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IRI Report No. 2327

HMX: Analysis in Plasma Obtained After 90 Day
Toxicity Studies with Rats and Mice

Final Report by:

M.S. Henderson

31 July 1985

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Inveresk Research International Limited
Musselburgh, EH21 7UB, Scotland

Contracting Officer's Technical Representative:

Jesse J. Barkley, Jr.
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

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Abstract

A sensitive method was developed for analysis of HMX in the plasma of laboratory rodents. It is based on reverse phase HPLC and has a limit of detection around 20 ng/ml⁻¹.

Analysis of blood samples from 13 week rat and mouse toxicity studies revealed that:

Rat plasma levels were very low and did not increase with increasing dose levels. It is therefore likely that most of the material was not absorbed.

Insufficient plasma was available from the 13 week mouse study for successful analysis.

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by other authorised documents.

FOREWORD

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."



A.B. Wilson, B.V.Sc., M.R.C.V.S.,
D.A.B.T.
Principal Investigator

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The title on the front cover is correct for
this report.
Per Ms. Virginia Miller, AMR&DC

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QUALITY ASSURANCE AUTHENTICATION

The conduct of this study has been subjected to periodic inspections by the IRI Quality Assurance Unit. The dates of inspection are given below.

IRI Project No. 415669 AD
415669 AR
415669 AM

Report No. 2327

Date of Q.A. Inspection

Date of Report to Management

11-14 January 1982

21 January 1982

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.

Signed:

Andrew Waddell
(Quality Assurance
Manager)

Date:

3rd March 1986

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SUMMARY

A sensitive and specific analytical procedure has been developed for the determination of HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in rat plasma. The method is based on reverse phase high performance liquid chromatography and indicates a limit of detection of around 20 ng.ml⁻¹ with good accuracy and precision. The procedure has been applied to the analysis of plasma samples collected at termination of the 90 day toxicity studies in rats and mice.

The principal findings were as follows:

- 1) The levels of HMX in plasma from rats were extremely low in relation to the dose levels.
- 2) No significant increase in plasma levels of HMX was observed with increasing dose.
- 3) The data may well indicate that most of the administered test material was not systemically absorbed but was excreted unchanged.
- 4) The presence of HMX in the plasma of the control group animals was also indicated. The levels were low but significant. They may approximate to those anticipated following a single dose of 13-15 mg.kg⁻¹. The reason why the plasma from the control group animals should have contained HMX are unknown.
- 5) Insufficient plasma was available for successful analysis of HMX in mouse plasma at the termination of the 90 day mouse toxicity study.

INTRODUCTION

Inveresk Research International have recently undertaken a series of toxicity studies in rats and mice with HMX under DAMD 17-80-C-0053 (IRI Project Nos. 415669 CR, 416877). The objective of the present study was to establish methodology to enable the determination of HMX in the plasma of rats and mice at termination of the 90 day programmes.

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited.

The analytical experiments were carried out at the Inveresk Gate site of Inveresk Research International Limited between May 1981 and May 1982.

EXPERIMENTAL PROCEDURES

Materials

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX) were supplied from the Royal Ordnance Factory, Bridgewater, England, as white suspensions containing approximately 20% (w/w) water. For the preparation of analytical standards both HMX and RDX were dried to constant weight in a water-heated oven.

Acetonitrile (S or HPLC grade) was purchased from Rathburn Chemicals Limited, Scotland.

Dichloromethane was of AnalaR grade (BDH) and was distilled at IRI prior to use.

Animal Diet

During the course of the study a laboratory Rodent Diet (BP Nutrition (Modified) Expanded Ground Maintenance Diet) was available to the animals ad libitum. A typical analysis for this diet is presented in Appendix 2.

Equipment

High performance liquid chromatography (HPLC) was performed on a system comprising an Altex model 110A pump and a Pye LC3 variable wavelength u.v. detector. Columns used for the analyses were slurry packed at IRI.

Final Analytical Method for the Analysis of HMX in Rat Plasma

Standard and internal standard solutions (at a concentration of approximately 1 mg.ml^{-1} in acetonitrile) were prepared in grade "A" volumetric flasks. Calibration solutions were prepared by appropriate dilutions of the stock solution. Standard samples were prepared by adding aliquots of these dilutions (100 μl) to control pooled plasma (100 μl). These samples when processed enabled the construction of a calibration curve in the range $0-5 \text{ }\mu\text{g.ml}^{-1}$. On each day of analysis, test samples and standards were processed and analysed concurrently.

Plasma samples (100 μl) were pipetted into test tubes to which was added RDX as internal standard (100 μl of a solution in acetonitrile, $6 \text{ }\mu\text{g.ml}^{-1}$). The tubes were briefly vortex mixed and redistilled dichloromethane (5 ml) added to each. Samples were hand-shaken (1 min) and centrifuged (10 min; 5000 rpm). After separation, the organic phase was taken to dryness under

a stream of nitrogen. Any residual dichloromethane was removed under vacuum. The residue was dissolved in mobile phase (50 μ l) and aliquots (25-50 μ l) taken for analysis. Calibration curves were constructed with peak height HMX/peak height internal standard plotted against plasma concentration of HMX. The lines of best fit were obtained from a linear regression programme on a Wang 2200s programmable desk calculator. The concentrations of HMX in test plasma samples were determined by interpolation from the standard curve.

Precision of analysis, based on the 95% confidence limits about the calibration curves, was determined from the standard deviations (σ) of the calibration values from their regression functions.

On each analytical occasion the assay was checked by analysing a series of quality control samples prepared by an independent operator at a concentration unknown to the analyst.

Equipment

The HPLC system used consists of:

Column:	100 x 5 mm i.d. stainless steel column packed with Hypersil-ODS (5 μ m)
Mobile Phase:	0.01% perchloric acid/acetonitrile (4:1 v/v)
Flow Rate:	2 ml.min ⁻¹
Pump:	Altex model 110A
Detector:	Pye LC3 variable wavelength
Wavelength:	228 nm
Range:	0.01 AUFS

The u.v. detector signal was recorded at both 50 and 20 mV full scale deflection to accommodate the full range of HMX concentrations.

RESULTS AND DISCUSSION

Assessment of the Performance of the Final Analytical Method

The final analytical method was assessed in terms of linearity, sensitivity, accuracy and precision.

Aliquots of rat plasma (200 μ l) containing HMX at concentrations of 0, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 μ g.ml⁻¹ and RDX as internal standard (2.41 μ g.ml⁻¹) were extracted into dichloromethane and the samples subsequently analysed by HPLC. Typical chromatograms are presented in Figure 1. A calibration curve was constructed with peak height ratio HMX/RDX plotted against plasma concentration HMX (Figure 2).

These data showed that HPLC offered a sensitive technique with linear response for the determination of HMX in plasma at concentrations down to approximately 20 ng.ml⁻¹. Calibration curves in the range 0-1 μ g.ml⁻¹ HMX similarly showed good sensitivity (Figure 3) and linearity (Figure 4).

The precision and accuracy of the assay was determined by analysing 2 sets (10 samples per set) of plasma containing added HMX at 1 μ g.ml⁻¹ and 100 ng.ml⁻¹. The co-efficients of variation were 6% and 10% respectively. The relative error in both instances was less than 10% (Table 1).

Analysis of HMX in Plasma Obtained During Toxicity Studies with Rats and Mice

The determination of HMX in the plasma of rats and mice at termination of the 90 day toxicity studies was undertaken using the final analytical method as described above. A single analysis was performed on plasma samples from 5 animals of each sex in all dose groups. The animal numbers used in these studies were detailed in Table 2.

The results for the rat study are shown in Table 2 and graphically in Figure 5.

Typical chromatograms are shown in Figure 6. Analysis of the plasma samples taken from the rat control groups 16 and 17 suggested the presence of small but significant quantities of HMX. Following a thorough review of the raw data by the analysts and by the IRI Quality Assurance Unit, further investigations were accordingly undertaken to substantiate or vitiate these observations. The results of these further investigations are described in Appendix 1.

The changes in plasma concentrations of HMX with respect to dose level are illustrated in Figure 5.

In relation to the doses administered the plasma levels of HMX are very low and show little change with increasing dose. In male animals the plasma levels from all dose groups are around $1.5 \mu\text{g.ml}^{-1}$. The females show slightly higher maximum plasma concentrations ($2.5\text{-}3.5 \mu\text{g.ml}^{-1}$). Some indication of increasing plasma concentrations with increasing dose is apparent in the female groups but only at the lowest dose levels.

The results suggest that the degree of systemic absorption of HMX is very low (probably due to the compound's poor aqueous solubility). The bulk of the administered dose is almost certainly not absorbed but is excreted unchanged.

The analysis of HMX in mouse plasma was unsuccessful. Despite the fairly high sensitivity of the assay the very small quantities of plasma obtained at post mortem rendered the generation of meaningful data impossible.

