

1. EPA (Mike Dellarco) description of Accession # 9 "DMP Deer + Elk Study" dated 1/23/[80].

Notes 3 of 6 deer positive, 7 of 9 elk positive for 2,3,7,8-TCDD.

Attached table (adipose tissue only) results from RTP + Wright State: up to 14 ppt in deer adipose up to 68 ppt in elk adipose.

2. Dec. 17, 1979 Results of Capillary column GC/HRMS analyses performed on deer + elk adipose tissue for 2378 TCDD residues, Robert Harless RTP: data report for results cited in Accession # 9 above.

3. Incomplete EPA Region ~~4~~X document, "Rationale and Study Design for Proposed TCDD Analysis of Region X Elk and Deer Adipose Tissue Samples" undated, c: 1977 or 1978. Described collecting methods, dates, locations, notes elk herd "has been experiencing an excess of reproductive problems, including still births and teratoma during recent years."

4. Thomas O. Tarnan, Wright State, Nov. 29, 1979 letter + attached table (attention to Sept. 23, 1979 report, below), reports additional 17 samples, up to 48 ppt.

5. Sept. 23, 1979 Report, Thomas O. Tarnan to EPA, 2378-TCDD levels in deer + elk adipose (similar results to those in Accession # 9, above).

6. July 13, 1979, Michael L. Gross ~~to~~ (UNL) to Hugh Black, USFS, California, with attached report on ²³⁷⁸TCDD in deer tissue [NOTE: This is a separate study from EPA studies above; Gross analysed ~~the~~ tissue from deer confined in a ²⁴⁵sprayed forest for 2 weeks after spraying. Note levels of 2378 TCDD in muscle as well as bone marrow liver fat]

DRAFT

1/23

Accession #: 9

Title of Project: DMP Deer and Elk Study

Status of Project: in progress

Location of Sampling: Oregon and Washington

Herbicide Use: forest

Sample Media: deer and elk adipose tissue

Range of Limit of Detection: RTP: 0.8 - 6.0 ppt
WSU: 8.0 - 34.0 ppt

Average Limit of Detection: RTP: 2.7 ppt
WSU: 14.8 ppt

of Samples Collected: deer: 6
elk : 9

of Samples Analyzed: 15

of Samples Suspected Positive: : deer: 3 of 6, 50.0%
elk: 7 of 9, 77.8%

Comments:

It is important to note that four samples were analyzed below 50% recovery of the 37Cl TCDD standard and therefore require re-extraction and re-analysis according to standard DMP procedures. These samples are # OR-D-1, WA-D-4, WA-D-8, and WA-E-7. Nevertheless, the data indicate the presence of 2,3,7,8 TCDD residues in both deer and elk.

Also it is important to note that RTP reported the presence of other minor isomers in addition to the major 2,3,7,8 TCDD isomer (based on the DMP analytical criteria).

The result of this study indicates that additional sampling may be useful in providing more information about the extent of dioxin contamination associated with the use of 2,4,5-T and silvex in this geographic area.

Preliminary Data

DRAFT

1/23

Research Triangle Park

Wright State University

Adipose Sample Description	Toxicant Analysis Center Sample #	ppt(c) Native TCDD Added to Sample	RTP #	TCDD Detected (ppt) (c)	TCDD Detection Limit (ppt) (c)	37CL(d) % Recovery	WSU #(f)	TCDD Detected (ppt) (c)	TCDD Detection Limit (ppt) (c)	37CL % Recovery
deer	OR-D-5		124(e)	12	2	89	1(g)	31	21	46
deer	WA-D-1		125	ND	2	50	2	ND	21	46
✓deer	OR-D-1		126	ND	4	34	3(g)	ND	34	29
deer	Control	7	127	9	2	78	4	14	13	51
deer	WA-D-4		128	ND	3	36	NA			
deer	OR-D-6		129(e)	7	2	84	5	14	13	59
✓deer	WA-D-8		130	7	4	31	6(g)	ND	24	33
Method Blank(a)		0	131	ND	3	50	7	ND	8	105
elk	WA-E-2		132(e)	9	2	67	NA			
elk	OR-E-11		133	ND	2	71	8	ND	8	97
elk	WA-E-6		134(e)	54	0.8	100	9	68	10	79
deer	Control	21	135(e)	34	6	50	10(g)	41	8	150
elk	OR-E-7		136	24	3	50	11	29	8	102
elk	OR-E-9		137(e)	5	3	100+	12(g)	ND	8	98
elk	WA-E-5		138	12	3	57	13(g)	ND	19	43
elk	OR-E-8		139(e)	4	1	87	14(g)	ND	10	84
Standard		14	140	23	2	100+	15	17	11	76
Solution(b)										
elk	WA-E-4		141(e)	21	2	76	16(g)	21	11	70
✓elk	WA-E-7		142	ND	4	36	17(g)	ND	25	32

- (a) Method ~~ank~~: extraction procedure using solvent only.
- (b) Standard Solution: a standard solution containing 2,3,7,8-tetrachloro^gdibenzo-p-dioxin
- (c) ppt: parts per trillion
- (d) corrected for recovery loss
- (e) two other isomers in addition to 2,3,7,8-tetrachloro^gdibenzo-p-dioxin, the major isomer, were observed
- (f) WSU analyses reported here are the average of two runs
- (g) evidence of PCB contamination in sample.

ND: Not Detected

NA: Not analyzed by WSU due to limited amount of sample

NB: Sample wt. for all samples was 5g.; 37CL TCDD addition to each sample is 5 nanograms (ng) except Control Sample RTP-135/WSU-10 which contained 1.33 ng.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

DATE: December 17, 1979

SUBJECT: Results of Capillary Column GC/HRMS Analyses Performed on Extracts of Deer and Elk Adipose Tissue for 2,3,7,8-TCDD Residues

FROM: Robert Harless *Robert Harless*
HERL, ETD, ACB (MD-69)

TO: Mike Dellarco, Dioxin Project Manager
Special Pesticide Review Division (TS-791)
Office of Toxic Substances
401 M Street, SW
US EPA
Washington, DC 20460

THRU: Dr. R. G. Lewis, Chief *RG Lewis*
Analytical Chemistry Branch (MD-69)
HERL, ETD

Per your request, these samples were not analyzed until the most high priority samples, "Vertac", etc. were analyzed.

This shipment of sample extracts was received from the EPA Pesticide Monitoring Laboratory, Bay-St. Louis, Mississippi, 9-6-79, and was assigned the identification number ACB-108. The sample extracts were subjected to a previously described capillary column GC/HRMS multiple ion monitoring method of analysis for 2,3,7,8-TCDD residues utilizing a Varian 311A mass spectrometer interfaced with an SE-30 WCOT glass capillary column.

