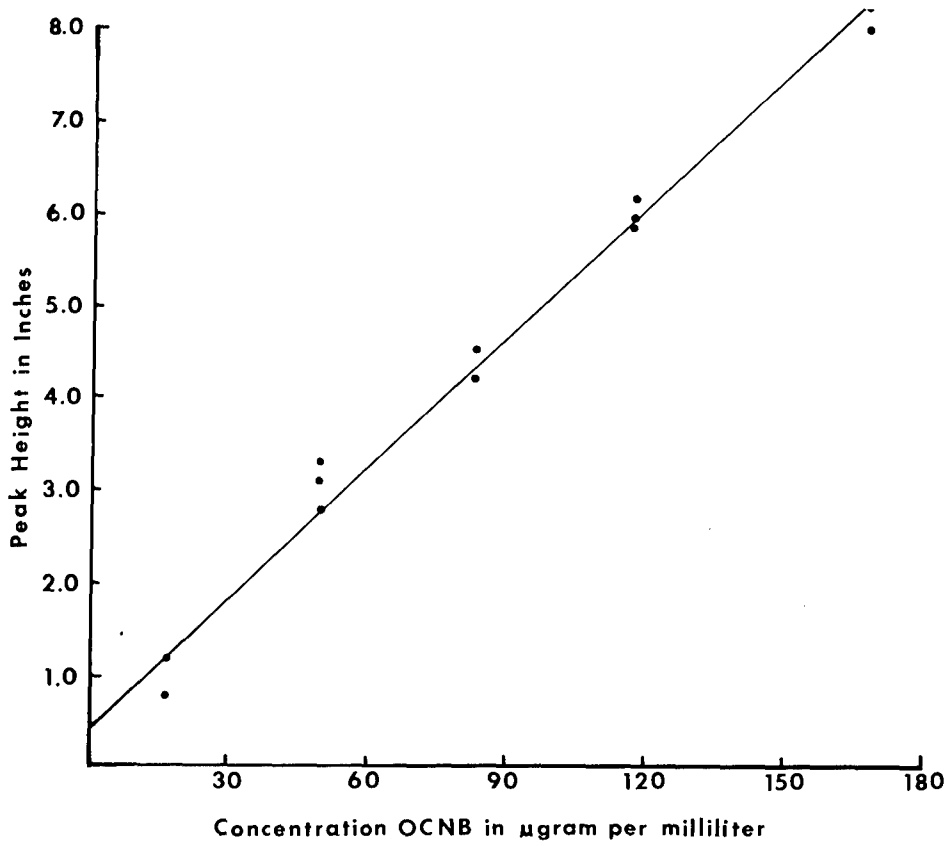

Figure 4. Standard calibration: o-Chloronitrobenzene in hexane; Peak height in inches vs. µgram OCNB per milliliter.

Table 2 is a summary of results obtained by measurement of the height of the OCNB peak on each chromatogram and calculation of micrograms (µg) per injection from the calibration information. The percent composition is percentage by mass assuming a density of 1.232 g/ml for neat fuel and 1.40 g/ml for the distillate and condensate (PGDN density is assumed for the latter two samples). The result of an Otto Fuel II sample spiked to a nominal OCNB concentration of 0.010% is also included.

The estimated OCNB concentrations in the domes as calculated from HPLC analysis of Otto Fuel distillate should be interpreted as maxima. This is because the vapor generation temperatures for the exposure chambers were lower than the distillate. Otto Fuel II vapor was generated at 60°C for the high concentration chamber, at slightly above room temperature for the low concentration chamber, and at 85°C for the distillate.

TABLE 2. ANALYSIS OF PERCENTAGE OF OCNB IN OTTO FUEL AND OTTO FUEL DERIVED SAMPLES

Source	Replicate Sample	Peak Height In Inches	OCNB%
Neat Otto Fuel	1	1.25	0.0015
	2	1.30	0.0016
	3	1.30	0.0016
	4	1.20	0.0014
	5	1.45	0.0018
	6	1.50	0.0019
Otto Fuel Distillate	1	0.95	0.0009
	2	0.95	0.0009
Condensate Trap	1	0.80	0.0006
	2	0.90	0.0008
Spiked Otto Fuel (0.010% OCNB)		5.60	0.0089
Mean Percentage (± 95% Confidence Limits) of OCNB in Neat Otto Fuel			0.0016 (±0.0003)
Mean Percentage of OCNB in Otto Fuel Distillate			0.0009
Mean Percentage of OCNB in Condensate Trap			0.0007
Percent Recovery of OCNB in Spiked Otto Fuel			89

The assumption that a higher percentage of less volatile OCNB would occur at higher temperatures is based on kinetic rather than thermodynamic principles. This was verified by analysis of another Otto Fuel II derivative, condensate from a vapor trap located in the chamber with the low level exposure. This trap was situated on the contaminant introduction line. As freshly generated Otto Fuel vapor was blown through the cooler introduction line, some condensation occurred. The trap acted as a reservoir which collected the condensate from the introduction line and prevented it being carried into the exposure chamber as an aerosol. Analysis of the trap condensate gave a value of 0.0007% OCNB as shown in Table 2.

CONCLUSIONS

OCNB IN OTTO FUEL II

The percent OCNB in Otto Fuel II as measured by HPLC agreed well with the values supplied by NMRI/TD of 0.00165%.

ESTIMATION OF OCNB IN EXPOSURE CHAMBER ATMOSPHERE

If Otto Fuel distillate is a good representation of the material introduced into the exposure chambers, one can estimate maximum chamber air concentrations of OCNB from the distillate value of 0.0009% shown in Table 2.

Dome	Contaminant Concentration In mg/m^3	OCNB Percentage	OCNB Concentration
7	240	0.0009	2.2×10^{-3} mg/m^3 (0.3 ppb)
8	1.4	0.0009	1.3×10^{-5} mg/m^3 (2.0 pptr)

The condensate from the trap yielded a mean OCNB percent composition of 0.0007% which is lower than that found in the distillate, as expected, supporting the interpretation that these exposure chamber estimates were maximum values.

ESTIMATE OF OCNB DOSE INHALED DURING THE YEAR-LONG EXPOSURE TO OTTO FUEL II

A total dose of OCNB can be calculated for animals exposed to 240 mg/m^3 Otto Fuel II for a 12-month period. With 249 6-hour exposures of rats breathing at a rate of 80 ml/min., the calculations are:

$$\text{Total Dose} = 2.2 \times 10^{-3} \times 80 \times 10^{-6} \times 60 \times 6 \times 249$$
$$= 0.016 \text{ mg/300 g. rat}$$
$$= 0.05 \text{ mg/kg OCNB}$$

This dose, which represented a maximum for absorption by rats, is insignificant compared to the 500 mg/kg found to be carcinogenic in the Weisberger study.

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Weisburger, E. K., A. B. Russfield, F. Homburger, J. H. Weisburger, E. Boger, C. G. VanDongen and K. C. Chu (1978), Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity, Journal of Environmental Pathology & Toxicology, 2:235-356.

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