TABLE 1

Determination of HMX in Plasma: Precision and Accuracy

Compound	Calibration Range	Internal Standard	Spiked Concentration of HMX	Mean Result \bar{x} (n = 10)	Standard Deviation σ	σ (%)	Mean Recovery (%)
HMX	0-10 $\mu\text{g}\cdot\text{ml}^{-1}$	RDX	1.10 $\mu\text{g}\cdot\text{ml}^{-1}$	1.19 $\mu\text{g}\cdot\text{ml}^{-1}$	0.07 $\mu\text{g}\cdot\text{ml}^{-1}$	5.9	108
HMX	0-1 $\mu\text{g}\cdot\text{ml}^{-1}$	RDX	103 $\text{ng}\cdot\text{ml}^{-1}$	108 $\text{ng}\cdot\text{ml}^{-1}$	11 $\text{ng}\cdot\text{ml}^{-1}$	10.2	105

TABLE 2

HMX: Analysis in Plasma Obtained After the 90 Day
Toxicity Study in Rats

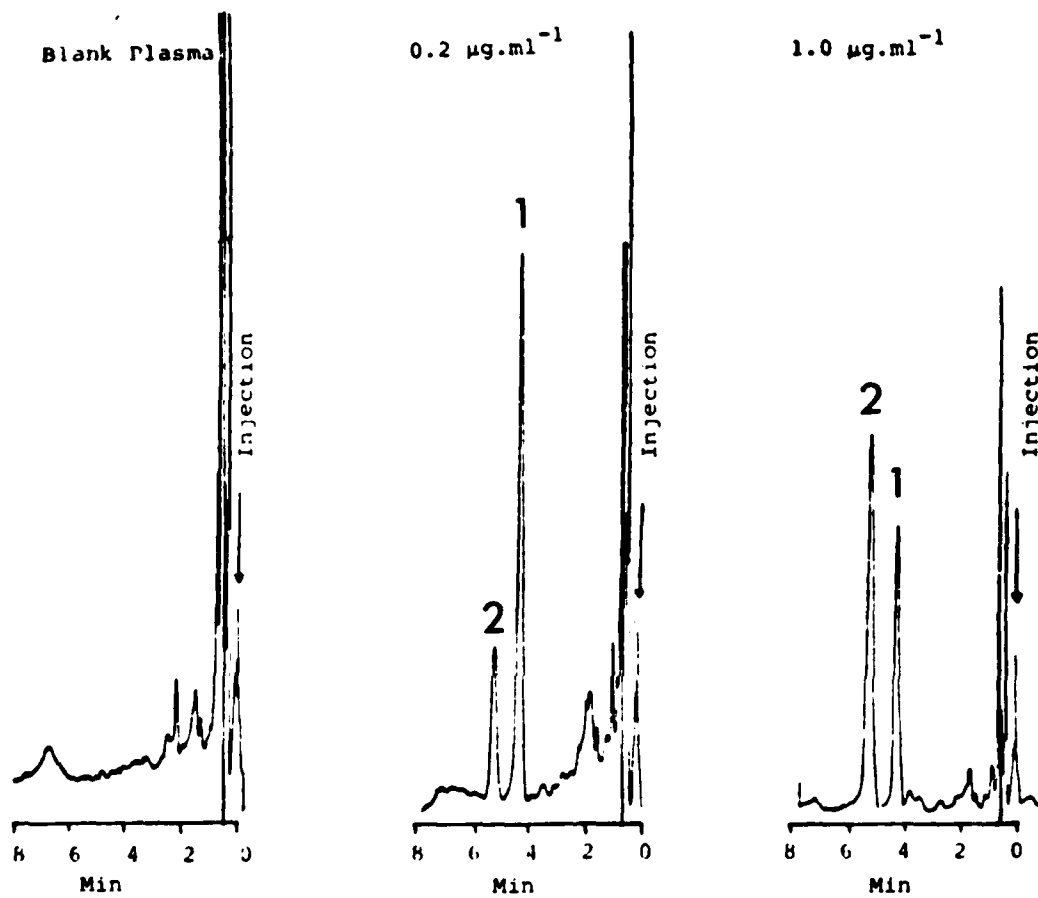
Group	Dose mg.kg ⁻¹ day ⁻¹	Animal Number	Conc HMX µg.ml ⁻¹	Mean µg.ml ⁻¹ ± σ
1	0	601♂	0.15	0.26 ± 0.13
		604♂	0.11	
		608♂	0.42	
		616♂	0.34	
		618♂	0.30	
		722♀	0.40	0.51 ± 0.28
		724♀	0.87	
		728♀	0.15	
		737♀	0.70	
		740♀	0.42	
2	50	621♂	1.50	1.14 ± 0.52
		624♂	0.79	
		628♂	1.63	
		636♂	0.40	
		638♂	1.39	
		745♀	1.13	1.65 ± 0.65
		748♀	1.14	
		756♀	1.27	
		758♀	2.20	
		759♀	2.50	
3	150	646♂	1.40	1.18 ± 0.83
		647♂	2.48	
		650♂	1.07	
		655♂	0.51	
		658♂	0.42	
	115	762♀	2.02	2.74 ± 0.69
		764♀	3.12	
		772♀	2.23	
		776♀	3.73	
		777♀	2.62	

TABLE 2 (continued)

Group	Dose mg. kg ⁻¹ day ⁻¹	Animal Number	Conc HMX μg. ml ⁻¹	Mean μg. ml ⁻¹ ± σ
4	450	664♂	0.52	0.91 ± 0.55
		666♂	0.51	
		667♂	0.72	
		675♂	1.84	
		679♂	0.98	
	270	784♀	1.68	1.62 ± 0.49
		785♀	1.69	
		792♀	1.11	
		796♀	2.37	
		800♀	1.27	
5	1350	681♂	1.18	0.96 ± 0.19
		684♂	0.98	
		690♂	0.92	
		691♂	1.05	
		697♂	0.67	
	620	802♀	3.40	3.76 ± 1.19
		804♀	3.84	
		806♀	5.14	
		814♀	1.97	
		815♀	4.43	
6	4000	704♂	0.85	1.43 ± 1.14
		707♂	1.05	
		709♂	3.45	
		711♂	0.76	
		719♂	1.04	
	1500	822♀	3.01	2.64 ± 0.87
		824♀	3.71	
		826♀	2.78	
		829♀	1.34	
		834♀	2.38	

FIGURE 1

Typical Chromatograms for the Analysis of HMX
in Rat Plasma ($0-10 \mu\text{g}\cdot\text{ml}^{-1}$)



1 = RDX
2 = HMX

FIGURE 2

Typical Standard Curve for the Analysis of HMX in Rat Plasma
(0-10 $\mu\text{g} \cdot \text{ml}^{-1}$)

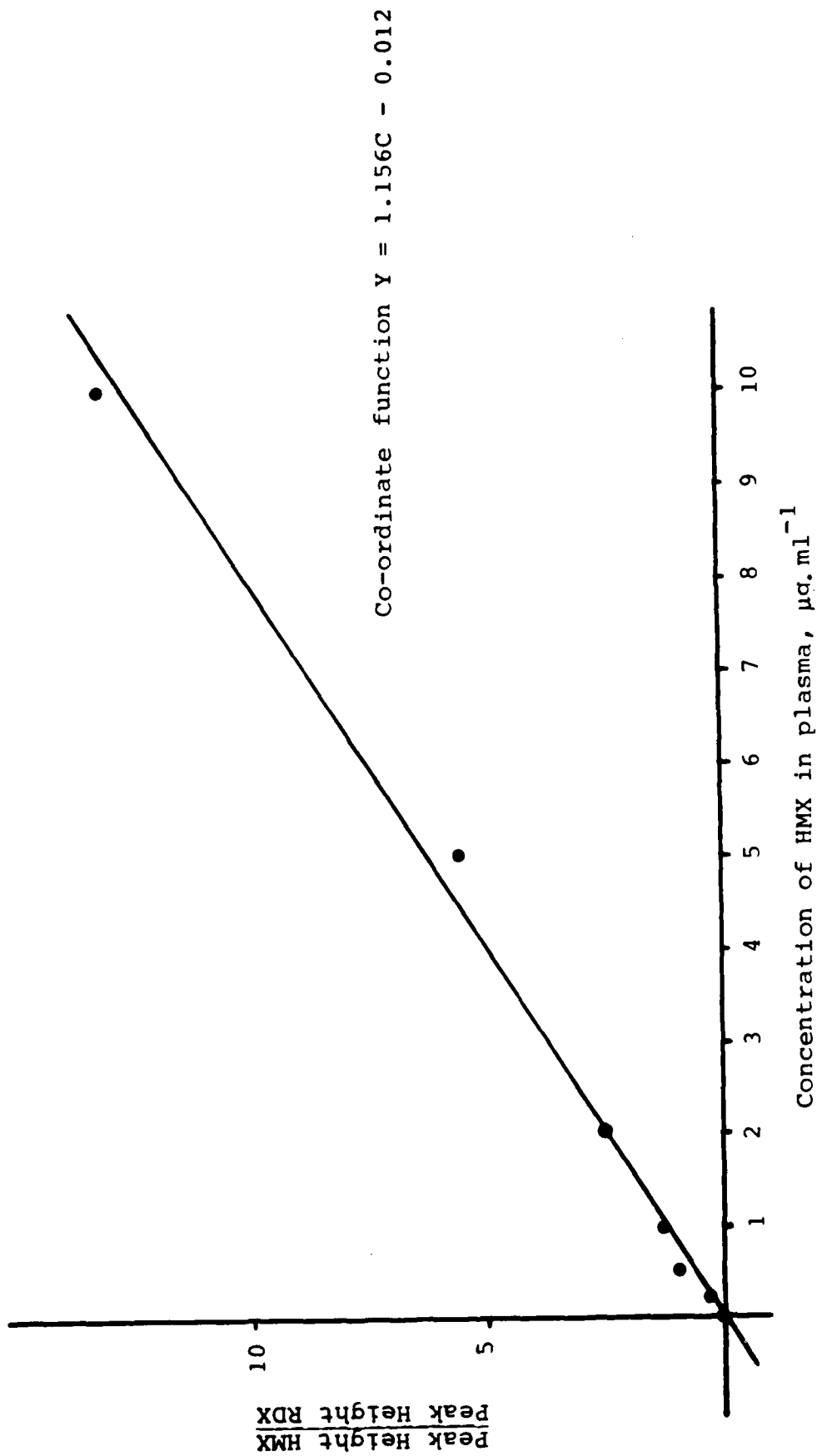


FIGURE 3

Typical Chromatograms for the Analysis of HMX in Rat Plasma
(0-1 $\mu\text{g}\cdot\text{ml}^{-1}$)