The criteria utilized for confirmation of 2,3,7,8-TCDD were:

1. Capillary column GC/HRMS retention time of 2,3,7,8-TCDD.
2. Co-injection of sample fortified with ³⁷Cl-TCDD and 2,3,7,8-TCDD standard.
3. Molecular ion chlorine isotope ratio (m/e 320 and m/e 322).
4. Capillary column GC/HRMS simultaneous multiple ion monitoring response (m/e 320, m/e 322, and m/e 328) for TCDD.
5. M/e 320 and m/e 322 MS response greater than 2.5 mm noise level.

The capillary column GC/HRMS retention time of 2,3,7,8-TCDD was 15 minutes $\pm$ 15 seconds. The MS mass resolution (8,500) was sufficient to resolve TCDD from contamination. The masses monitored during these analyses were:

1. m/e 318.9793 PFK reference
2. m/e 319.8965 TCDD
3. m/e 321.8935 TCDD
4. m/e 327.8847 ³⁷Cl-TCDD

Results

The results are shown in Table 1. The recovery of ³⁷Cl-TCDD was below 50% for specific sample extracts. Therefore, the results should not be reported. These specific samples should be subjected to analytical clean-up and GC/HRMS analysis again to confirm the reported results. TCDD isomers (3) were detected in RTP-124, 129, 132, 134, 135, 137, 139, and 141. The isomer having exact GC/HRMS retention time as 2,3,7,8-TCDD was the major isomer detected (high concentration). No problems were encountered in these analyses.

Summary

2,3,7,8-TCDD and two (2) TCDD isomers were detected in deer and elk adipose tissue samples.

CC: Dr. William Durham, Director
Environmental Toxicology Division (MD-66)

Dr. Nancy Wilson, Chief
Chemical Characterization Section (MD-69)

TABLE 1 ACB #108
ANALYTICAL RESULTS FOR 2,3,7,8-TCDD RESIDUES

Sample ID	Sample Weight (g)	³⁷ Cl-TCDD Fortification Level (ng)	³⁷ Cl-TCDD % Recovery	TCDD Detection Limit (ppt)*	TCDD Detected (ppt) *	Comments
RTP-124	5	5	89	2	12	Deer Adipose
RTP-125	5	5	50	2	ND	" "
RTP-126	5	5	34	4	ND	" "
RTP-127	5	5	78	2	9	" "
RTP-128	5	5	36	3	ND	" "
RTP-129	5	5	84	2	9	" "
RTP-130	5	5	31	4	7	" "
RTP-131	5	5	50	3	ND	" "
RTP-132	4	4	67	2	9	Elk Adipose
RTP-133	5	5	71	2	ND	" "
RTP-134	5	5	100	0.8	54	" "
RTP-135	5	1.33	50	6	34	" "
RTP-136	5	5	50	3	24	" "
RTP-137	5	5	100+	3	5	" "
RTP-138	5	5	57	3	12	" "
RTP-139	5	5	87	1	4	" "
RTP-140	5	5	100+	2	23	" "
RTP-141	5	5	76	2	21	" "
RTP-142	5	5	36	4	ND	" "

ND = not detected

ppt = parts per trillion

* = corrected for recovery losses

Rationale and Study Design for Proposed TCDD Analysis of Region X Elk and Deer Adipose Tissue samples.

1. Reasons why such a study is needed:

- A. Because of so many variables, mother's milk TCDD levels alone do not provide much valuable information about TCDD in the environment. Utilizing human subjects presents great diversity in terms of diet, mobility in the environment, medication, and exposure history. Because of the nature of the TCDD molecule, it should be more concentrated in adipose tissue than elsewhere. Milk with its butterfat content would thus not be an unreasonable sample type, but adipose tissue would be much better. The difficulties of obtaining adipose biopsies from a selected population of humans are obvious.

- B. A TCDD monitoring study of carefully selected samples of elk and deer will provide the following advantages:

Elk and deer live in discrete forest areas all of their lives. The herds are closely monitored by professional wildlife biologists. Exposure information from past spraying histories of the areas can be obtained. This localized nature of the herds enhances research design.

Elk and deer are browsers and grazers, eating only vegetation from the forest areas they inhabit. Their diet thus reflects their environment (in terms of residues of any environmental contaminants) far more accurately than does a typical diet of an "exposed" human.

Elk and deer are consumed by large numbers of people. Knowledge of possible contaminants in these animals is thus of public health importance. In California, State agencies have interpreted the precautions on the 2,4,5-T label to apply to both deer (California does not have appreciable elk populations) and livestock. The state thus opposes 2,4,5-T spraying 2 weeks prior to and during the deer season.

Elk and deer herds in Region X coastal forests often consist of sufficient quantities of older animals to allow for sampling them preferentially as indicators of any bioaccumulation in an "exposed" environment over a several year timeframe. As explained later, several of our elk samples are from animals in the 10 to 13 year age class.

1/14/85

were labeled as to: species, date sampled, sex, age, site and date of kill, name, address and telephone of donating hunter, name, address and telephone of biologist making the collection, and miscellaneous comments by the sampling officer. They were then frozen and taken to a central site where they were then picked up by EPA Region X. Labels were made of paper and affixed to the bottles with strong twine.

Collecting dates were as follow: Oregon deer, 11-5-77; Oregon elk, 12-3-77; Washington deer, 10-22 and 10-23-77; and Washington elk, 10-31-77 through 11-21-77.

- At present, the samples are stored frozen at the EPA Region X Laboratory in Seattle. They are labelled and sealed since their collection, and can be shipped at once whenever a suitable analytical laboratory can be found and utilized.

E. Sample data are as follow:

Oregon elk totalled 15. All of these animals were taken from the Coos Bay to Rosenberg area. Specifically, all of the animals came from the Millacoma and Coos River drainages, stretching across predominantly Weyerhaeuser land eastward from the vicinity of Allegany. The elk herd is monitored closely by game biologists, and has been experiencing an excess of reproductive problems, including stillborns and teratisms during recent years. Ages range from 2 to 13 years (mean: 7.5 years). Mean size of fat sample is 43.5 grams. All are female, because only cows are sacrificed and studied from this herd annually by the game biologists.

Oregon deer totalled 10. All were taken from hunters at check stations in the Tillamook area. Age ranged from 1.5 to 5.0 years (mean: 2.8 years). Mean size of fat sample is 58.8 grams. Seven males and three females were sampled.

Washington deer totalled 9. They were taken in the general area stretching from Aberdeen toward the Pacific Coast. Mean sample size was 22.3 grams. Ages ranged from 0.5 to 5 years (mean: 1.9 years). Seven males and two females were sampled.

Region X began this study in the Fall of 1977 as a corollary to the mother's milk sampling study for TCDD, for reasons already outlined at the beginning of this paper. We have informed OSPR of this effort since its inception and have requested assistance numerous times in the analysis of the samples for TCDD, chiefly through Mr. Reising of OSPR. We have received no response whatsoever from OSPR.

