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INTERLABORATORY METHOD VALIDATION STUDY FOR DIOXIN

AN INTERIM REPORT

by Robert G. Heath
Human Effects Monitoring Branch
OPP, OTS, EPA

January 5, 1979

Interlaboratory Method Validation Study for Dioxin

A. Introduction and Scope

B. Study Design

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Acknowledgements page to be prepared.

A. Introduction and Scope

The Interlaboratory Method Validation Study for Dioxin was undertaken to measure the accuracy and precision with which 2,3,7,8-tetrachlorodiobenzo-p-dioxin (TCDD), when added to beef fat and human milk at low parts-per-trillion concentrations, can be extracted and quantified by methods of gas chromatography-mass spectrometry (GC-MS). Method validation also included quantitation of equivalent amounts of TCDD standards. In particular, the study was undertaken to develop regression statistics for converting reported TCDD concentrations to "best estimates" of actual (but unknown) concentrations and for expressing the reliability of such estimates in terms of statistical confidence limits. The study was also intended to determine the lowest concentration of TCDD that was identified with practicable consistency and the frequency of "false positive" and "false negative" reports.

All samples were prepared and extracted at the EPA Pesticide Monitoring Laboratory, Bay St. Louis, Mississippi. Analytical laboratories participating in all or part of the GC-MS quantitation were those of Dow Chemical Company, Harvard University, University of Nebraska, Wright State University and the EPA Health Effects Research Laboratory (HERL),

Research Triangle Park, North Carolina. Analytical laboratories are identified only as laboratory A,B,C,D, or E throughout the report; alphabetical order is independent of the above laboratory order. The number of samples, by type, quantified by each laboratory is shown in Table A-1.

TABLE A-1.

Number of Samples, by Type, Quantified by Participants

	Sample Description				
		Acid/base cleanup		Neutral Extraction	Lab
Laboratory	Standard	Beef fat	Human milk	Beef fat	Totals
A	26	26	26	0	78
B	25	26	26	0	77
C	26 + 3	26	0	16	71
D	0	6	0	16	22
E	1	0	11	0	12
Type totals	81	84	63	32	260

B. Study Design

Beef fat and human milk samples were "spiked" with ^{35}Cl TCDD at levels ranging from 0 to 81 ppt. Standards were prepared so as to contain equivalent amounts of the chemical. Samples were prepared from one of two pools of rendered beef fat (pools F and G) and one of two pools of human milk (pools M and N). Fat was from cattle without potential exposure to dioxin; the milk had been collected in regions where use of pesticides potentially contaminated with TCDD was incidental. Pools were constructed using equal amounts of fat or milk from each animal or donor, using a separate set of animals or donors for construction of each pool.

Eleven samples each were prepared from fat pool F and milk pool M--the major pools. The samples from each pool were spiked individually at 0, 0.5, 1, 4, 9, 16, 25, 36, 49, 64 or 81 ppt. The samples were then extracted by procedures developed/refined at PML, and the extract was divided into the required numbers of equal aliquots for shipment to the analytical laboratories. Spiking levels, excepting 0.5 ppt, were systematically incremented as the squares of the digits 0 through 9 to provide close spacing at low levels and a moderate, systematic increase in spacing with increasing levels.

To test the precision of the extraction methodology, five samples each were prepared from the minor pools (fat pool G and milk pool N). These samples were spiked individually at 0, 9, 25, 49, and 81 ppt, extracted by the same procedures used for samples from pools F and M, and divided into the required number of equal aliquots for shipment to the analytical laboratories. Thus, fat pools F and G and milk pools M and N provide replicate samples at the above levels of spiking for testing extraction precision.

To test the precision of GC-MS quantitation for comparison with that of extraction, laboratories were provided two aliquots of the G- and N-pool extracts, along with two aliquots from each of the matching extracts from pools F and N, so as to obtain duplicate analyses of the same extract. Labs also received four standards at each equivalent of 0, 9, 25, 49, and 81 ppt, as well as single standards at 0.5, 1, 4, 16, 36, and 64 ppt. Standards are denoted as S.

All samples--fat, milk and standards--were required to be prepared and shipped in random order, and laboratories were to analyze the samples in the order in which they were received. Samples were identified only by shipment number, so that laboratories knew neither the type of sample nor the TCDD level at the time of analysis.

A variation in the above procedure was developed for a set of beef fat samples analyzed at Lab D. The fat samples in that set were spiked at PML but were extracted at Lab D using a neutral extraction procedure rather than the acid/base procedure utilized throughout the study.

Accuracy (the degree of constant tendency to either under-report or over-report the true level) and precision (variation among repeated measurements of the same extract) have been measured by methods of regression analysis; comparisons of extraction vs quantitation precision are by analysis of variance based on those spiking levels for which there were duplicate analyses of replicate extractions. The analytical schedule is presented in Table B-1.

Table B-1.

Design Diagram for Phase II Dioxin Study

TCDD Level (ppt)	Beef Fat Measurements			Human Milk Measurements			Standard Measurements		
	Pool Code	Lab A	Lab B etc.	Pool Code	Lab A	Lab B etc.	Pool Code	Lab A	Lab B etc.
0	F	2	2	M	2	2	S	4	4
0	G	2	2	N	2	2			
1/2	F	1	1	M	1	1	S	1	1
1	F	1	1	M	1	1	S	1	1
4	F	1	1	M	1	1	S	1	1
9	F	2	2	M	2	2	S	4	4
9	G	2	2	N	2	2			
16	F	1	1	M	1	1	S	1	1
25	F	2	2	M	2	2	S	4	4
25	G	2	2	N	2	2			
36	F	1	1	M	1	1	S	1	1
49	F	2	2	M	2	2	S	4	4
49	G	2	2	N	2	2			
64	F	1	1	M	1	1	S	1	1
81	F	2	2	M	2	2	S	4	4
81	G	2	2	N	2	2			

C. General Results

Analytical results for the quantitation of standards are presented in Tables C-1 through C-3. Figures C-1 through C-3 (each figure follows its respective table) show the plotted results and the least squares regression lines and equations for reported values on spiked values. The theoretical line $y=x$, for perfect extraction and quantitation is also shown for comparison.

Equivalent results for beef fat samples are presented in Tables and Figures C-4 through C-9, and those for human milk appear in Tables and Figures C-10 through C-12.

An explanation of the types of reporting errors and an enumeration of those errors are presented in Tables C-13 through C-16. In this report, the reporting of a positive value in an unspiked sample is identifed as a "False Positive" (FP), and a positive report given when the detection limit exceeds the level of spiking is identified as a "false positive" (fp). A "false not detected" (fnd) is defined as a report of "nd" when, in fact, the level of detection is less than the level of spiking.