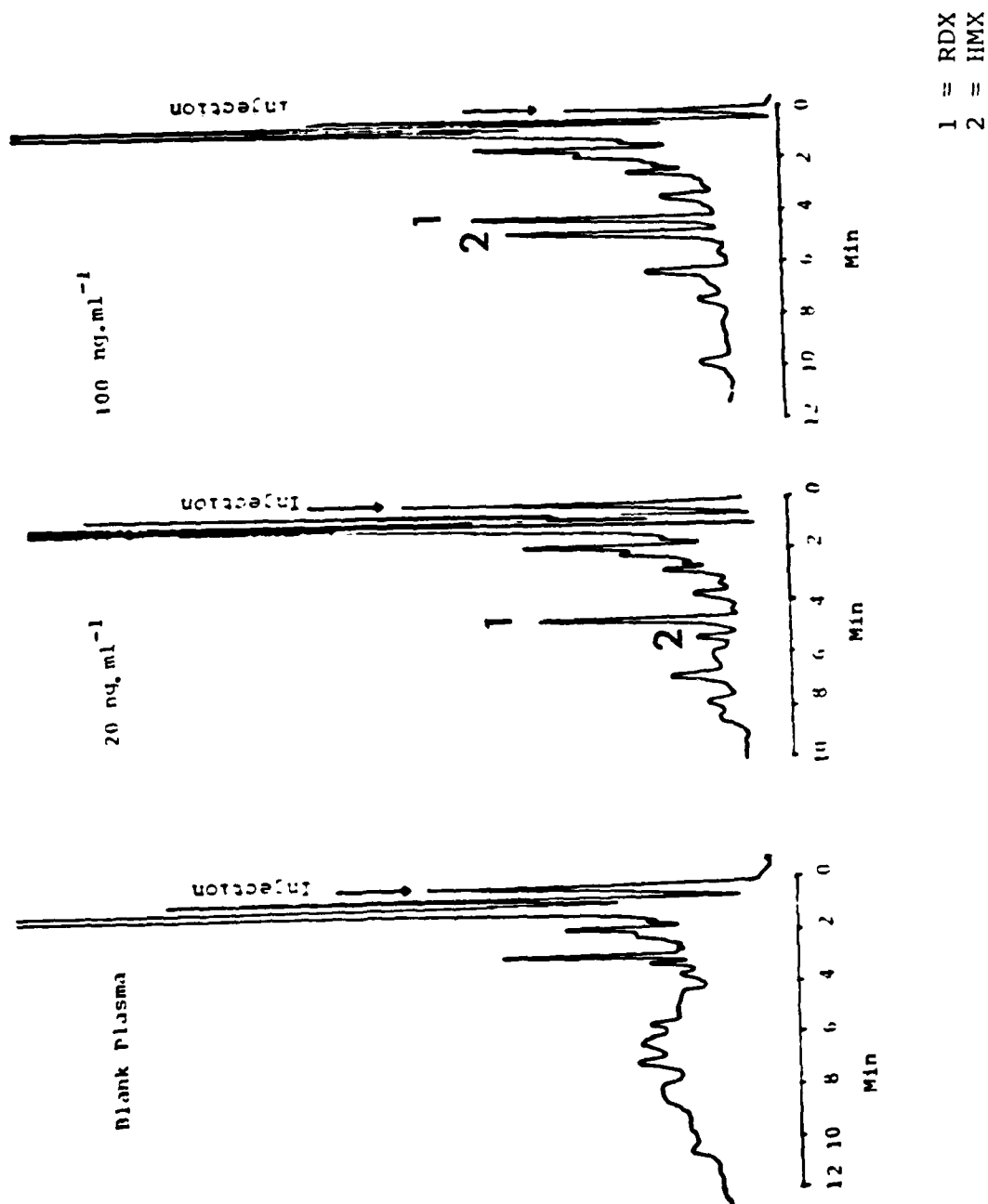


FIGURE 4

Typical Standard Curve for the Analysis of HMX
in Rat Plasma ($0-1 \mu\text{g}\cdot\text{ml}^{-1}$)

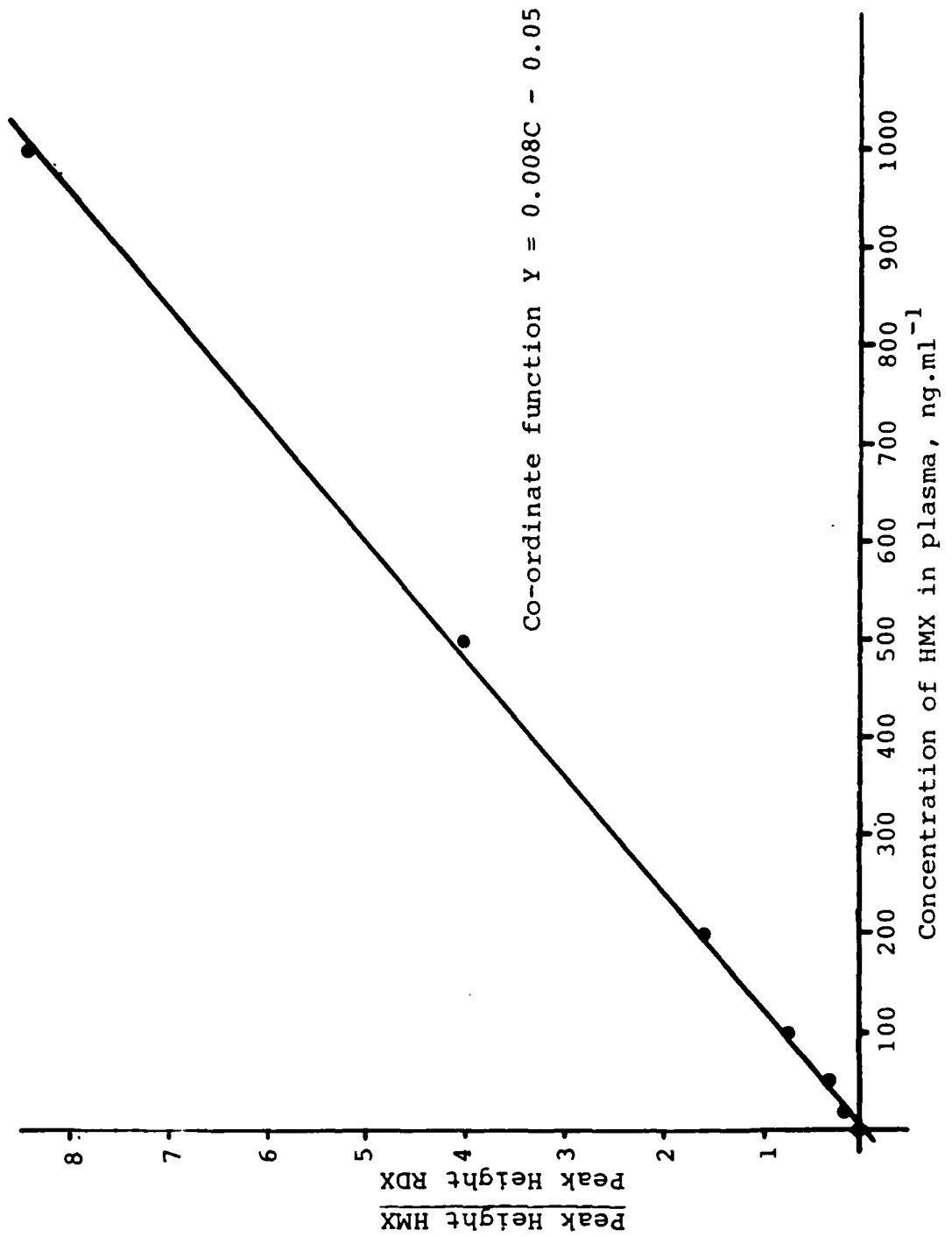


FIGURE 5

HMX: Analysis in Plasma Obtained After the 90 Day
 Toxicity Study in Rats
 The Effect of Increasing Dose Levels