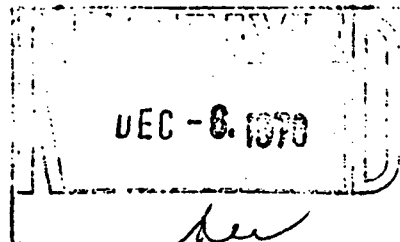
Wright State University



Dayton, Ohio 45431

November 29, 1979

Mr. W. T. Hollaway
U.S. Environmental Protection Agency
Office of Toxic Substances
Special Pesticide Review Div. (TS-791)
Crystal Mall #2
1921 Jefferson Davis Highway, Rm. 728
Arlington, Virginia 22202



Dear Mr. Hollaway:

Attached is a table showing the results of analyses of the remaining samples in the batch of 17 which we have been analyzing. Results for the first nine samples were reported in our recent Quarterly Report. We are proceeding as rapidly as possible with the MS-30 modifications to permit isomer-specific TCDD determinations, and will provide you with a status report on those developments in the near future.

Sincerely,

Thomas O. Tiernan, Ph.D.
Professor of Chemistry and
Director of the Brehm Laboratory

TOT/aam
Attachment

TABLE 1

RESULTS OF GC-HIGH RESOLUTION MS ANALYSES OF SAMPLE EXTRACTS PROVIDED BY EPA
FOR TETRACHLORODIBENZO-p-DIOXIN (TCDD) BY WRIGHT STATE UNIVERSITY

EPA Sample No.	Run 1			Run 2			Average Native TCDD (ppt)	Average M.D.C. (ppt)	Average % Recovery	Notes
	Native TCDD Detected (parts-per-trillion)			Native TCDD Detected (parts-per-trillion)						
	m/e 320	m/e 322	Average	m/e 320	m/e 322	Average				
WSU-10	31	43	37	48	43	45	41	8	150	a.,b.
WSU-11	25	23	24	35	32	34	29	8	102	--
WSU-12	0	0	0	0	0	0	0	8	98	a.
WSU-13	0	0	0	0	0	0	0	19	43	a.
WSU-14	0	0	0	0	0	0	0	10	84	a.
WSU-15	15	20	18	14	16	15	17	11	76	--
WSU-16	18	26	22	17	20	19	21	11	70	a.
WSU-17	0	0	0	0	0	0	0	25	32	a.

a. PCB contamination of sample is evident.

b. This sample was supposedly spiked with only 1.33 ng total ³⁷Cl-TCDD. The indicated recovery however indicates a possible error in spiking.

QUANTITATION OF PARTS-PER-TRILLION LEVELS OF
2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN EPA
FURNISHED EXTRACTS OF BIOLOGICAL MATERIALS

EPA Cooperative Agreement No. CR806846-01

Submitted by

Brehm Laboratory
Wright State University
Dayton, Ohio 45435

Thomas O. Tiernan, Joseph G. Solch, Garrett VanNess,
Michael L. Taylor, and Thomas Mazer

Quarterly Report

September 23, 1979

Submitted to

William T. Hollaway
Coordinator - Dioxin Implementation Plan
U.S. Environmental Protection Agency
Office of Toxic Substances
Special Pesticide Review Division (TS-791)
Crystal Mall #2
1921 Jefferson Davis Highway, Rm. 728
Arlington, Virginia 22202

I. INTRODUCTION

Analyses of EPA-furnished extracts of deer and elk adipose for tetrachlorodibenzo-p-dioxin content were accomplished by Wright State University during the third quarter of the program being conducted under EPA Cooperative Agreement No. CR806846-01. Results of these analyses are reported herein and other pertinent topics are discussed.

II. EXPERIMENTAL PROCEDURES

The experimental procedures applied for the analyses reported herein are essentially the same as those described previously in the Quarterly Report of March 23, 1979.

III. RESULTS AND CONCLUSIONS

The results of analyses of nine extracts of deer and elk adipose are listed in Table 1. Since these extracts were based on five gram samples, whereas the Cooperative Agreement specified that ten gram samples would be extracted by EPA, it was not possible to achieve the specified detection sensitivity for TCDD. However, the minimum detectable TCDD levels in most of the samples are quite reasonable, being 13 parts-per-trillion (ppt) or less for five of the nine samples. In all cases where higher detection limits were imposed, the recoveries, based on the ^{37}Cl -TCDD internal standard, were extremely poor. It is noteworthy that recoveries were less than 50% for four of the nine samples, and were in the acceptable range for only three samples. This suggests that the EPA extraction and cleanup methodology is not really adequate for these types of samples, and that improvements are desirable. Three of the nine samples analyzed exhibited very large

apparent polychlorinated biphenyl (PCB) interferences, and the recoveries for these samples were the lowest observed in this set, as shown in Table 1.

IV. PROJECT STATUS AND PLANS

In order to be consistent with the Cooperative Agreement schedule, Wright State would have been required to analyze twenty-one (21) sample extracts during the present third quarter reporting period. As noted in the previous Quarterly Report submitted to EPA, Wright State analyzed nine (9) samples in excess of the requirements during the previous quarter, which reduces the normal sample analysis load (30 samples/quarter) to twenty-one (21), as just mentioned. However, EPA supplied Wright State with only seventeen (17) sample extracts during the present reporting period, and these were not received until September 20, 1979, only three days before the end of the third quarter of the Cooperative Agreement. In view of this late shipment of samples, it was not possible to complete analyses of all seventeen (17) extracts prior to the end of the reporting period. Analyses of nine of the seventeen were completed and are reported herein, as noted above.

It must be emphasized that EPA has not provided Wright State with the numbers of samples specified in the Cooperative Agreement for the present quarter, and that the sample shipment which was made was not timely. It is urged that attempts be made to rectify this situation in the coming quarter, and that the appropriate number of samples be shipped as soon as possible. Wright State should receive a minimum of thirty-four samples from EPA for analyses during the fourth quarter (prior to December 23, 1979) in order to satisfy the projected schedule.

During this reporting period, authorization was received from EPA (letter of September 13, 1979 from W. T. Hollaway) to accomplish an internal budget-transfer of funds for the Cooperative Agreement. Accordingly, \$8000 was transferred from the equipment line to the supply line of expenditures for the Agreement, as authorized.

Also during this reporting period, Dr. T. O. Tiernan attended the National Meeting of the American Chemical Society in Washington, D.C., where he participated in a Symposium on the Chemistry of Chlorinated Dibenzodioxins and Dibenzofurans. Dr. Tiernan also visited Mr. Hollaway and Mr. DeLarco, at their EPA offices during this trip, to discuss the status of the Cooperative Agreement and future plans. In the course of these discussions, Dr. Tiernan reviewed some recent data obtained by Wright State, relevant to the detection of TCDD in combustion samples. Preliminary data showing separation and determination of TCDD isomers using capillary-column gas chromatography in conjunction with low-resolution mass spectrometry were presented by Dr. Tiernan, and the detection of TCDD in fireplace soot by Wright State was noted. Dr. Tiernan further emphasized the desirability of proceeding with the proposed modifications to our MS-30 Mass Spectrometer to incorporate capillary-column GC, in view of these findings and the reports by Dow Chemical of TCDD in combustion samples.