As might have been expected, the highest frequency of errors occurred at spiking levels below 9 ppt.

Table C-1.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Standard (5g equivalent)
Preparation Lab: PML
Quantitation Lab: Lab A WS

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37} (%)	Added	320	322	Avg.	320	322
$S\text{-}0_1$	ST-0	2	88	0		nd			0.3
$S\text{-}0_2$	ST-0	40	101	0		nd			6
$S\text{-}0_3$	ST-0	45	83	0		2			2
$S\text{-}0_4$	ST-0	49	94	0		nd			2
S-0.5	ST-.5	15	95	0.5		1			1
S-1	ST-1	19	141	1		nd			2
S-4	ST-4	10	77	4		2			2
$S\text{-}9_1$	ST-9	21	68	9		7			2
$S\text{-}9_2$	ST-9	28	93	9		.4			2
$S\text{-}9_3$	ST-9	46	113	9		7			7
$S\text{-}9_4$	ST-9	47	69	9		10			3
S-16	ST-16	22	86	16		6			2
$S\text{-}25_1$	ST-25	16	93	25		nd			0.7
$S\text{-}25_2$	ST-25	42	90	25		19			3
$S\text{-}25_3$	ST-25	48	64	25		8			2
$S\text{-}25_4$	ST-25	52	131	25		18			2
S-36	ST-36	5	81	36		16			4
$S\text{-}49_1$	ST-49	13	67	49		23			2
$S\text{-}49_2$	ST-49	32	109	49		26			2
$S\text{-}49_3$	ST-49	44	98	49		18			1
$S\text{-}49_4$	ST-49	50	98	49		68			4
S-64	ST-64	17	107	64		nd			0.5
$S\text{-}81_1$	ST-81	12	46	81		44			2
S-8	ST-81	29	98	81		40			2
$S\text{-}81_3$	ST-81	43	102	81		44			2
$S\text{-}81_4$	ST-81	51	106	81		56			3

Figure C-1

Standards
Lab A (322 m/e) WS

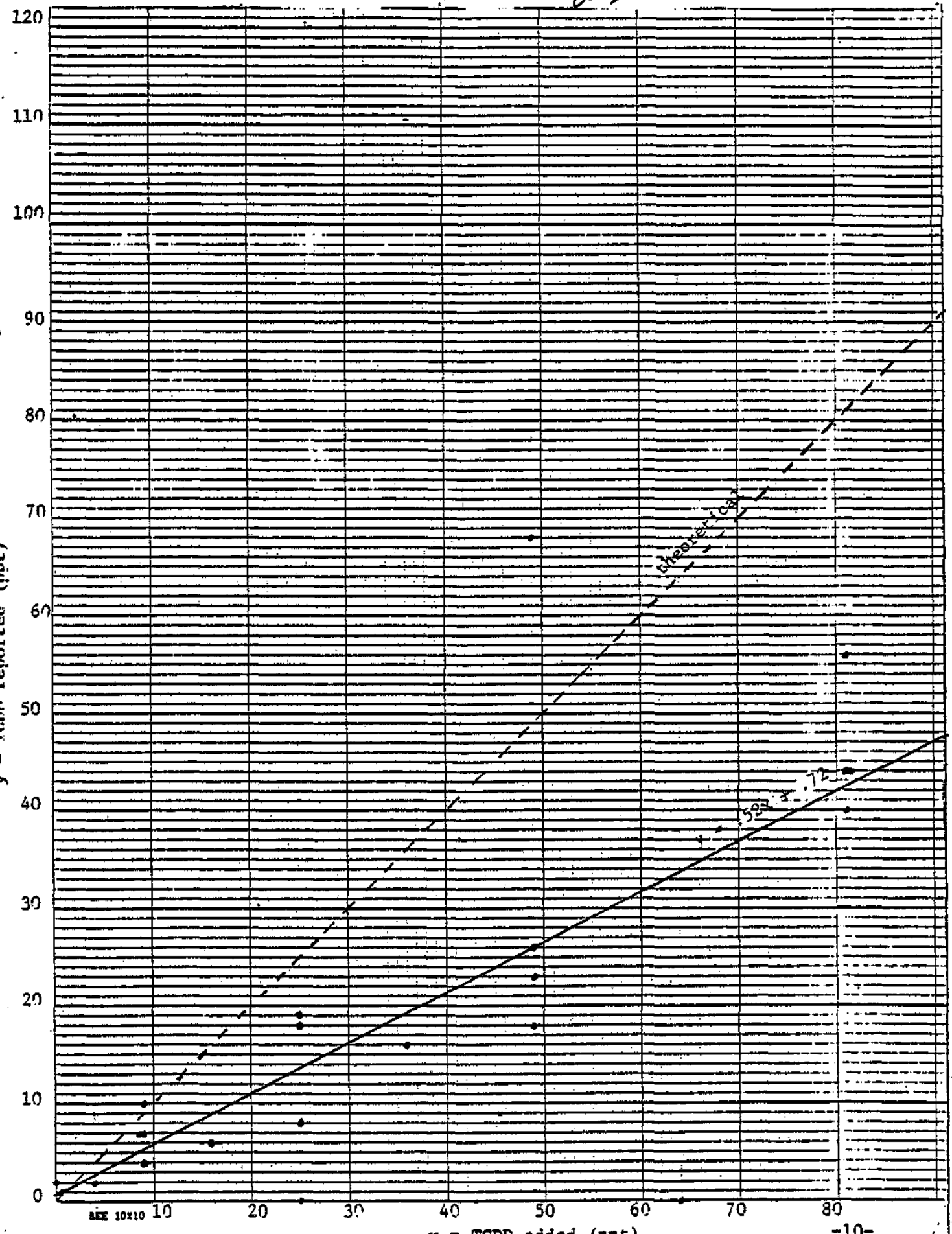


Table C-2.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Standard (5g equivalent)
Preparation Lab: PML
Quantitation Lab: Lab B