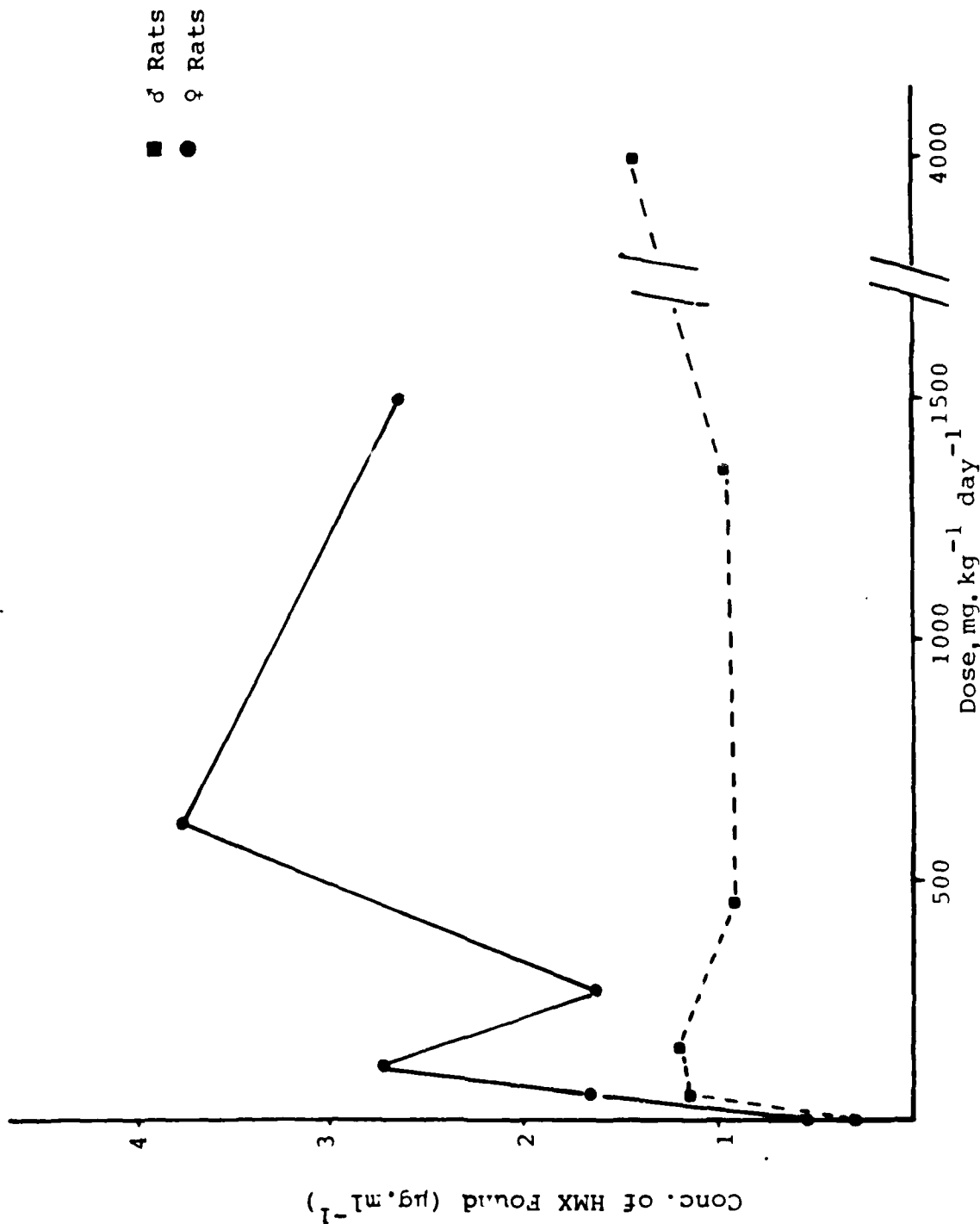
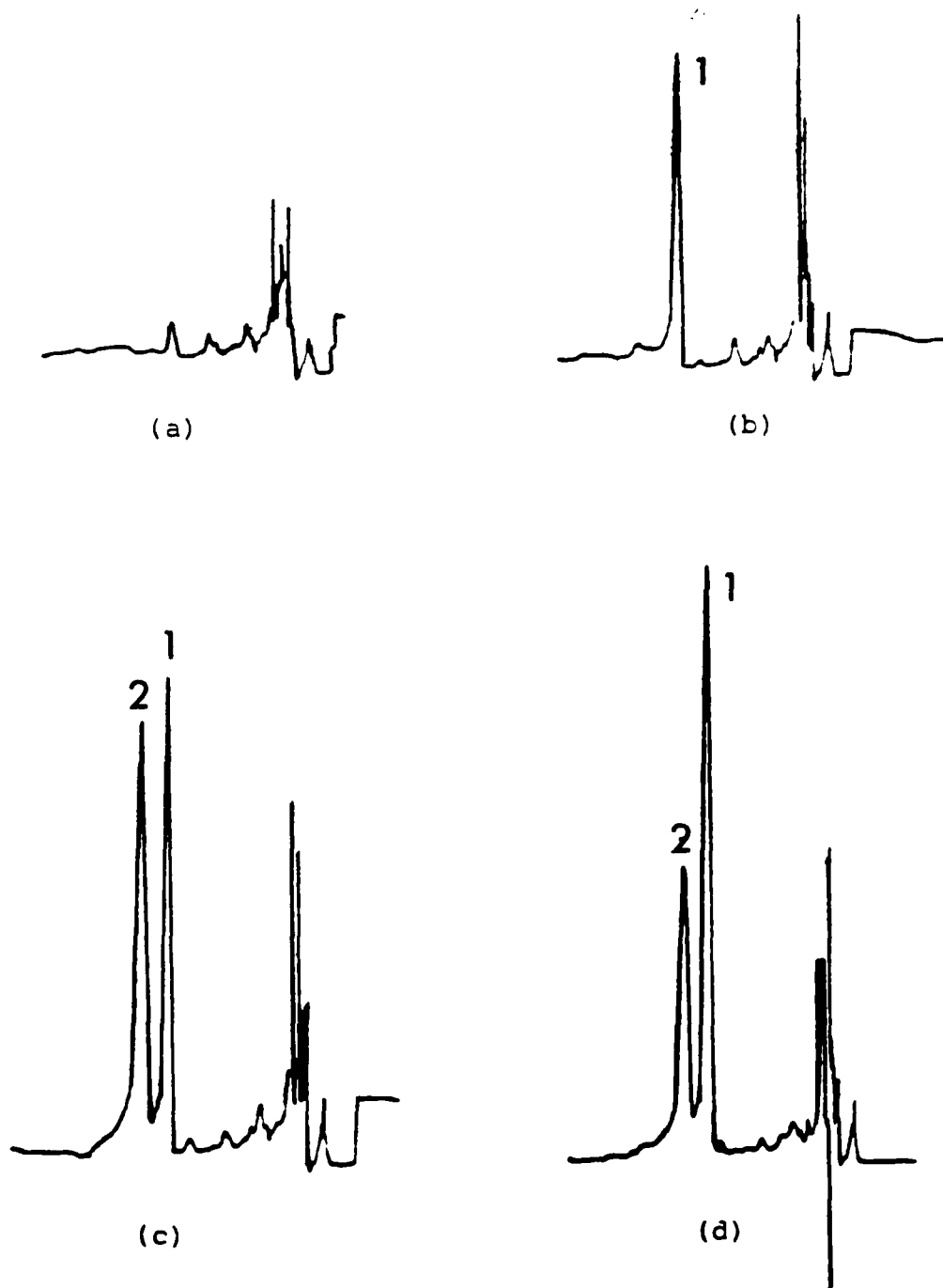


FIGURE 6

Typical Chromatograms for the Analysis of HMX in Rat Plasma
Obtained After the 90 Day Toxicity Study



a) double blank
b) single blank
c) $2.0 \mu\text{g HMX.ml}^{-1}$ standard
d) sample 707 δ

1 = RDX
2 = HMX

APPENDIX 1Further Investigations of the Occurrence of HMX in Control
Group PlasmaIntroduction

Analysis of the plasma samples taken from the control group 1♂ and 1♀ rats in the 90 day toxicity study indicated the presence of small but significant quantities of HMX. Further investigation has therefore been undertaken to verify that the chromatographic peak observed was indeed due to HMX and was not due to an endogenous plasma component.

In liquid chromatography, compounds can be separated either by adsorption on a polar surface (normal phase) or by partition from a non-polar surface (reverse phase). Although it is possible for 2 or more compounds to have the same retention volume on a particular column, it is very improbable that the same compounds will behave identically in both normal and reverse phase modes.

It was thus proposed that the plasma samples be re-analysed using both forms of liquid chromatography.

Experimental Procedures

Two male and 2 female Fischer S344 rats were fed control diet (for typical analysis see page 19) for a period of 2 weeks. They were then bled and the plasma collected and stored at -20°C. Blank plasma was also obtained from a CD rat.

The group 1 plasma samples collected were pooled according to sex. To 100 µl aliquots of plasma were added internal standard (RDX), HMX and acetonitrile as shown in Appendix Table 1.

Methylene chloride (5 ml) was added to each sample and the tubes were hand-shaken (2 min). The samples were centrifuged (10 min; 5000 rpm) and the supernatant transferred to tapered tubes, taking care not to transfer the precipitated proteins. The solvent was then evaporated at room temperature under nitrogen. Residual amounts of methylene chloride were removed under vacuum.