TAB. 1

RESULTS OF GC-HIGH RESOLUTION MS ANALYSES OF
EPA-SUPPLIED SAMPLE EXTRACTS BY WRIGHT STATE UNIVERSITY

EPA Sample No.	Run 1 Native TCDD Detected (parts-per-trillion)			Run 2 Native TCDD Detected (parts-per-trillion)			Average Native TCDD (ppt)	Average Minimum Detectable Concentration (ppt)	Average % Recovery
	m/e 320	m/e 322	Average	m/e 320	m/e 322	Average			
WSU-1 ^a . (deer adipose)	30	25	28	46	21	34	31	21	46
WSU-2 (deer adipose)	0	0	0	0	0	0	0	21	46
WSU-3 ^a . (deer adipose)	0	0	0	0	0	0	0	34	29
WSU-4 (deer adipose)	14	14	14	14	13	14	14	13	51
WSU-5 (deer adipose)	14	11	13	16	14	15	14	13	59
WSU-6 ^a . (deer adipose)	0	0	0	0	0	0	0	24	33
WSU-7 (deer adipose)	0	0	0	0	0	0	0	8	105
WSU-8 (elk adipose)	0	0	0	0	0	0	0	8	97
WSU-9 (elk adipose)	72	64	68	65	70	68	68	10	79

a. PCB contamination of samples is evident.

W

THE UNIVERSITY OF NEBRASKA-LINCOLN
LINCOLN, NEBRASKA 68598

DEPARTMENT OF CHEMISTRY

July 13, 1979

Ex. 228

Copy 4
FILE 105

JUL 19 '79

Dr. Hugh Black
U.S. Forest Service
Fisheries and Wildlife
Management Staff
630 Sansome Street
San Francisco, CA 94111

Info	Act	Re
DIRECTOR		
WILDLIFE		
W.L.		
Fac. Sec.		
CLERK		
RECORDS		
MR. LACROIX		
SEC. SER.		
FILES		

Dear Hugh:

Enclosed is a copy of the final report for analysis of TCDD in deer tissue and in other samples (mylar panel and leaf washings). In the report, I outline sample work-up (only briefly since Dr. Dupuy did the work and he should report on this), sample custody, and the details of the GC/MS analysis. Some typical outputs are also given as well as a tabular report of all the results. If there are questions, errors, or sections which require amplification, please let me know and I will submit a revised report.

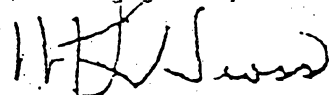
In my judgments, all "positives" (see Table IV) are indeed "positive". You can see from the Figures 2-3 that the data for a sample at ca. 5 parts-per-trillion is rather unambiguous. Of course, there is always a possibility for contamination in the work-up or sample handling.

I have taken the liberty of sending Dr. Dupuy a copy of the report because he has served as an important coworker in this project.

Upon your approval of the report, I will ask my University Grants and Contracts Office to send a bill to you for full payment. Although the contract arrangements were made for sample work-up, we have done a number of additional analyses (particularly the mylar panel and leaf washings). In addition, each "positive" (and others) were validated by means of a full, second analysis monitoring m/z 320 and 322. From these data, we could check the ratio of isotopic cluster signals and quantitate by a second, absolute method. Neither of these research activities were required by the contract. Therefore, I propose that they be considered as a full substitute for the workups.

Thank you for the opportunity to assist you with this interesting project. I hope the results will be of value to you in understanding the possible bio-accumulation of dioxin in wildlife. Finally, I would appreciate the opportunity to study the sample code and comment on our performance on control samples.

Sincerely yours,


Michael L. Gross
Professor

PRELIMINARY DRAFT
SUBJECT TO REVISION

FINAL REPORT

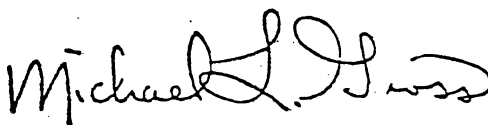
Ultratrace Analysis of Tetrachlorodibenzo-p-dioxin
in Samples of Deer Tissue by Gas Chromatography/
High Resolution Mass Spectrometry
(Contract No. 53-91SB-8-6133)

to

United States Department of Agriculture
Forest Service (Region 5)
630 Sansome St.
San Francisco, CA 94111

Dr. Hugh Black
Project Coordinator

by



Michael L. Gross
Professor of Chemistry
University of Nebraska
Lincoln, NE 68588

July 10, 1979

I. Introduction

This is a report of the analytical support work for a project to examine the possible accumulation of tetrachlorodibenzo-p-dioxin (TCDD) in the tissue of deer exposed to TCDD via spraying of 2,4,5,-T. The project was organized and conducted by the U.S. Forest Service, Dr. Hugh Black, project coordinator.

II. Sample Preparation and Custody

The various deer tissue samples and controls were digested in base, extracted with hexane, washed with sulfuric acid and submitted to small scale liquid chromatography ("acid-base" work-up) by Dr. Aubry Dupuy and his coworkers at the Pesticide Monitoring Laboratory, USEPA, in Bay St. Louis, Mississippi. The extracts along with any controls were coded without regard to origin and shipped frozen in dry ice to our laboratory. Upon reception, the samples were stored in a freezer until they were analyzed. All arrived in perfect condition.

Various samples of washings of 6 Mylar panels and 5 leaf samples were passed through a silica gel column and concentrated by Dr. Anthony S. Wong of California Analytical Laboratories. These samples were collected after the 2,4,5-T spraying at the deer confinement area.

Sample Coding	Date Shipped	Date Received
FS-1 through FS-14	February 14, 1979	February 15, 1979
Mylar 2,4,34,38,42,44 and 7672-1, 7635-2,7635-1, 7696-1, 7635-3	March 1, 1979	March 2, 1979
FS-15 through FS-27	March 26, 1979	March 27, 1979
FS-28 through FS-48	May 9, 1979	May 10, 1979

III. Analysis of TCDD

At the time of analysis, each sample tube provided by Dr. Dupuy was opened, the total volume measured using a 100 μ l syringe, and an aliquot removed (usually between 20 and 35% of the total sample) for GC/HRMS. The Mylar Panel and leaf washing were analyzed by simply removing an aliquot (the total volume was measured by Dr. Wong and not by us) and injecting onto the GC/HRMS.

Each of the extracts was analyzed for tetrachlorodibenzo-p-dioxin using gas chromatography/high resolution mass spectrometry (GC/HRMS). The GC was a Perkin Elmer Sigma II and the mass spectrometer was a Kratos MS-50. The interface consisted of a single glass lined capillary tube followed by a capillary leak directly into the mass spectrometer source. The source region was pumped with a 1300 l/sec diffusion pump and, thus, no molecular separator was employed. Typical source pressures were about 1×10^{-5} torr at the ion gauge located on the 6" pump tube.