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37}(%)	Added	320	322	Avg.	320	322
$S\text{-}0_1$	ST-0	2		0	nd	nd	nd	4	3
$S\text{-}0_2$	ST-0	40		0	nd	nd	nd	2	3
$S\text{-}0_3$	ST-0	45		0	3	nd	-	2	2
$S\text{-}0_4$	ST-0	49		0	nd	2	-	3	1
S-0.5	ST-.5	15		0.5	nd	nd	nd	3	4
S-1	ST-1	19		1	nd	nd	nd	4	5
S-4	ST-4	10		4	-	-	-	-	-
$S\text{-}9_1$	ST-9	21		9	4	5	4.5	3	2
$S\text{-}9_2$	ST-9	28		9	9	6	7.5	4	1
$S\text{-}9_3$	ST-9	46		9	4	6	5	2	2
$S\text{-}9_4$	ST-9	47		9	10	7	8.5	3	1
S-16	ST-16	22		16	10	10	10	2	1
$S\text{-}25_1$	ST-25	16		25	16	22	19	4	4
$S\text{-}25_2$	ST-25	42		25	17	13	15	2	1
$S\text{-}25_3$	ST-25	48		25	7	19	13	1	2
$S\text{-}25_4$	ST-25	52		25	29	21	25	3	1
S-36	ST-36	5		36	6	12	9	4	5
$S\text{-}49_1$	ST-49	13		49	13	22	17.5	5	3
$S\text{-}49_2$	ST-49	32		49	52	29	40.5	5	1
$S\text{-}49_3$	ST-49	44		49	40	18	29	1	1
$S\text{-}49_4$	ST-49	50		49	8	25	16.5	2	1
S-64	ST-64	17		64	28	33	30.5	3	2
$S\text{-}81_1$	ST-81	22		81	59	50	54.5	5	2
S-8	ST-81	29		81	21	23	22	1	1
$S\text{-}81_3$	ST-81	43		81	77	47	62	6	2
$S\text{-}81_4$	ST-81	51		81	83	43	63	3	2

Figure C-2a

Standards
Lab B (322 m/e)

Dow

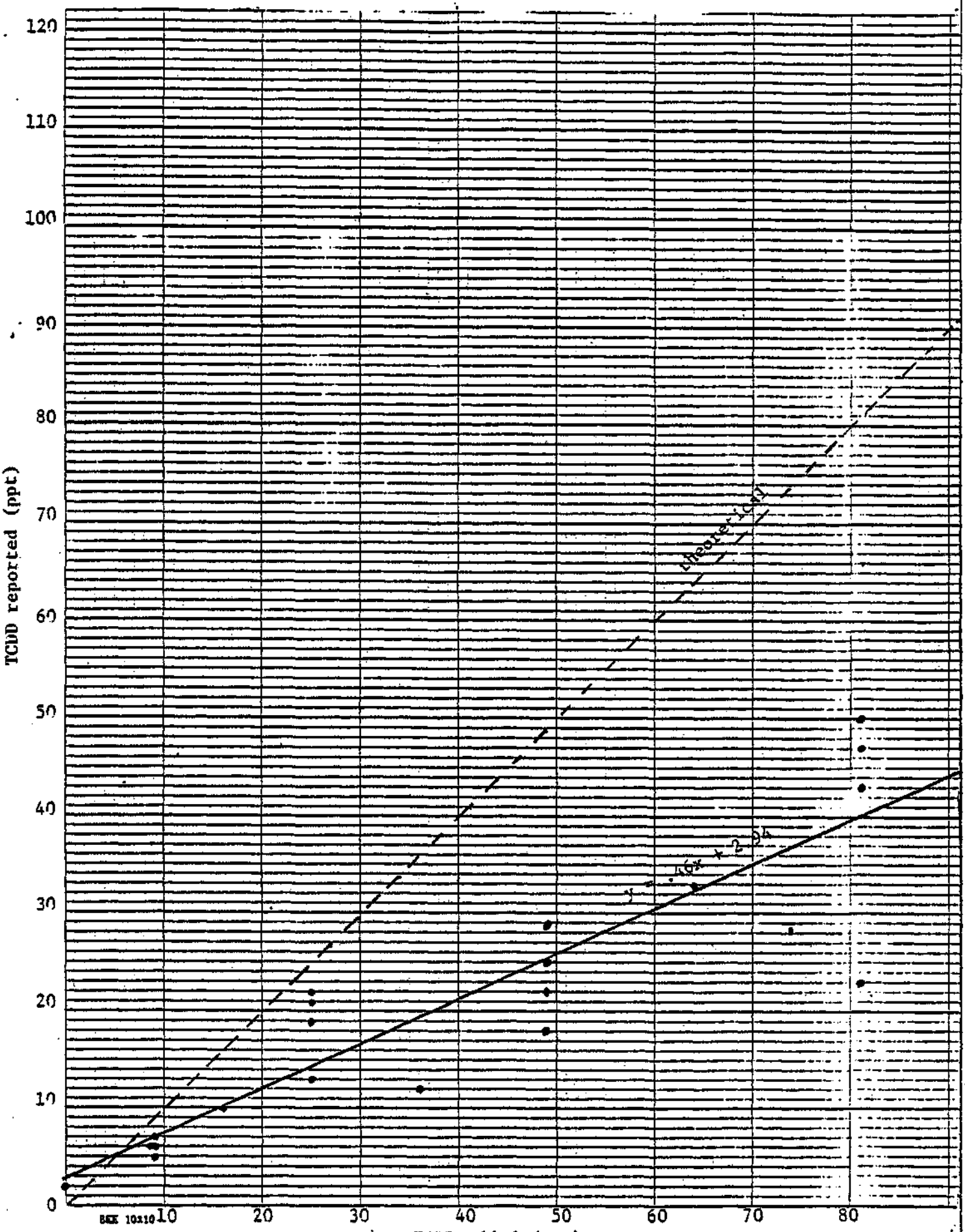


Figure C-2b

Standards
Lab B (320 m/e)