The residue was dissolved in mobile phase (50 µl) and a 25 µl aliquot chromatographed. In Appendix Table 1 the samples marked "A" were chromatographed using the normal phase mode and all those marked "B" were chromatographed using the reverse phase system.

HPLC conditions were as follows:

Normal Phase

Column: Hypersil 3 μm (100 x 5 mm i.d.)

Mobile Phase: Hexane/propan-2-ol (55:45, v/v)

Reverse Phase

Column: ODS-Hypersil 5 μm (100 x 5 mm i.d.)

Mobile Phase: 0.01% perchloric acid/acetonitrile
(80:20, v/v)

Detector: Ultraviolet at 228 nm 0.01 AUFS

Pump: Altex Model 110A

Results and Discussion

In the normal phase mode, quantitative results were not possible as the internal standard peak was not resolved from the solvent front. However, the results can be viewed qualitatively. Although there was a small endogenous peak eluting near HMX in plasma from Fischer male rats (to which no HMX had been added, Appendix Figure 1) this was negligible compared to the peak representing $0.47 \mu\text{g}\cdot\text{ml}^{-1}$ HMX in the standard sample (Appendix Figure 1C). Appendix Figure 2 shows the chromatogram of the Group 1 σ sample which contains a significant peak at the retention volume of HMX. When HMX (4.7 ng) was added to the same sample, this peak increased in size (Appendix Figure 2B) indicating that the compound in (A) behaves identically to HMX. Similar results were obtained for the Fischer female and Group 1 ρ samples (Appendix Figures 3 and 4) except that the size of the HMX peak is much larger.

In the reverse phase mode, the CD rat, Fischer female and Fischer male samples (to which no HMX had been added) also showed no significant peak at the HMX retention volume (Appendix Figures 5 and 6). The Group 1 σ and 1 ρ samples both showed peaks at the HMX position and, when spiked with HMX (4.7 ng), showed increased peak height ratio (Appendix Table 3) indicating that the compound observed in the chromatograms (Appendix Figures 7 and 8) of the Group 1 σ and Group 1 ρ samples behaves identically to HMX in the reverse phase mode. Appendix Tables 2 and 3 show quantitative results obtained while Appendix Table 4 compares them with the results found previously. As can be seen, there is good agreement between the analyses.

Conclusions

As the compound present in the Group 1♂ and Group 1♀ samples co-chromatographs with HMX on both normal and reverse phase systems, it is concluded that these samples indeed contain HMX.

APPENDIX TABLE 1

Preparation of Plasma Samples for Analysis of HMX

Sample		Volume of acetonitrile added (μ l)	Volume of HMX added (μ l)*	Volume of RDX added (μ l)+
CD	1A	200	-	-
	1B	200	-	-
	2A	100	-	100
	2B	100	-	100
	3A	-	100	100
	3B	-	100	100
Fischer φ	1A	200	-	-
	1B	200	-	-
	2A	100	-	100
	2B	100	-	100
	3A	-	100	100
	3B	-	100	100
Fischer δ	1A	200	-	-
	1B	200	-	-
	2A	100	-	100
	2B	100	-	100
	3A	-	100	100
	3B	-	100	100
Group 1 φ	1A	-	-	100
	1B	-	-	100
	2A	-	-	100
	2B	-	-	100
Group 1 δ	1A	-	-	100
	1B	-	-	100
	2A	-	-	100
	2B	-	-	100

* = Concentration of HMX solution = 475 ng HMX.ml⁻¹+ = Concentration of RDX solution = 667 ng RDX.ml⁻¹

APPENDIX TABLE 2

Peak Heights of HMX and RDX in Control Plasma Extracts
(Reverse Phase System)

Species	Sample	Peak Height (mm)		Peak Height Ratio
		HMX	RDX	
CD	Double blank	0	0	0
	Single blank	0	46.0	0
	Standard	53.0	60.5	0.876
Fischer 2	Double blank	2.0	0	
	Single blank	0	55.0	0
	Standard	34.0	35.0	0.971
Fischer 3	Double blank	0	0	0
	Single blank	0	50.5	0
	Standard	29.0	10.5	2.762

Standard } 47.5 ng HMX added
 } 66.7 ng RDX added

APPENDIX TABLE 3

Peak Heights of HMX and RDX in Group 1 (♂ ♀) Plasma Extracts

Group	Sample	Peak Height (mm)		Peak Ratio HMX/RDX	Calculated* Concentration ($\mu\text{g}.\text{ml}^{-1}$)	Mean Concentration ($\mu\text{g}.\text{ml}^{-1}$)
		HMX	RDX			
1 ♀	1	70.0	51.0	1.373	0.744	0.81
	1 spiked	57.0	30.5	1.869		
	2	79.0	49.0	1.612	0.874	
	2 spiked	79.0	42.0	1.881		
1 ♂	1	32.0	61.0	0.525	0.284	0.25
	1 spiked	21.5	37.0	0.581		
	2	20.0	49.0	0.408	0.221	
	2 spiked	28.0	43.0	0.651		

* = Based on peak height ratio of CD rat extract

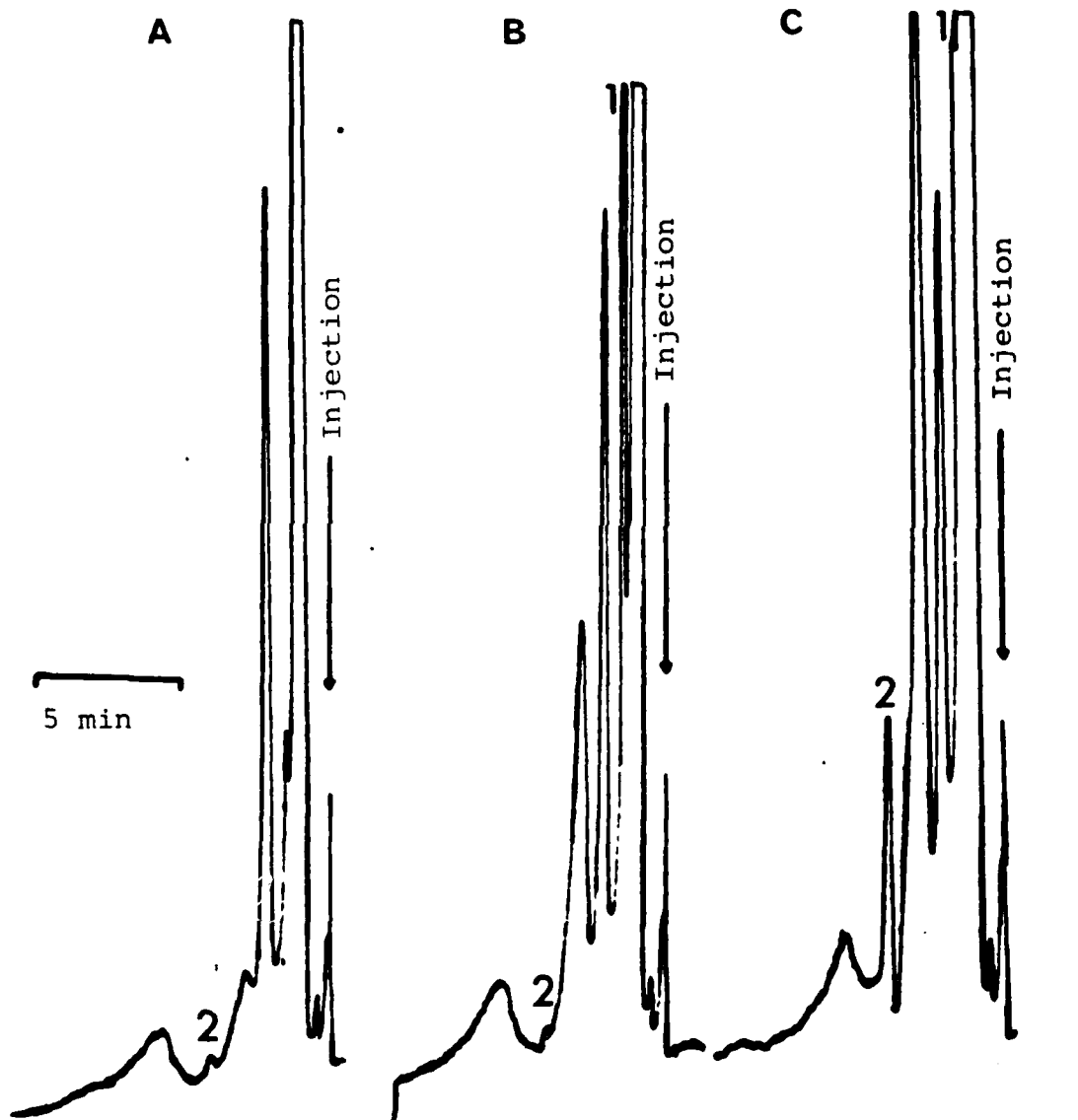
APPENDIX TABLE 4

Comparison of HMX Concentrations in Group 1(3 ?) Samples
with Original Analysis

Group	Mean Concentration of HMX Found ₋₁ ($\mu\text{g}.\text{ml}^{-1}$)	Mean Concentration of HMX Originally Found ₋₁ ($\mu\text{g}.\text{ml}^{-1}$)
1?	0.81	0.51
1?	0.25	0.30