The gas chromatographic column (180 cm x 2mm ID. glass) contained 0.60% OV-17 + 0.40% Poly S-179 coated on 100% methyl silicone bonded to 80/100 mesh Chromosorb W-AW, and was operated with a helium flow rate of 15 ml/min. The temperature was held at 250° C for 1.5 min and then increased linearly to 300°C at a rate of 10°C/min; the retention time of authentic TCDD was 3.3 min. The entire effluent from the gas chromatographic column was admitted to the mass spectrometer ion source and ionized using 70eV electrons. The source temperature was 250°C, the accelerating voltage 8kV, and the mass spectral resolution was 10,000 (10% valley definition). TCDD was quantitated by dual ion monitoring using peak matching. One channel was centered at m/z 327.8848 ($^{37}\text{Cl}_4$ -TCDD, the internal standard) and the other at m/z 321.8936

(the most abundant molecular ion of TCDD having natural isotopic elemental abundances). The complete peak profiles were acquired by scanning at a frequency of 2Hz, corresponding in each case to a mass range of 300 ppm (0.096 amu). The output was accumulated using a Nicolet Model 1170 signal averager and the resulting signals were submitted to a 3-point smoothing routine prior to output to an X-Y recorder.

The concentration of TCDD was calculated using the ratio of the intensities (maximum peak heights) at m/z 327.8848 and 321.8936; if no signal was observed, the detection limit was set at 2.5 times the noise amplitude (a 2.5:1 S/N criterion). The calculation is made possible by obtaining the slope of a calibration plot based on various standard mixtures of TCDD and TCDD- $^{37}\text{Cl}_4$. These standard mixtures were interspersed between unknowns and were analyzed in the same manner. The value of the slope was 1.5 ± 0.1 .

6.77 $\pm$ 0.10
0.67-87
1/ Positives were validated by analyzing a second aliquot, this time monitoring 319.8966 (TCDD- $^{35}\text{Cl}_4$) and 321.8936 (TCDD- $^{35}\text{Cl}_3$ ^{37}Cl). The theoretical ratio
1/ for the intensities of these two ions is 0.77. We anticipate the uncertainty in this measurement is $\pm 10\%$ relative. Certainly, those "positives" giving a ratio less than 0.60 should not be considered as validated, and, therefore, they are judged to be "negatives". The validation also presented the opportunity to quantitate the amount of TCDD in each "positive" sample. This was done using absolute mass spectrometer response factors determined by injection of known amounts of TCDD (native) and measurement of peak heights at m/z 321.8936. Because most recoveries are not 100%, the concentration of TCDD determined in this absolute manner will be too low, and it has been corrected by dividing the concentration by the recovery expressed as a fraction. The agreement between the concentration determined by this method and by the ratio method is usually quite good.

When the validation studies were initiated, the results using the internal standard method had not yet been worked up carefully. Consequently, some

samples which turned out to be "negative" by the internal standard method had been subjected to the validation study. They were "negative" by this method (see FS-5, 10, 15, 22, 26, 32, 44, and 48), also.

The concentration of TCDD in the Mylar and leaf washing was determined only by this method as no standard TCDD-³⁷Cl₄ had been added to the solution. Thus the internal standard method was not applicable.

The results are reported in Tables I, II, and III. Each Table represents data taken during separate analysis periods beginning on the dates specified below.

<u>Table</u>	<u>Samples</u>	<u>Date</u>
Table I	FS-1 through FS-27	April 20, 1979
Table II	FS-28 through FS-48	May 17, 1979
Table III	Mylar and Leaf Washings	April 20, 1979

Typical data representing a standard mixture, an unknown "positive", validation of that "positive", and a typical "negative" are shown in Figures 1-4 respectively.

IV. Conclusions:

- A. Precision and Accuracy: No detailed study of precision and accuracy below levels of 10 parts-per-trillion (ppt) has been made to date. Consequently, it is difficult to estimate these parameters. However, errors and precision on the order of $\pm 100\%$ (relative) would not be surprising in view of the difficulty in working at these low levels.

It should be pointed out that our work at levels greater than 9 parts-per-trillion has been validated by an EPA study conducted in 1977. Reports of that study have been made public and may be obtained from the Dioxin Project manager of EPA. Dr. Ralph Ross of USDA has

Figure Legends

Figure 1: Typical output for calibration mixture of 64 picograms (pg) of native 2,3,7,8-TCDD and 1100 pg of 2,3,7,8-TCDD- $^{37}\text{Cl}_4$ (internal standard). The gain x30/x3" refers to the gain of the low and high mass ions respectively, and "at 2K" pertains to the attenuator on the signal averager. Note that the slope of the calibration plot based on this single analysis is 1.49. "RT" is retention time in minutes.

Figure 2: A typical output for an unknown (FS-23) giving a positive result. m/z 321.8936 is on the right.

Figure 3: Validation study for FS-23 monitoring m/z 319.8966 and m/z 321.8936. The observed intensity ratio is 0.72 (theory=0.77)

Figure 4: A typical output for an unknown (FS-38) giving a "not detected" (nd) for TCDD. Note that the detection limit is taken by multiplying the observed noise level at 321.8936 by a factor of 2.5 (see output on left).

Figure 1

$$m = \frac{\frac{64}{1100}}{\frac{4.8/30}{122/3}} = \frac{.058}{.16/4.07} = \frac{.058}{.039} = 1.49$$

MCMS/UNI-L 322/323	
RUN NO. <u>71021</u>	SAMPLE <u>Std</u>
GAIN <u>X30/12</u> AT <u>2 K</u>	
VOL. RATIO <u>64/1100</u> PL <u>R.T. 3.1</u>	
SWEEPS <u>128</u>	<u>Green label spike</u> <u>White label spike</u>