Dow

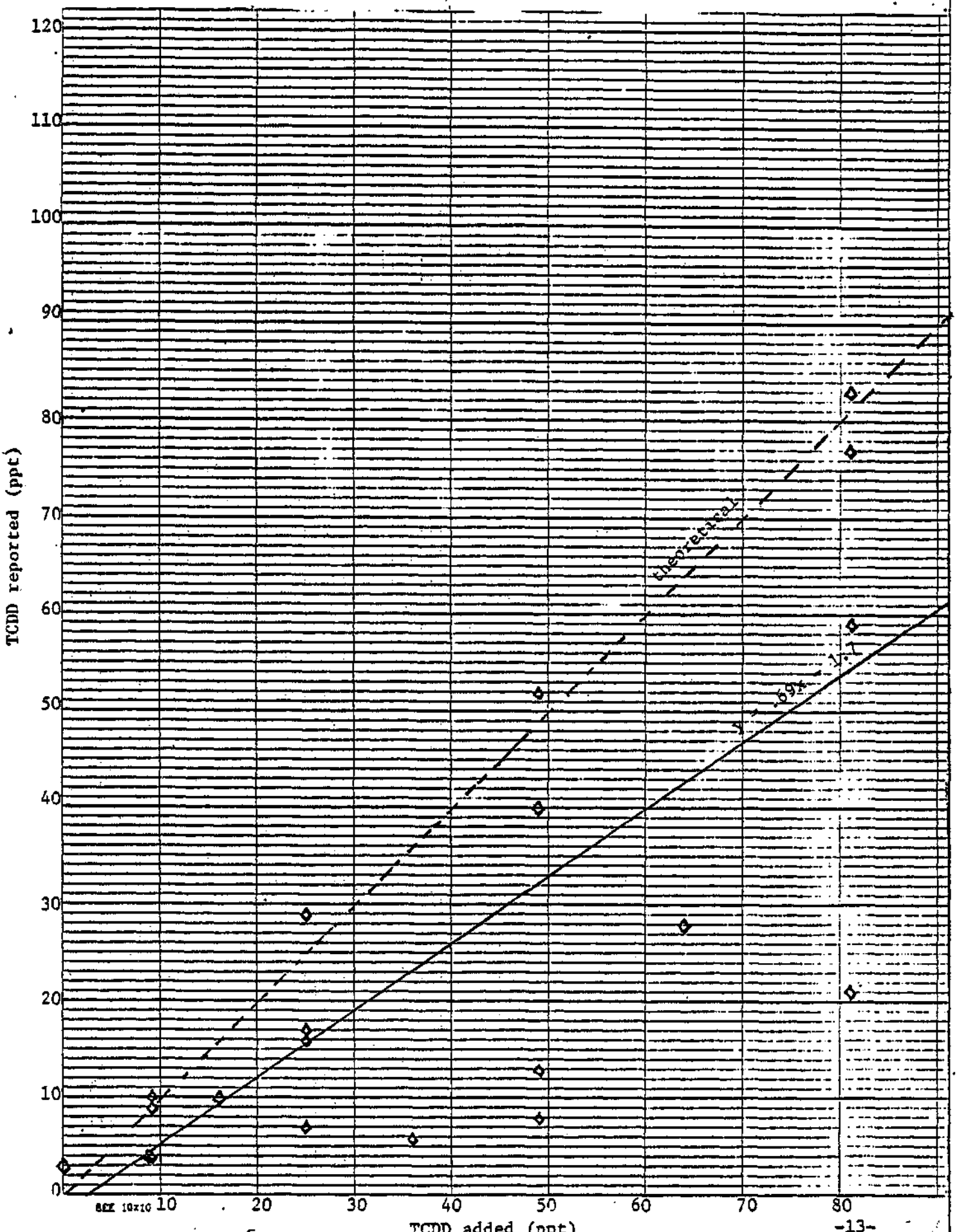


Table C-3.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Standard (5g equivalent)
Preparation Lab: PML
Quantitation Lab: Lab C Nebraska

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37}(%)	Added	320	322	Avg.	320	322
$S\text{-}0_1$	ST-0	2		0		nd			10
$S\text{-}0_2$	ST-0	48		0		nd			4
$S\text{-}0_3$	ST-0	57		0		nd			4
$S\text{-}0_4$	ST-0	62		0		nd			4
S-0.5	ST-.5	15		0.5		nd			6
S-1	ST-1	19		1		nd			12
S-4	ST-4	10		4		nd			6
$S\text{-}9_1$	ST-9	21		9		nd			10
$S\text{-}9_2$	ST-9	56		9		11			5
$S\text{-}9_3$	ST-9	60		9		9			4
$S\text{-}9_4$	ST-9	64		9		6			4
S-16	ST-16	22		16		16			10
$S\text{-}25_1$	ST-25	16		25		21			4
$S\text{-}25_2$	ST-25	55		25		17			4
$S\text{-}25_3$	ST-25	63		25		25			4
$S\text{-}25_4$	ST-25	69		25		24			4
S-36	ST-36	5		36		34			16
$S\text{-}49_1$	ST-49	13		49		41			12
$S\text{-}49_2$	ST-49	51		49		51			3
$S\text{-}49_3$	ST-49	52		49		47			9
$S\text{-}49_4$	ST-49	70		49		47			5
S-64	ST-64	17		64		65			6
$S\text{-}81_1$	ST-81	12		81		76			6
S-8	ST-81	49		81		77			3
$S\text{-}81_3$	ST-81	59		81		80			3
$S\text{-}81_4$	ST-81	67		81		80			5