APPENDIX FIGURE 1

Normal Phase Chromatograms of Plasma Extracts from
Fischer ♂ Rats

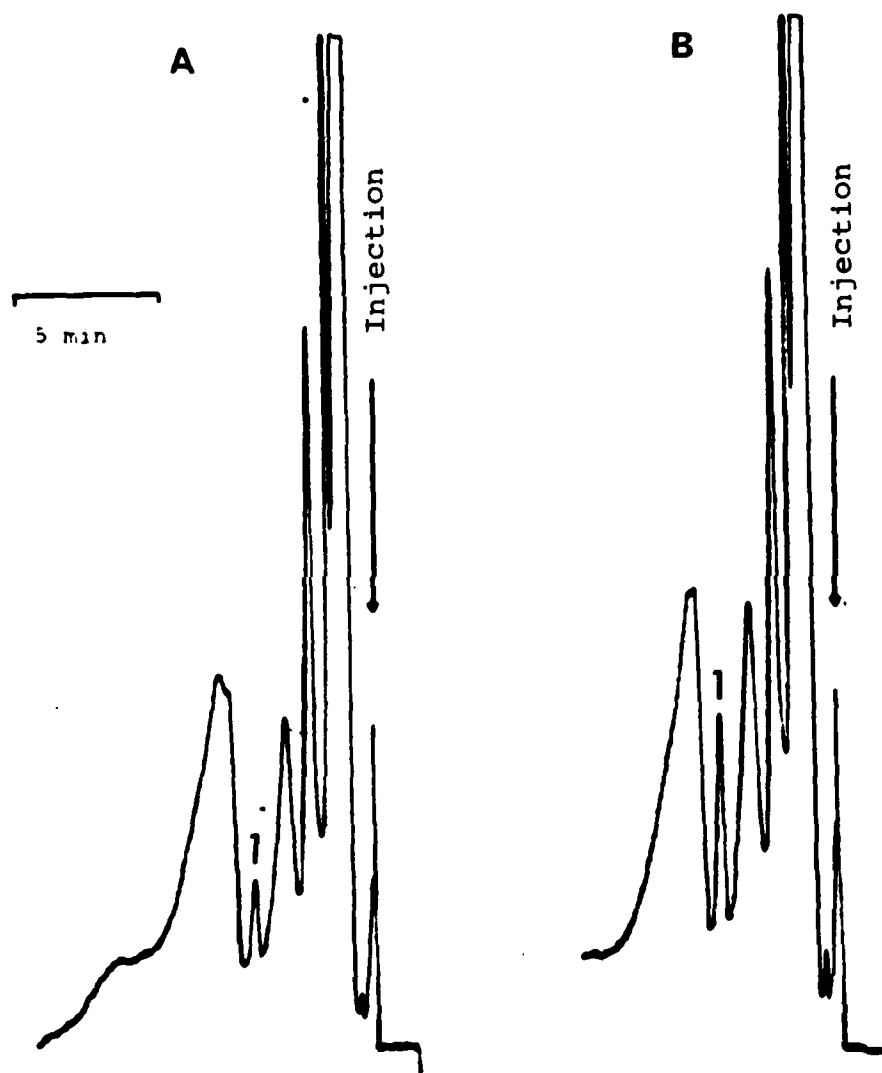


A) No HMX or RDX added to plasma
B) 66.7 ng RDX added to plasma
C) 47.5 ng HMX, 66.7 ng RDX added to plasma

1 = RDX
2 = HMX

APPENDIX FIGURE 2

Normal Phase Chromatograms of Plasma Extracts from
Group 1♂ Rats

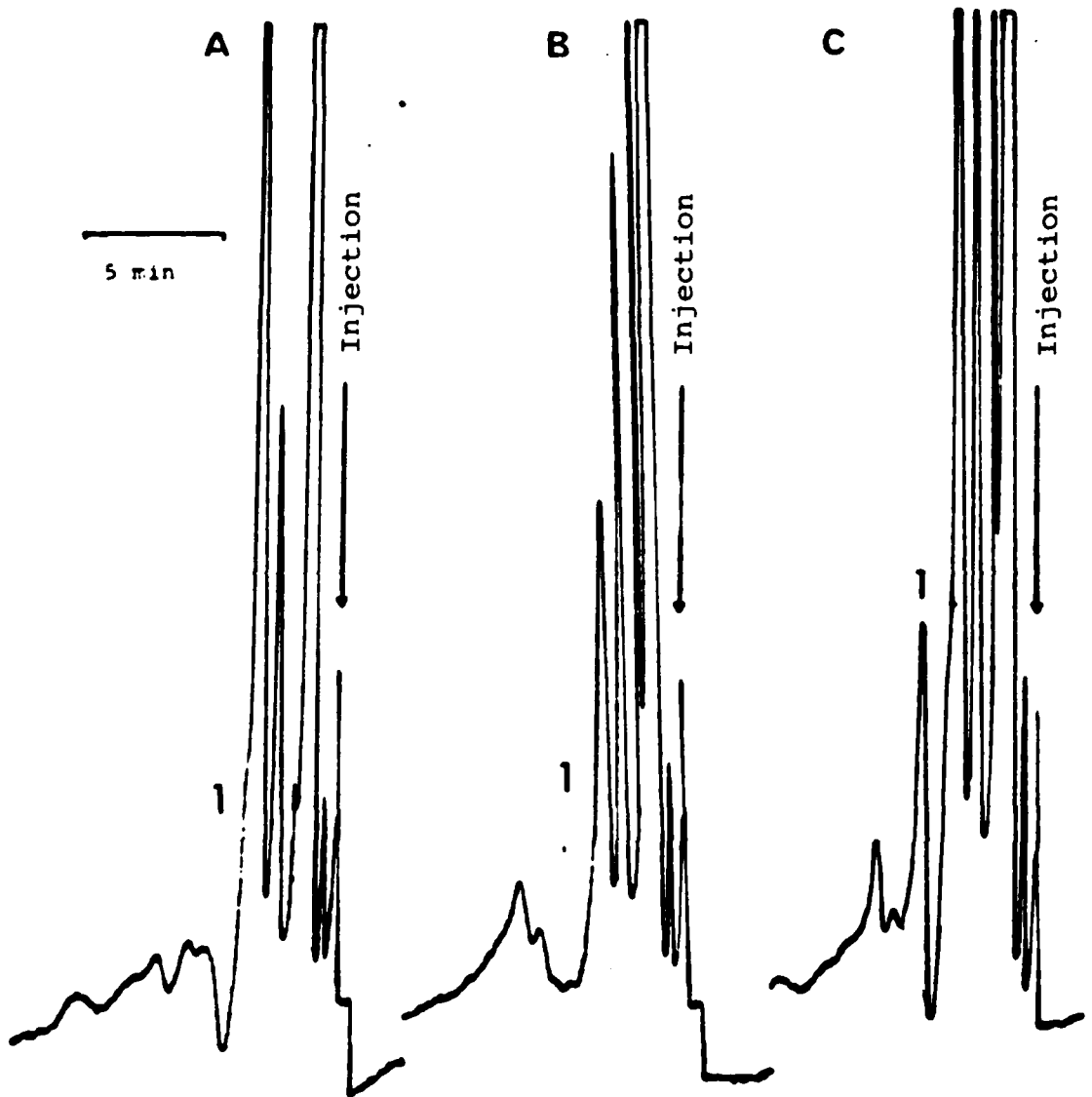


- A) No HMX added to extract
B) 4.7 ng HMX added to extract

1 = HMX

APPENDIX FIGURE 3

Normal Phase Chromatograms of Plasma Extracts from
Fischer ♀ Rats

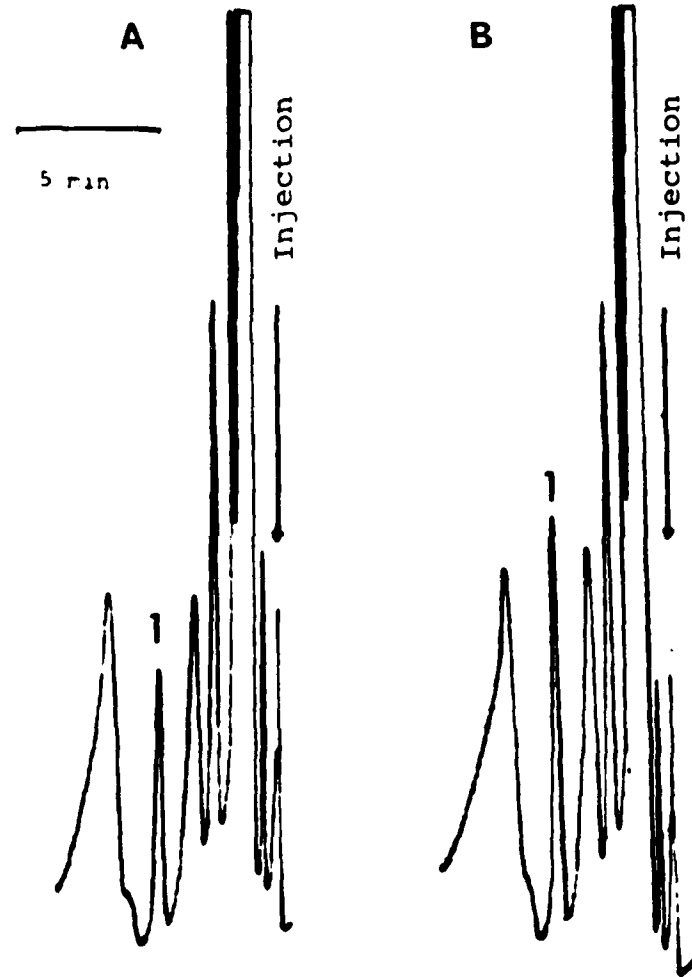


- A) No HMX or RDX added to plasma
B) 66.7 ng RDX added to plasma
C) 47.5 ng HMX, 66.7 ng RDX added to plasma