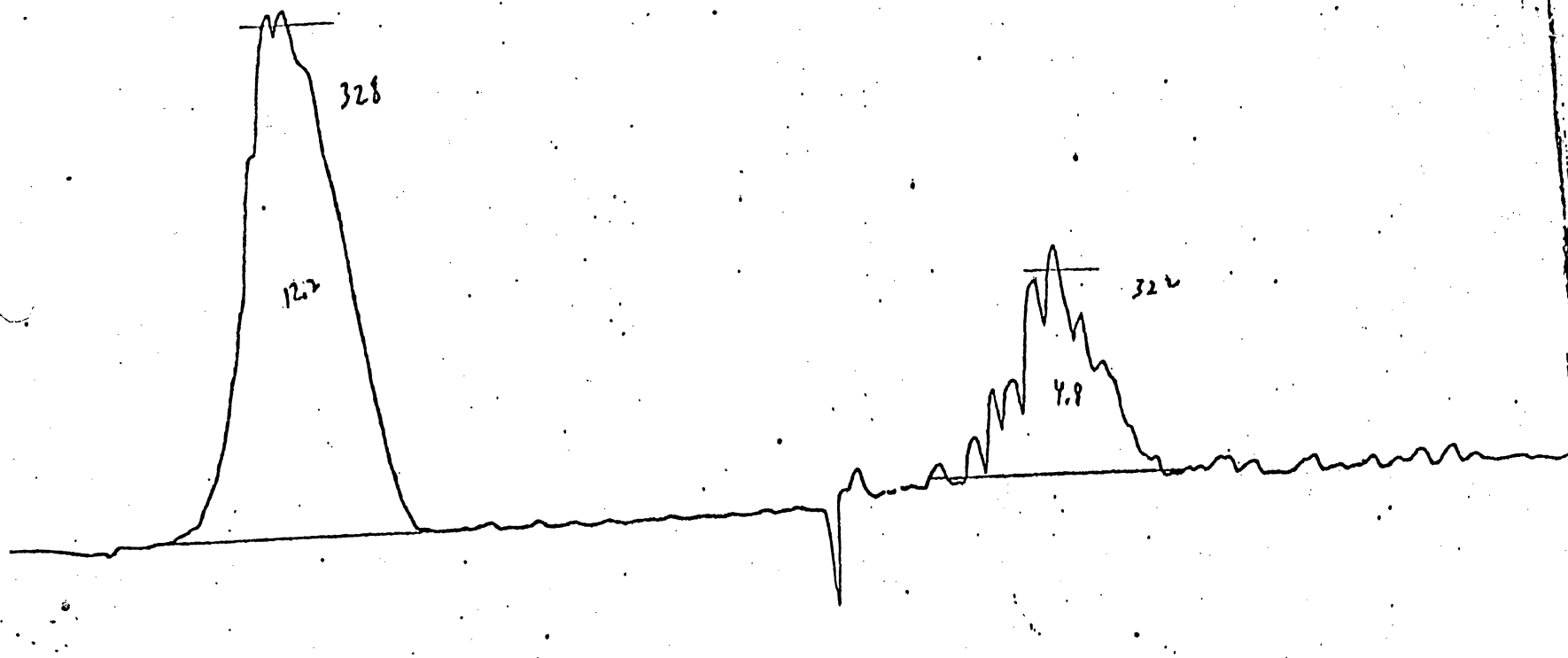


Figure 2

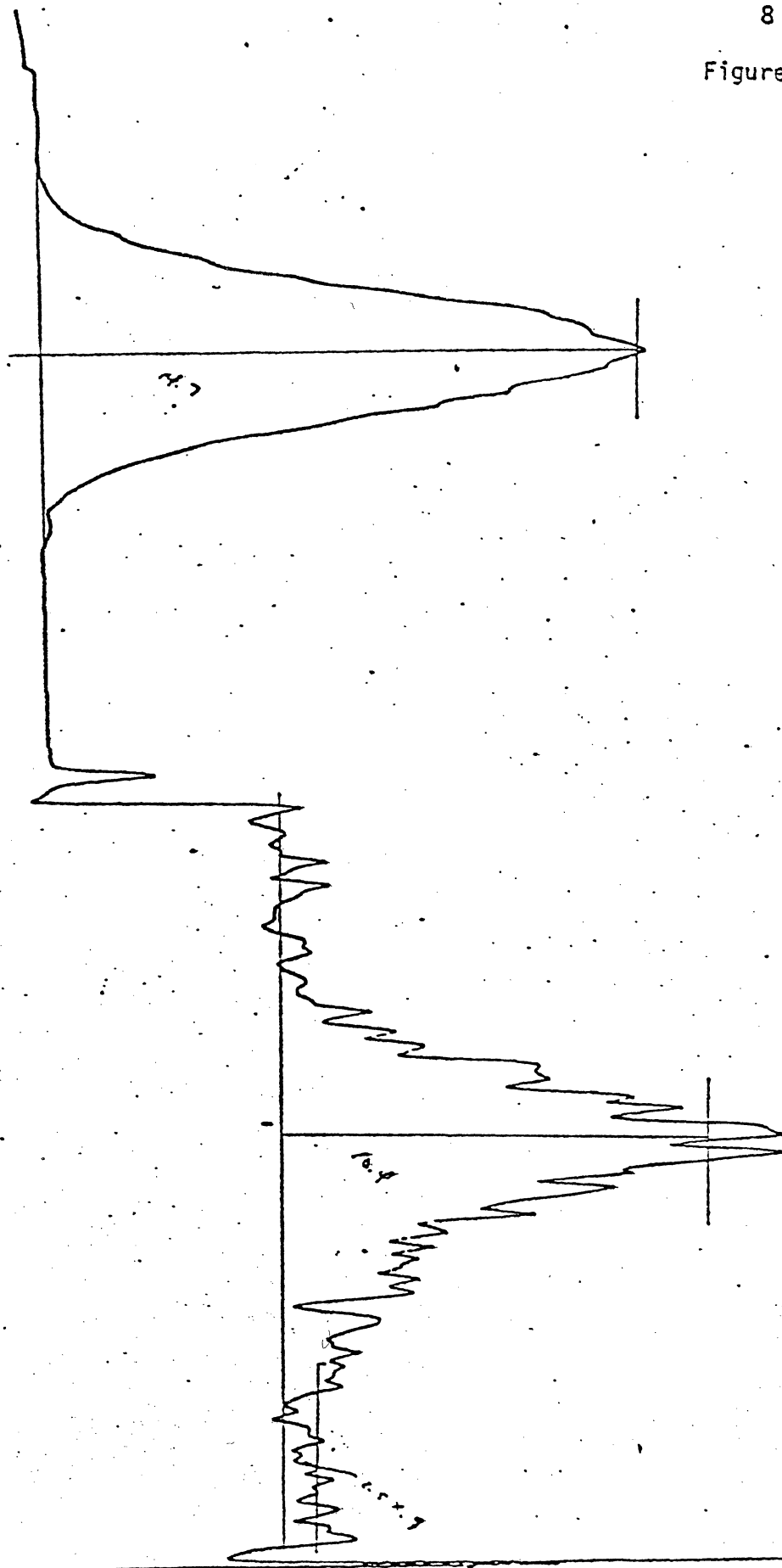
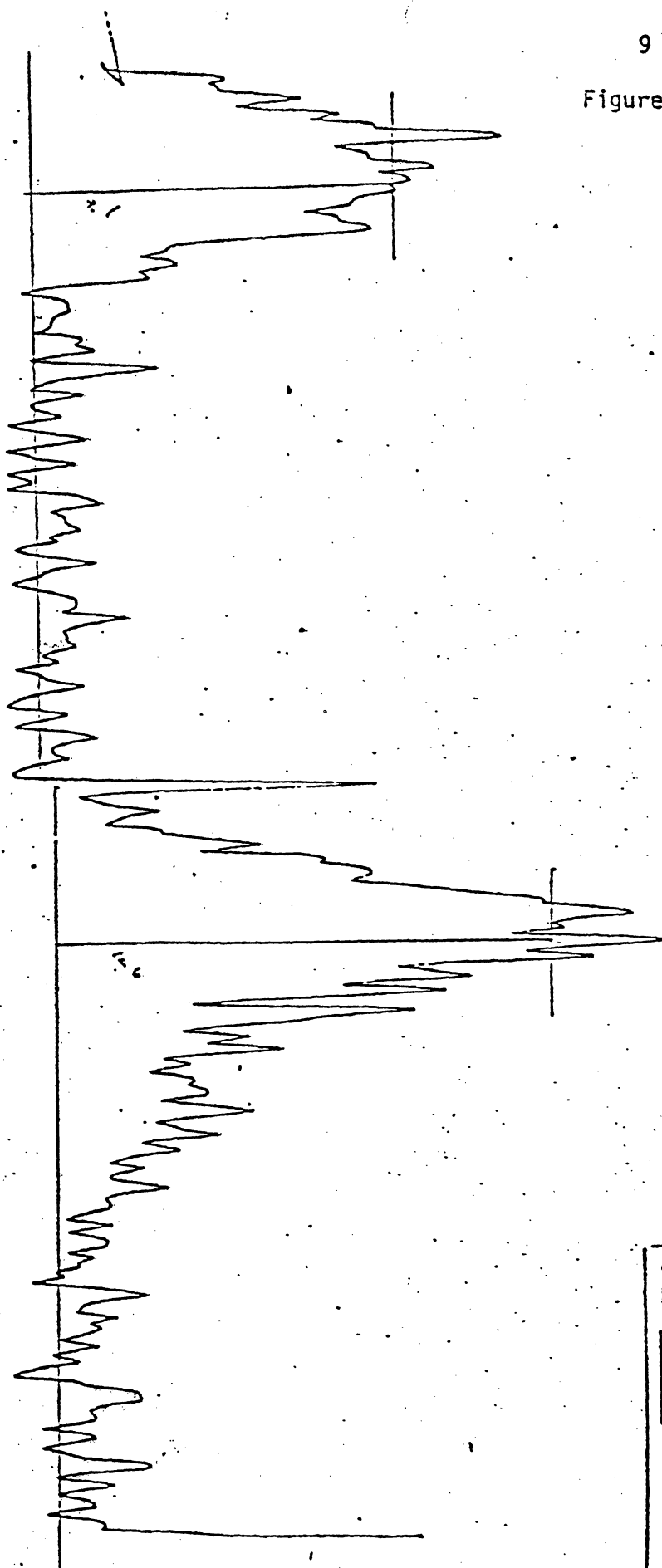