Figure C-3

Standards

Lab C (322 m/e) Nebraska

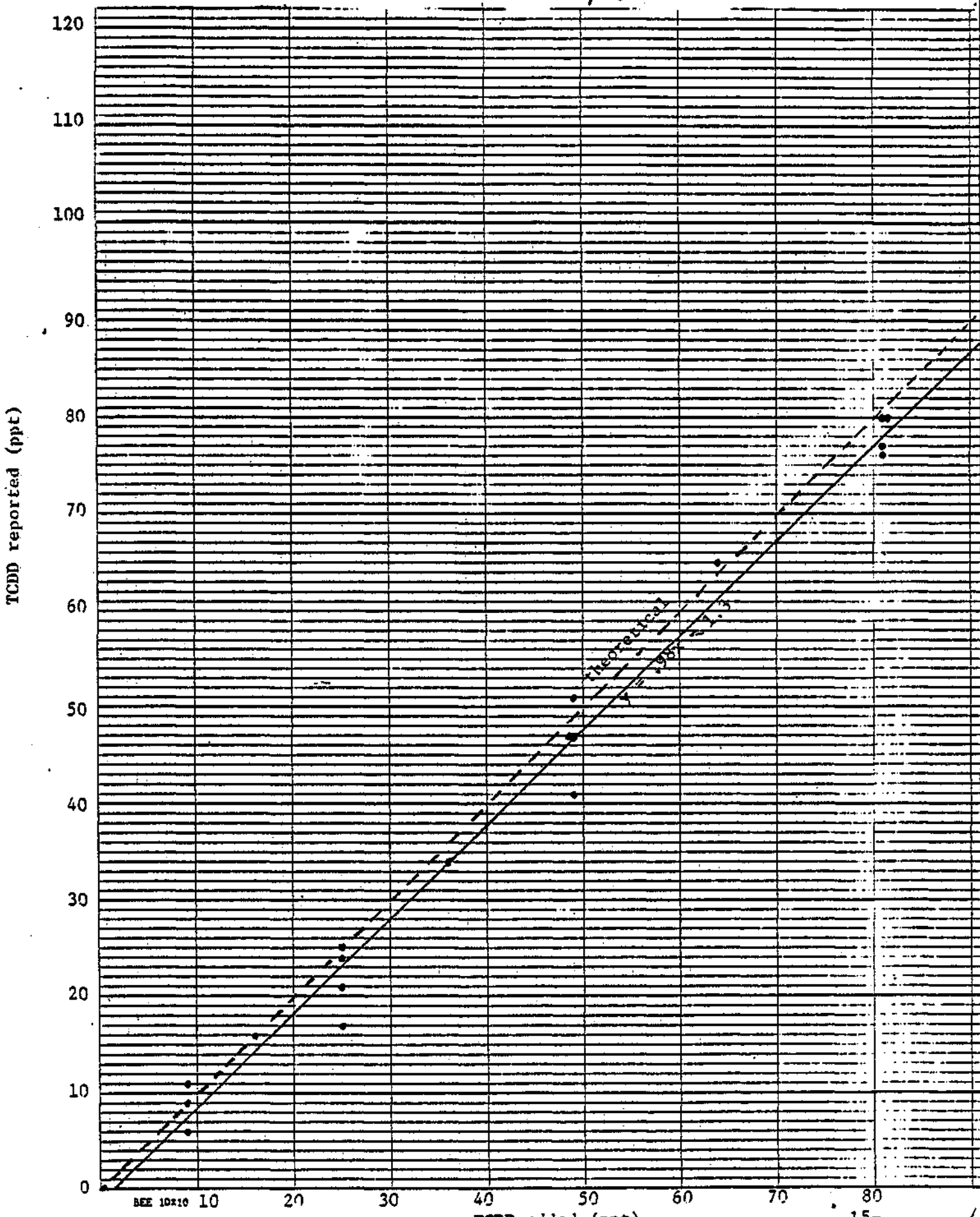


Table C-4.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat (5g sample)
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab A WS

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
		Ship-			Reported				
Study	PML	ment	Cl^{37} (%)	Added	320	322	Avg.	320	322
F-0_1	FE	11	71	0		103			7
F-0_2	FE	37	72	0		nd			5
G-0_1	GB	24	73	0		nd			4
G-0_2	GB	39	53	0		2			2
F-0.5	FG	1	69	0.5		12			0.4
F-1	FC	7	72	1		3			2
F-4	FI	6	70	4		28			5
F-9_1	FK	25	64	9		4			2
F-9_2	FK	41	75	9		9			1
G-9_1	GC	20	93	9		2			1
G-9_2	GC	30	68	9		nd			1
F-16	FL	26	72	16		8			4
F-25_1	FD	3	71	25		110			1
F-25_2	FD	34	77	25		22			6
G-25_1	GE	18	73	25		5			5
G-25_2	GE	38	68	25		8			1
F-36	FA	8	80	36		34			3
F-49_1	FH	27	73	49		18			2
F-49_2	FH	33	99	49		7			2
G-49_1	GA	14	87	49		6			0.8
G-49_2	GA	35	68	49		32			5
F-64	FB	9	101	64		86			3
F-81_1	FJ	23	53	81		25			2
F-81_2	FJ	31	89	81		22			4
G-81_1	GD	4	69	81		110			1
G-81_2	GD	36	71	81		40			2

Figure C-4

Beef Fat Samples
Lab A (322 m/e) WS

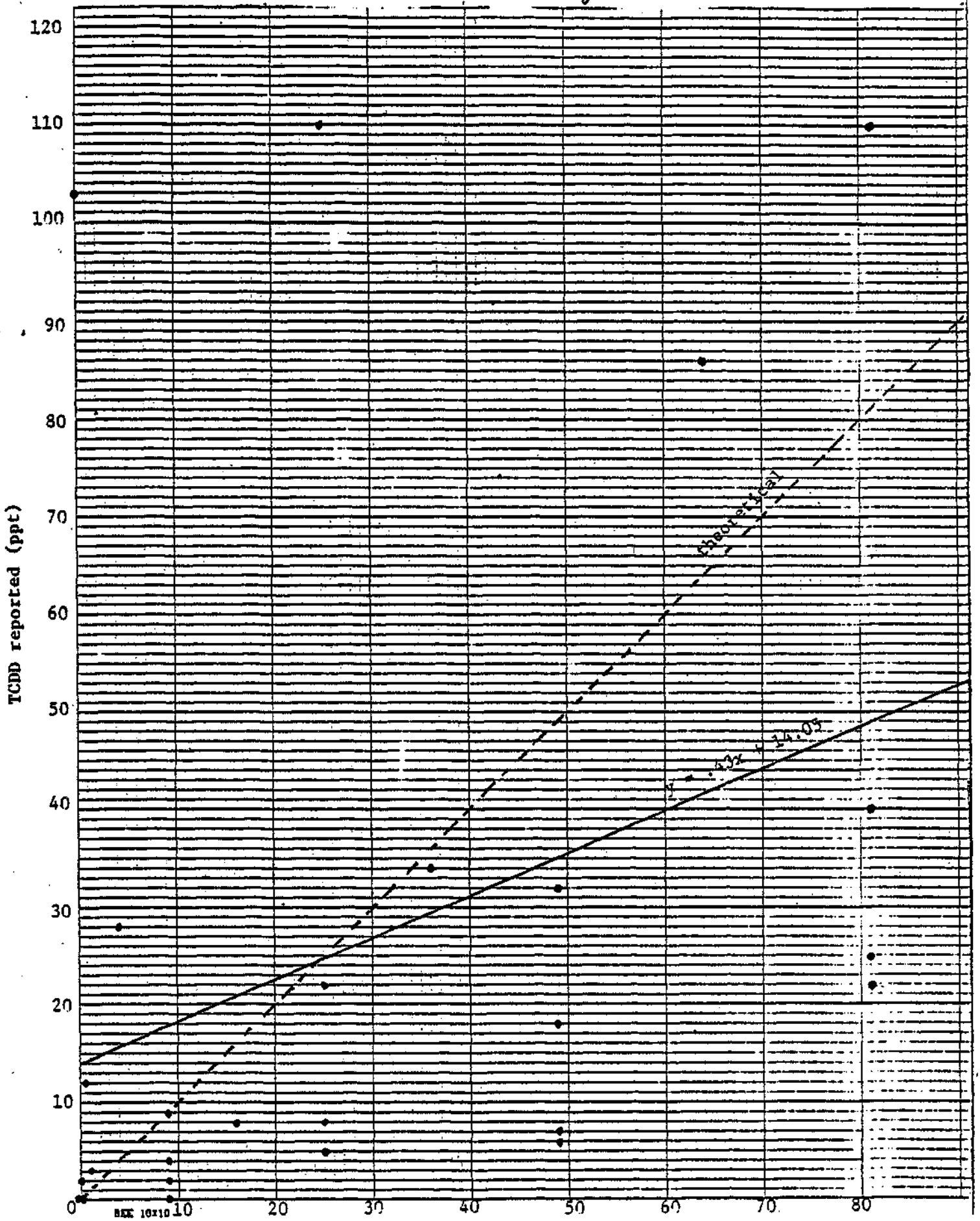


Table C-5.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat (5g sample)
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab B Dow