1 = HMX

APPENDIX FIGURE 4

Normal Phase Chromatograms of Plasma Extracts from
Group 1♀ Rats

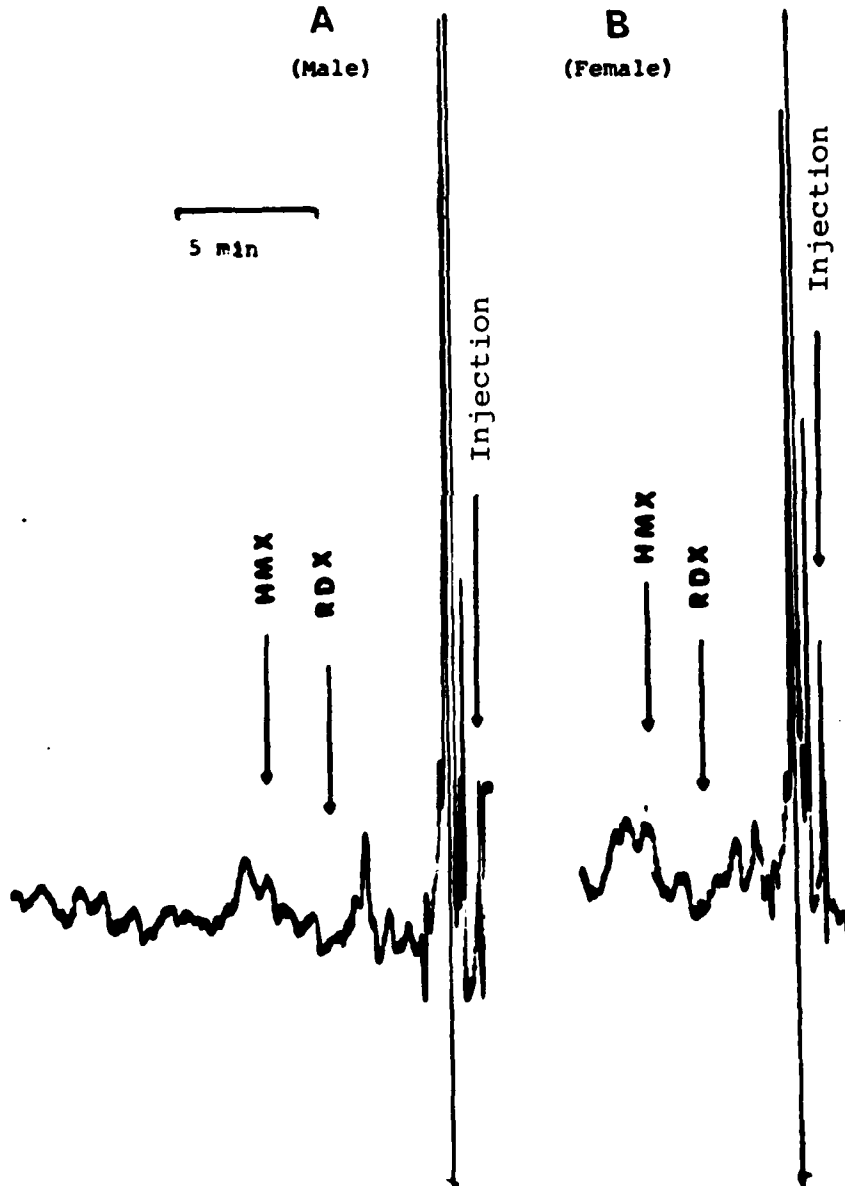


- A) No HMX added
B) 4.7 ng HMX added

1 = HMX

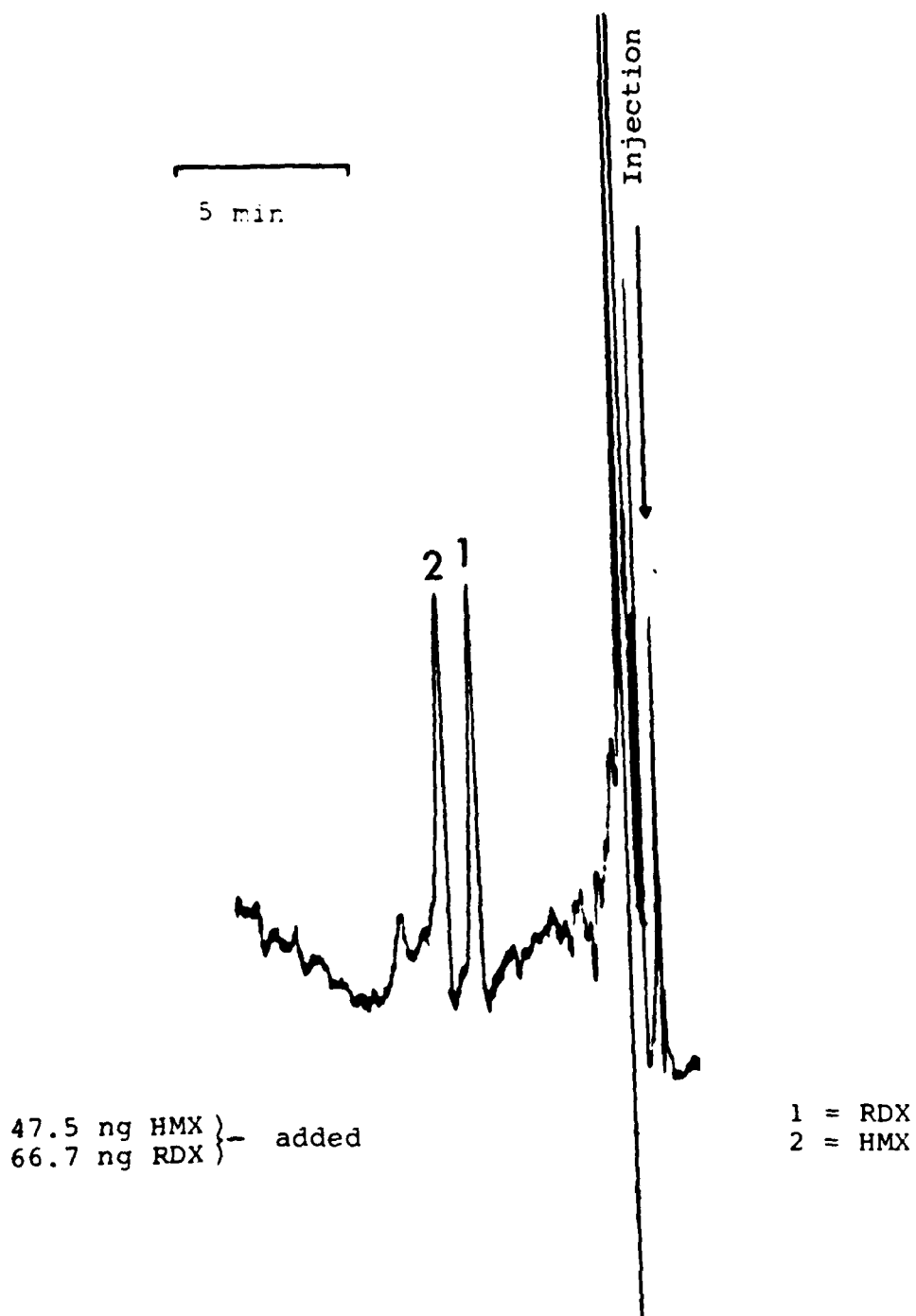
APPENDIX FIGURE 5

Reverse Phase Chromatograms of Plasma Extracts
from Fischer Rats



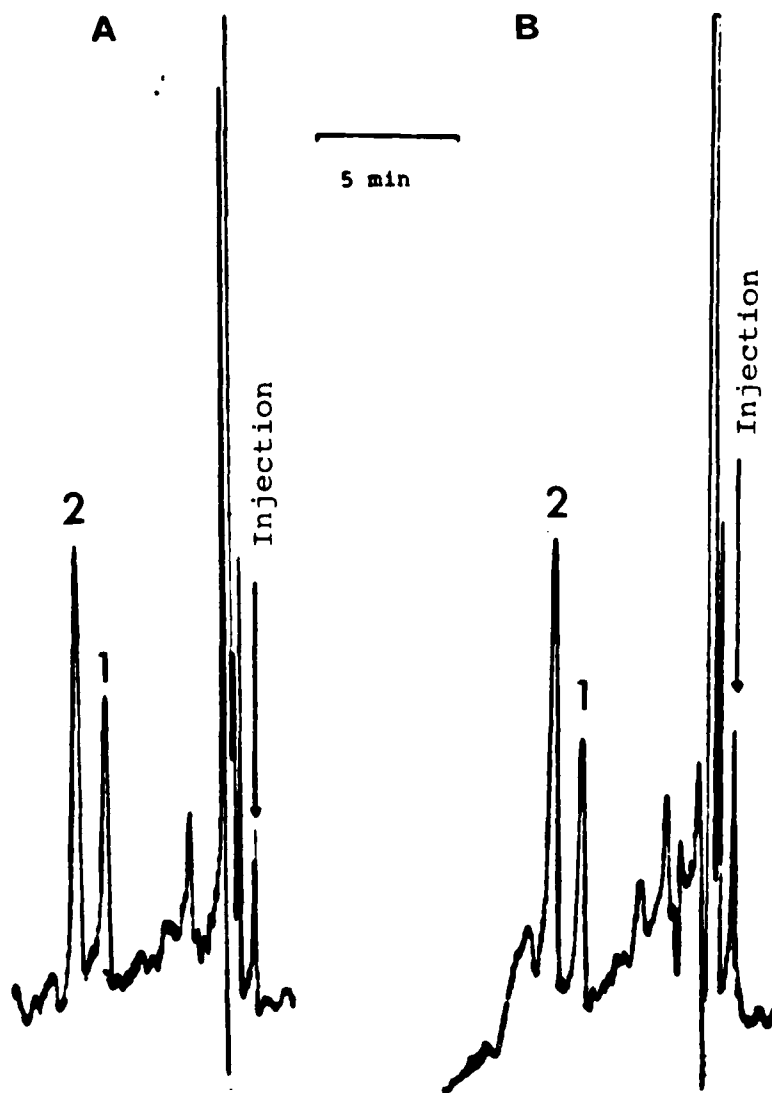
APPENDIX FIGURE 6

Reverse Phase Chromatogram of a Plasma Extract from
a CD Rat (containing added HMX and RDX)



APPENDIX FIGURE 7

Reverse Phase Chromatograms of Plasma Extracts from
Group 1♂ Rats

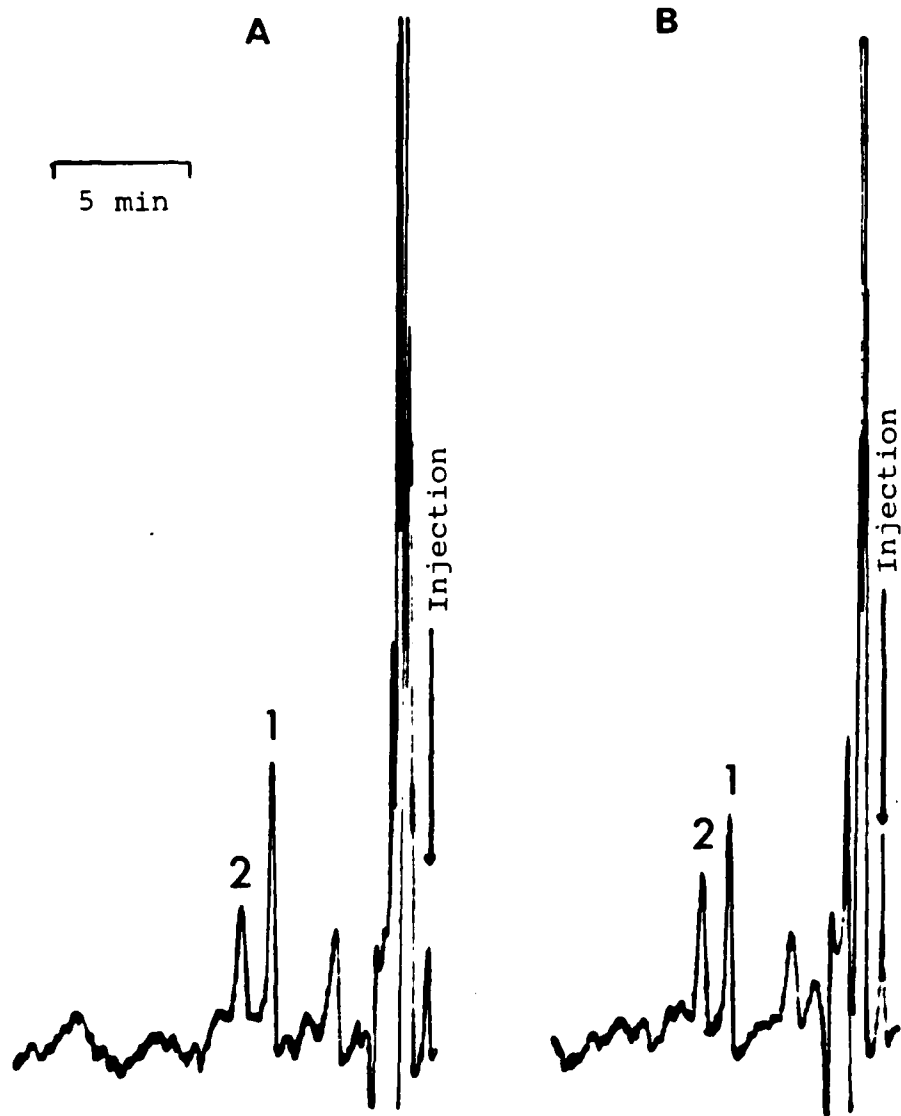


A) No HMX added to extract
B) 4.7 ng HMX added to extract

1 = RDX
2 = HMX

APPENDIX FIGURE 8

Reverse Phase Chromatograms of Plasma
Extracts from Group 1♀ Rats



A) No HMX added to extract
B) 4.7 ng HMX added to extract

1 = RDX
2 = HMX

APPENDIX 2

Analysis of Untreated Diet

Special Diets Services Limited
SPECIAL QUALITY CONTROL OF SMALL ANIMAL DIETS

CERTIFICATE OF ANALYSIS

RECEIVED
18 MAY 1982

PRODUCT RAT & MOUSE NO.1 (MODIFIED) EXPANDED

BATCH NO 1539

PREMIX BATCH NO P192

DATE OF MANUFACTURE 19th April, 1982

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	9.4	%	Fluorine	3.2	mg/kg	1.0 mg/kg
Crude Fat	3.1	%	Nitrate as NaNO ₃	8.0	mg/kg	1.0 mg/kg
Crude Protein	15.5	%	Nitrite as NaNO ₂	<1.0	mg/kg	1.0 mg/kg
Crude Fibre	4.2	%	Lead	<0.25	mg/kg	0.25 mg/kg
Ash	4.9	%	Arsenic	<0.2	mg/kg	0.2 mg/kg
Calcium	0.92	%	Cadmium	0.55	mg/kg	0.05 mg/kg
Phosphorus	0.64	%	Mercury	0.03	mg/kg	0.01 mg/kg