Figure 2

RUN NO. 1751 SAMPLE FS 23
 GAIN X300 AT 1.512 K
 VOL. RATIO 17.8-158.0 R.T. 3.3
 SWEEP 76

22005

Figure 3



RUN NO. 1764 SAMPLE F5 23
GAIN 1300/1200 AT 256 K
VOL. RATIO 15.4/42.8 R.T. 3.4
SWEEPS 17

TABLE I
ANALYSIS OF TCDD
BY
GLC HIGH RESOLUTION MASS SPECTROMETRY

ID	Nature of Sample	Conc.	Detection Limit	Percent Recovery	Conc. (i ₃₂₂)	Conc. (i ₃₂₂) [*]	i ₃₂₀ /i ₃₂₂
-1	Deer Muscle	nd	1	50	-	-	-
-2	"	nd	2	75	-	-	-
-2	"	nd	.8	105	-	-	-
S-3 ^{*1}	" 15 ppt	3.3	1	85	4.1	4.8	.60
S-4 ^{*1}	" positive	1.6	1	125	3.0	3.0	.64
S-5	"	nd	.9	85	< .1	-	-
S-6	"	nd	1	130	-	-	-
S-7	" 30 ppt	7.4	.8	95	5.6	5.9	.79
S-8	" positive	3.4	1	95	1.9	2.0	.69
S-9 ^{*2}	"	nd	1	95	1.2	1.3	.48
S-10 ^{*2}	"	nd	.5	80	-	-	.36
S-11	" positive	4.6	.7	85	4.7	5.5	.77
S-12	"	nd	.2	75	-	-	-
S-13	"	nd	.6	85	-	-	-
S-14	"	nd	.4	100	-	-	-
S-15 ^{*2}	deer adipose was fine	2.0	2	45	<2	-	.54 < 0.6
S-16	"	nd	.9	65	-	-	-
S-17 ^{*2}	"	1.9	1	70	1.8	2.6	.57 < 0.6
S-18	" 43 ppt	12	2	75	6.1	8.3	.83
S-19	" positive	2	2	135	3.0	3.0	.67

TABLE II
ANALYSIS OF TCDD
BY
GLC HIGH RESOLUTION MASS SPECTROMETRY

ID	Nature of Sample	Conc.	Detection Limit	Percent Recovery	Conc. (i ₃₂₂)	Conc. (i ₃₂₂) [*]	i ₃₂₀ /i ₃₂₂
FS-28	Deer Liver	nd	2	40	-	-	-
FS-29	" 48 ppt	14	.8	75	-	-	-
FS-30	"	nd	2	70	-	-	-
FS-31	" positive	2.5	1	75	1.1	1.5	.80
FS-32	"	nd	2	75	<1	-	-
FS-33	"	nd	3	35	-	-	-
FS-34	" positive	2.1	1	65	1.3	2.0	.76
FS-35	"	nd	2	55	-	-	-
FS-36	" 14 ppt	4.5	2	55	2.9	5.3	.75
FS-37	"	nd	5	30	-	-	-
FS-38	"	nd	2	75	-	-	-
FS-39	"	nd	2	70	-	-	-
FS-40	" 28 ppt	7.8	2	65	9.2	14	.82
FS-41	"	nd	1	80	-	-	-
FS-42	" positive	3	3	130	6.4	6.4	.69
FS-43	Bone Marrow	nd	5	65	-	-	-
FS-44 ^{*4}	" negative	2	2	60	<1	-	-
FS-45	" 7 ppt	3.9	3	80	2.8	3.5	.71
FS-46	"	nd	7	70	-	-	-
FS-47	"	nd	7	70	-	-	-
FS-48	"	nd	3	70	<3	-	-

TABLE III
ANALYSIS OF TCDD
BY
GLC HIGH RESOLUTION MASS SPECTROMETRY

ID	Conc.	i_{320}/i_{322}
Mylar 2	110 pg/ μ l	.80
Mylar 4	61 pg/ μ l	.76
Mylar 34	<1.4 pg/ μ l	---
Mylar 38	18 pg/ μ l	.81
Mylar 42	9.4 pg/ μ l	.70
Mylar 44	99 pg/ μ l	.84
7635-1	.7 pg/ μ l	.52
7635-2	<.3 pg/ μ l	---
7635-2	<.4 pg/ μ l	---
7635-3	< 2 pg/ μ l	---
7672-1	<.1 pg/ μ l	---
7696-1	<.3 pg/ μ l	---