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37} (%)	Added	320	322	Avg.	320	322
$F\text{-}0_1$	FE	11		0	4	7	5.5	4	2
$F\text{-}0_2$	FE	37		0	13	6	9.5	2	3
$G\text{-}0_1$	GB	24		0	nd	10	-	4	4
$G\text{-}0_2$	GB	39		0	9	5	7	2	2
F-0.5	FG	1		0.5	7	12	9.5	4	3
F-1	FC	7		1	nd	nd	nd	4	4
F-4	FI	6		4	7	20	13.5	5	5
$F\text{-}9_1$	FK	25		9	7	13	10	5	3
$F\text{-}9_2$	FK	41		9	6	13	9.5	2	2
$G\text{-}9_1$	GC	20		9	8	21	14.5	7	5
$G\text{-}9_2$	GC	30		9	7	11	9	2	2
F-16	FL	26		16	13	18	15.5	7	2
$F\text{-}25_1$	FD	3		25	12	10	11	4	2
$F\text{-}25_2$	FD	34		25	12	17	14.5	2	3
$G\text{-}25_1$	GE	18		25	18	27	22.5	6	6
$G\text{-}25_2$	GE	38		25	12	8	10	3	2
F-36	FA	8		36	27	29	28	5	7
$F\text{-}49_1$	FH	27		49	21	22	21.5	3	2
$F\text{-}49_2$	FH	33		49	35	26	30.5	3	2
$G\text{-}49_1$	GA	14		49	38	43	40.5	7	8
$G\text{-}49_2$	GA	35		49	15	28	21.5	2	2
F-64	FB	9		64	54	25	39.5	8	2
$F\text{-}81_1$	FJ	23		81	42	34	38	5	1
$F\text{-}81_2$	FJ	31		81	27	32	29.5	2	3
$G\text{-}81_1$	GD	4		81	60	64	62	9	5
$G\text{-}81_2$	GD	36		81	39	32	35.5	2	2

Figure C-5a

Beef Fat Samples
Lab B (322 m/e)

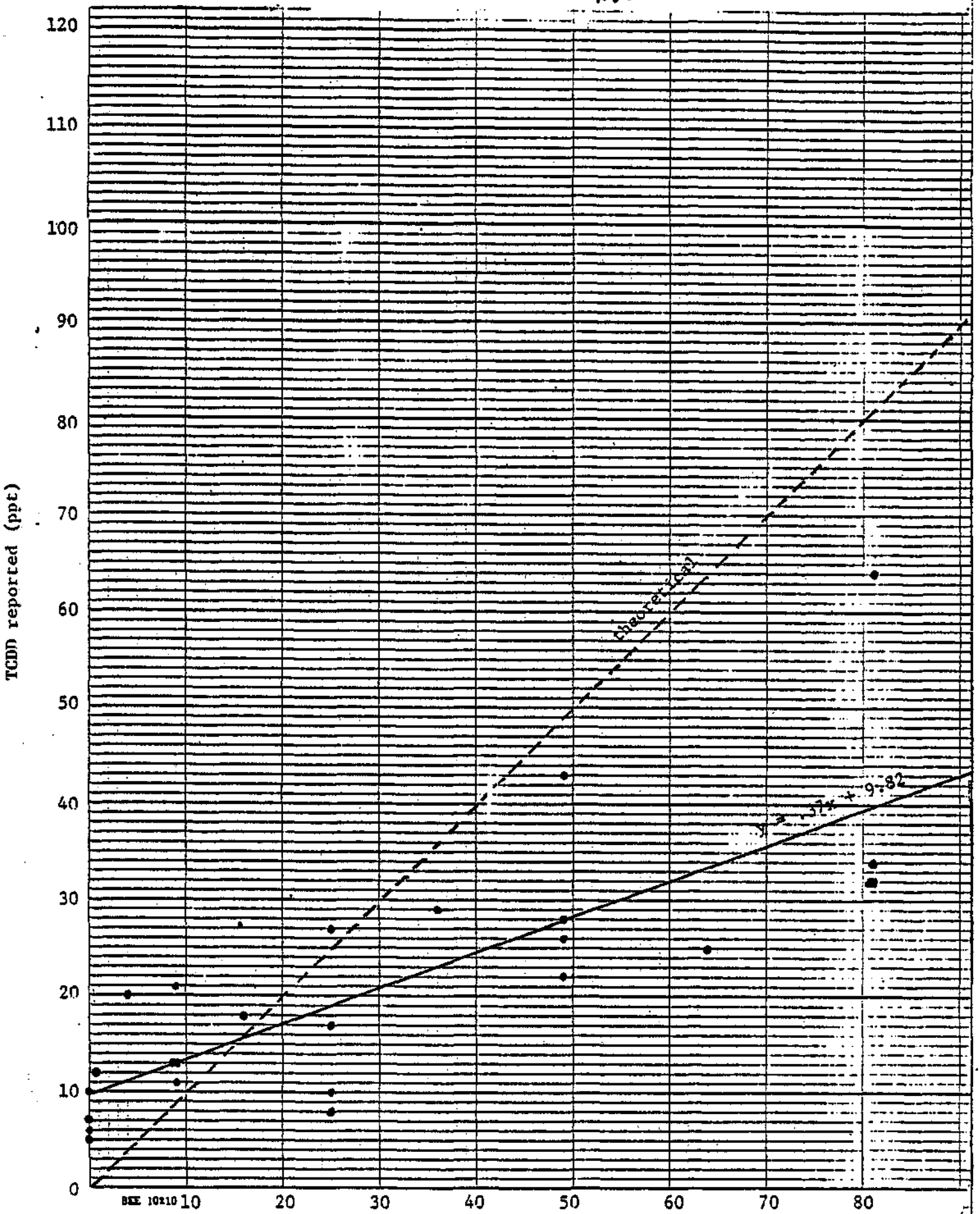


Figure C-5b

Beef Fat Samples

Lab B (320 m/e)