dium	0.30	%	Selenium	0.17	mg/kg	0.02 mg/kg
Chlorine	0.57	%				
Potassium	0.88	%				
Magnesium	0.20	%	Total Aflatoxins	N.D.	mg/kg	1 mg/kg each of B1,B2,G1,G2
Iron	121	mg/kg				
Copper	13	mg/kg				
Manganese	64	mg/kg	Total PCB	N.D.	mg/kg	0.001 mg/kg
Zinc	47	mg/kg	Total DDT	0.035	mg/kg	0.001 mg/kg
			Dieldrin	0.003	mg/kg	0.001 mg/kg
			Lindane	0.002	mg/kg	0.001 mg/kg
			Heptachlor	N.D.	mg/kg	0.001 mg/kg
			Malathion	N.D.	mg/kg	0.02 mg/kg
Vitamin A	6,300	IU/kg	Total Viable Organisms	2.75 X 10 ³	per gram	1000/g
Vitamin E	67.5	mg/kg	Mesophilic Spores	7.5 X 10 ²	per gram	100/g
Vitamin C		mg/kg	Salmonellae Species	N.D.	per gram	Absent in 20 gram
			Presumptive E. coli	N.D.	per gram	Absent in 10 gram
			E. coli Type 1	N.D.	per gram	Absent in 10 gram
			Fungal Units	N.D.	per gram	Absent in 10 gram
			Antibiotic Activity	N.D.		

N.D. = None Detected

Signed

Dated

C. R. POPPLESTONE M.Sc., Ph.D., C.Chem., M.R.S.C.
Quality Control Manager

Special Diets Services Limited
1 Stepfield
Witham
Essex, CM8 3AB
Telephone (0376) 513651

PERSONNEL INVOLVED IN PROJECT 415669

Principal Investigator:	A.B. Wilson, B.V.Sc., M.R.C.V.S., D.A.B.T.
Department Manager: Analytical Chemistry:	J.D. Gilbert, B.Sc., Ph.D., C. Chem., F.R.C.S.
Senior Analyst:	M.S. Henderson, B.Sc., Ph.D.
Analysts:	D.W. Carmichael, B.Sc. A.R. Gambie, M.Sc.
Quality Assurance:	A.W. Waddell, B.Sc., Ph.D. E.M. Baxendine, B.Sc. C. Young, B.Sc.

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