Table IV

Summary of Positives
in Deer Tissue

<u>ID No.</u>		<u>Sample Type</u>	
FS-3	3	Deer Muscle	Fortified
✓ FS-4		" "	+
FS-7		" "	Fortified
FS-8		" "	+
FS-11		" "	+
FS-18	3	Deer Adipose	Fortified
FS-19		" "	+
FS-20		" "	+
FS-23		" "	Fortified
FS-24		" "	+
FS-27		" "	Fortified
FS-31	3	Deer Liver	+
FS-34		" "	+
FS-36		" "	Fortified
FS-40		" "	Fortified
FS-42		" "	+
FS-45		Bone Marrow	Fortified
FS - 29		Deer Tissue	Fortified

ID	Nature of Sample	Conc.	Detection Limit	Percent Recovery	Conc. (i ₃₂₂)	Conc. (i ₃₂₂) [*]	i ₃₂₀ /i ₃₂₂
FS-20	Deer adipose ^{positive}	2.9	1	.85	3.0	3.5	.75
FS-21 ^{*2}	" ^{positive}	3	3	.95	5.4	5.7	.57
FS-22 ^{*3}	" ^{negative} **	1.6	1	50	<1	-	.62
FS-23	" ^{16 ppt}	4.9	1	60	4.6	7.7	.72
FS-24	" ^{positive}	2.2	1	55	4.6	8.4	.68
FS-25	" nd		1	65	-	-	-
FS-26	" ^{negative} **	2.3	1	65	<2	-	-
FS-27	" ^{10 ppt}	3.6	.7	75	3.1	4.1	.73

~~**~~ See p 5

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Figure 4

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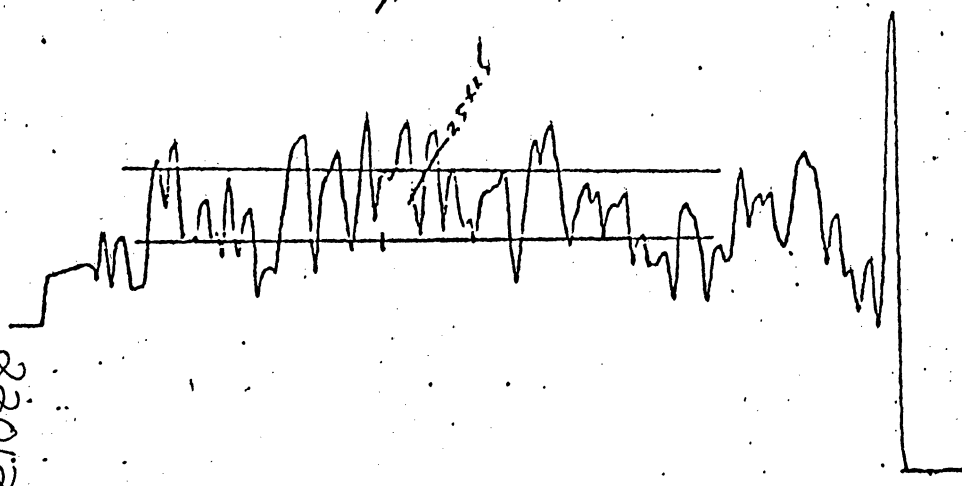
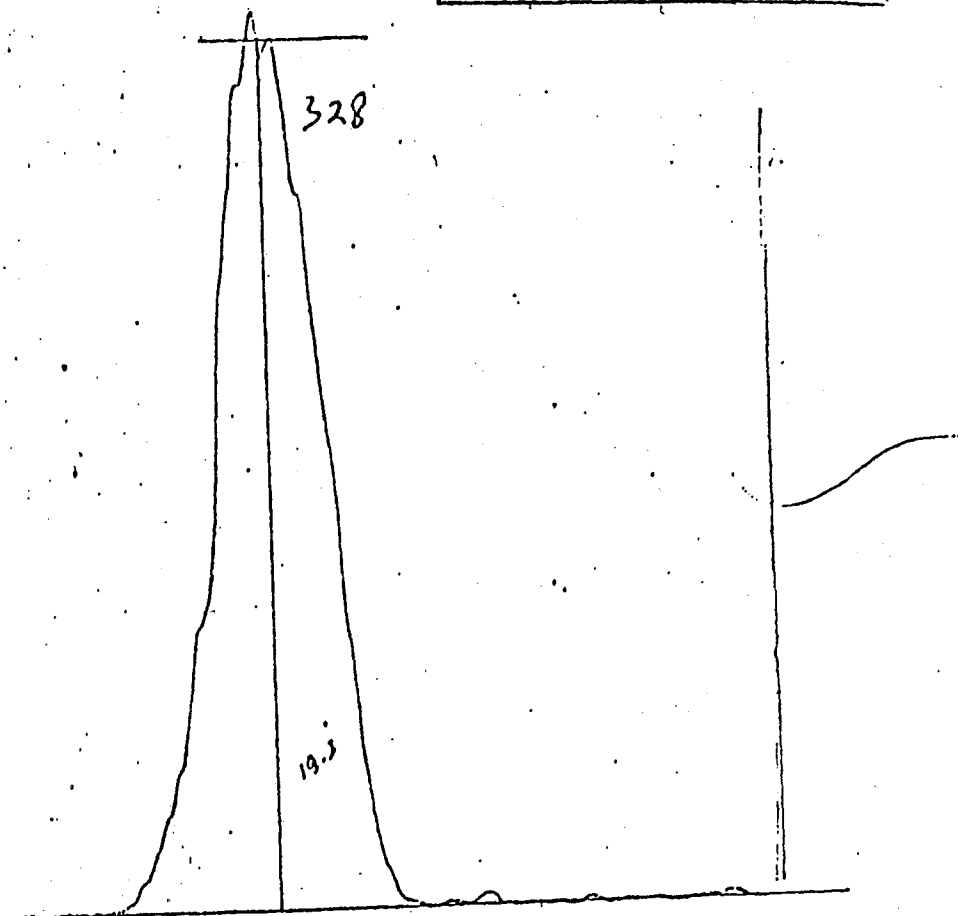


Figure 4-

MCMS/UN-L 322/328	
RUN NO: <u>T/C38</u>	SAMPLE <u>FS-38</u>
GAIN <u>X300/X3AT</u>	<u>1</u> K
VOL. RATIO <u>17 of 57</u>	R.T. <u>3.1</u>
SWEEPS <u>91</u>	



notes: i_{322} corrected from less than 100% recovery to 100% recovery.

1. Ratio distorted due to large PCB enhancement at m/e 322 decreasing the ratio i_{320}/i_{322} .
2. The ratios of 0.60 or less are suggestive of residuals in the initial standard and should be considered negative.
3. S/N poor on validation, however i_{320}/i_{322} is extractable from the data.
4. S/N poor on validation, and peak heights not measurable. Should be considered as negative.
5. Isotope ratio not in accord with a positive.

studied this report.

- B. Samples giving "Positive" Results: The validity of our assignment of "positives" can be judged by examining the control samples. At this writing, these are unknown to us. The following Table IV is a summary of samples judged definitely to be positive based on the internal standard method and the validation work. All other samples should be considered "negative" at the reported detection limit.