Dow

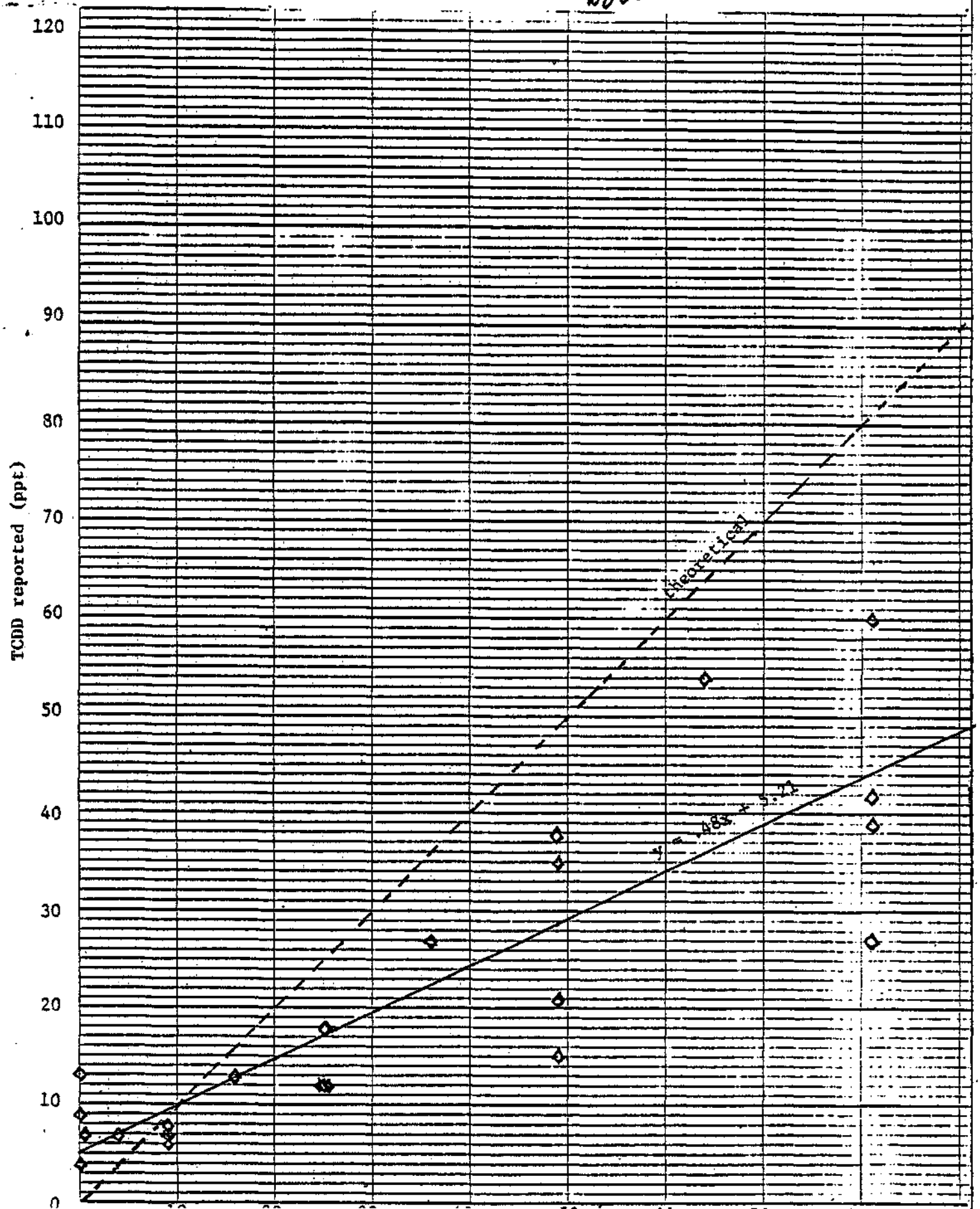


Table C-6.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat (5g sample)
Extraction Lab: FML; Method: Acid/base
Quantitation Lab: Lab C

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
Study	PML	Shipment	Cl^{37} (%)	Added	320	322	Reported Avg.	320	322
$F\text{-}0_1$	FE	11		0		nd			8
$F\text{-}0_2$	FE	54		0		nd			5
$G\text{-}0_1$	GB	24		0		nd			6
$G\text{-}0_2$	GB	65		0		nd			6
F-0.5	FG	1		0.5		nd			14
F-1	FC	7		1		nd			10
F-4	FI	6		4		nd			18
$F\text{-}9_1$	FK	25		9		9			4
$F\text{-}9_2$	FK	68		9		11			5
$G\text{-}9_1$	GC	20		9		nd			14
$G\text{-}9_2$	GC	50		9		12			5
F-16	FL	26		16		19			10
$F\text{-}25_1$	FD	3		25		(63)*			10
$F\text{-}25_2$	FD	47		25		24			2
$G\text{-}25_1$	GE	18		25		26			12
$G\text{-}25_2$	GE	71		25		27			4
F-36	FA	8		36		31			6
$F\text{-}49_1$	FH	27		49		50			6
$F\text{-}49_2$	FH	58		49		48			4
$G\text{-}49_1$	GA	14		49		39			10
$G\text{-}49_2$	GA	66		49		48			6
F-64	FB	9		64		58			4
$F\text{-}81_1$	FJ	23		81		76			6
$F\text{-}81_2$	FJ	61		81		74			4
$G\text{-}81_1$	GD	4		81		76			14
$G\text{-}81_2$	GD	53		81		77			3

*Aberrant value both included and excluded in calculations.

Figure C-6a

Beef Fat (5 gm)
Lab C (322 m/e)
(n = 17)

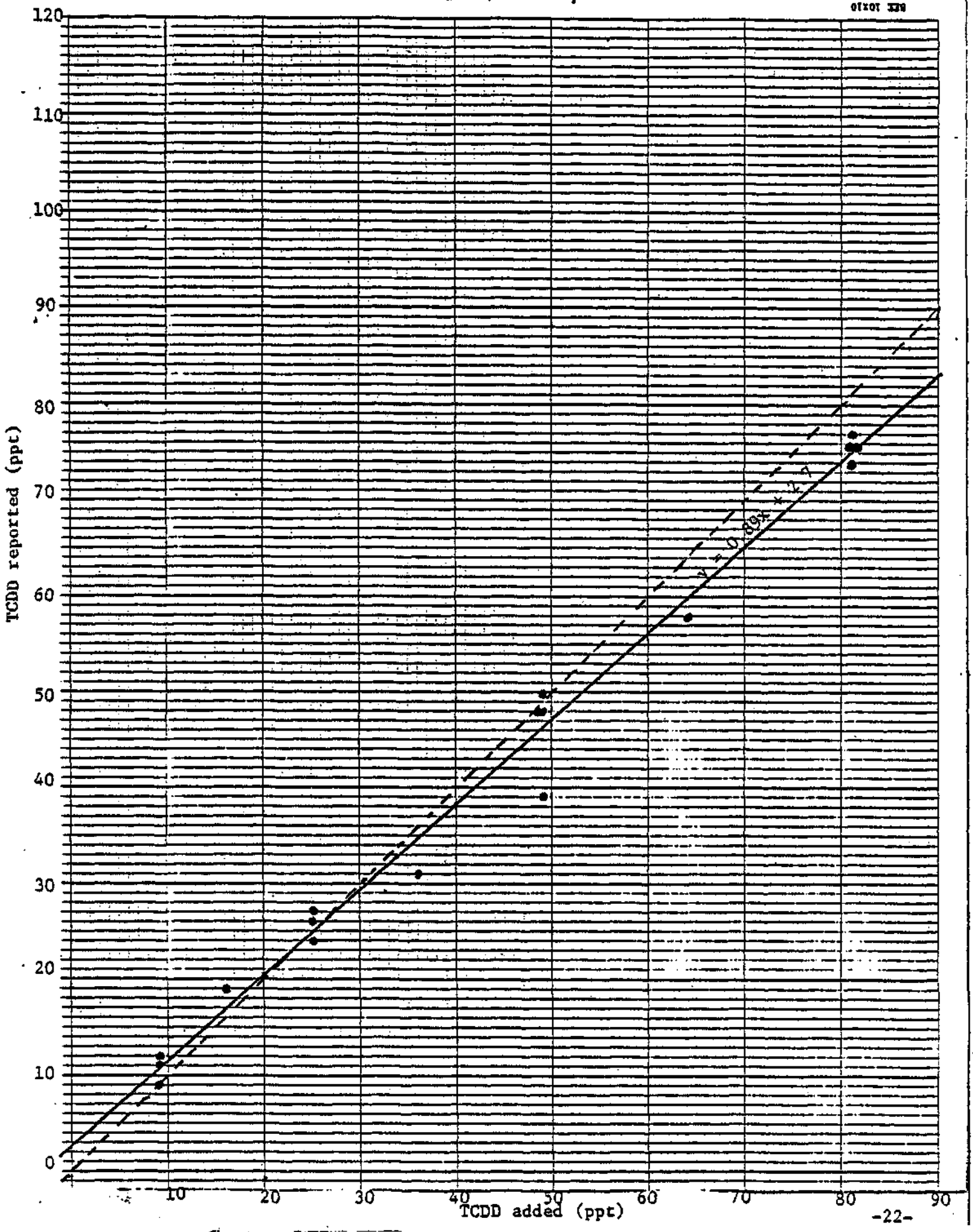


Figure C-6b

Beef Fat (5 gm)
Lab C (322 m/e)
(n = 18)

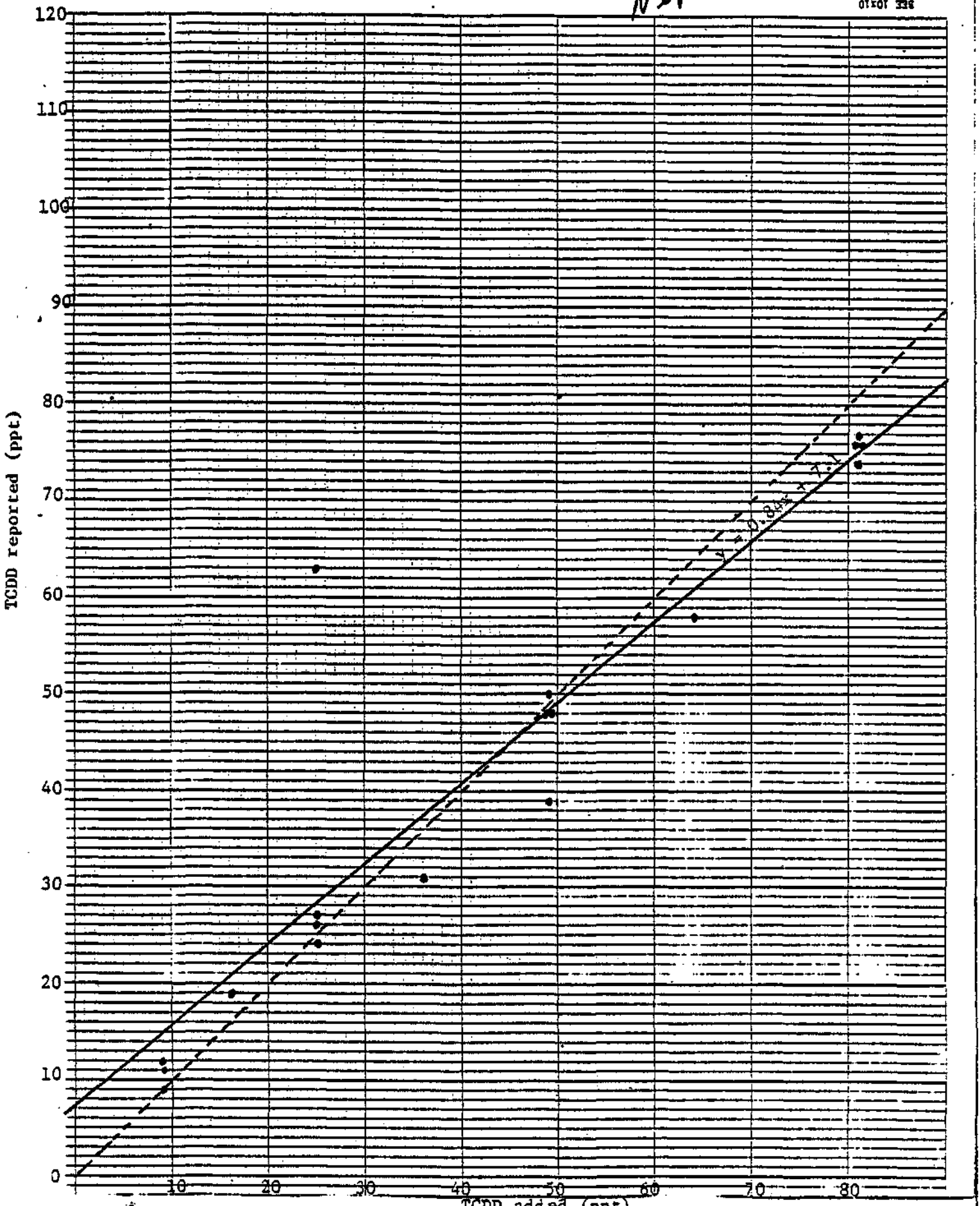


Table C-7.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat (2.5g sample)
Extraction Lab: Lab D; Method: Neutral extraction
Quantitation Lab: Lab C

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37}(%)	Added	320	322	Avg.	320	322
F'-0_1	S-12	41		0		nd			6
G'-0_1	S-1	28		0		nd			6
F'-0.5	S-4	31		0.5		nd			6
F'-1	S-9	37		1		nd			6
F'-4	S-2	29		4		17			6
F'-9_1	S-10	38		9		nd			8
G'-9_1	S-13	42		9		10			5
F'-16	S-3	30		16		12			6
F'-25_1	S-6	34		25		24			5
G'-25_1	S-11	40		25		25			10
F'-36	S-16	45		36		31			6
F'-49_1	S-15	44		49		45			9
G'-49_1	S-8	36		49		70			5
F'-64	S-14	43		64		52			5
F'-81_1	S-5	33		81		76			8
G'-81_1	S-7	35		81		70			3

Figure C-7

Beef Fat Samples
Lab C (322 m/e)
Neutral Extraction, 2.5 gm

Neb

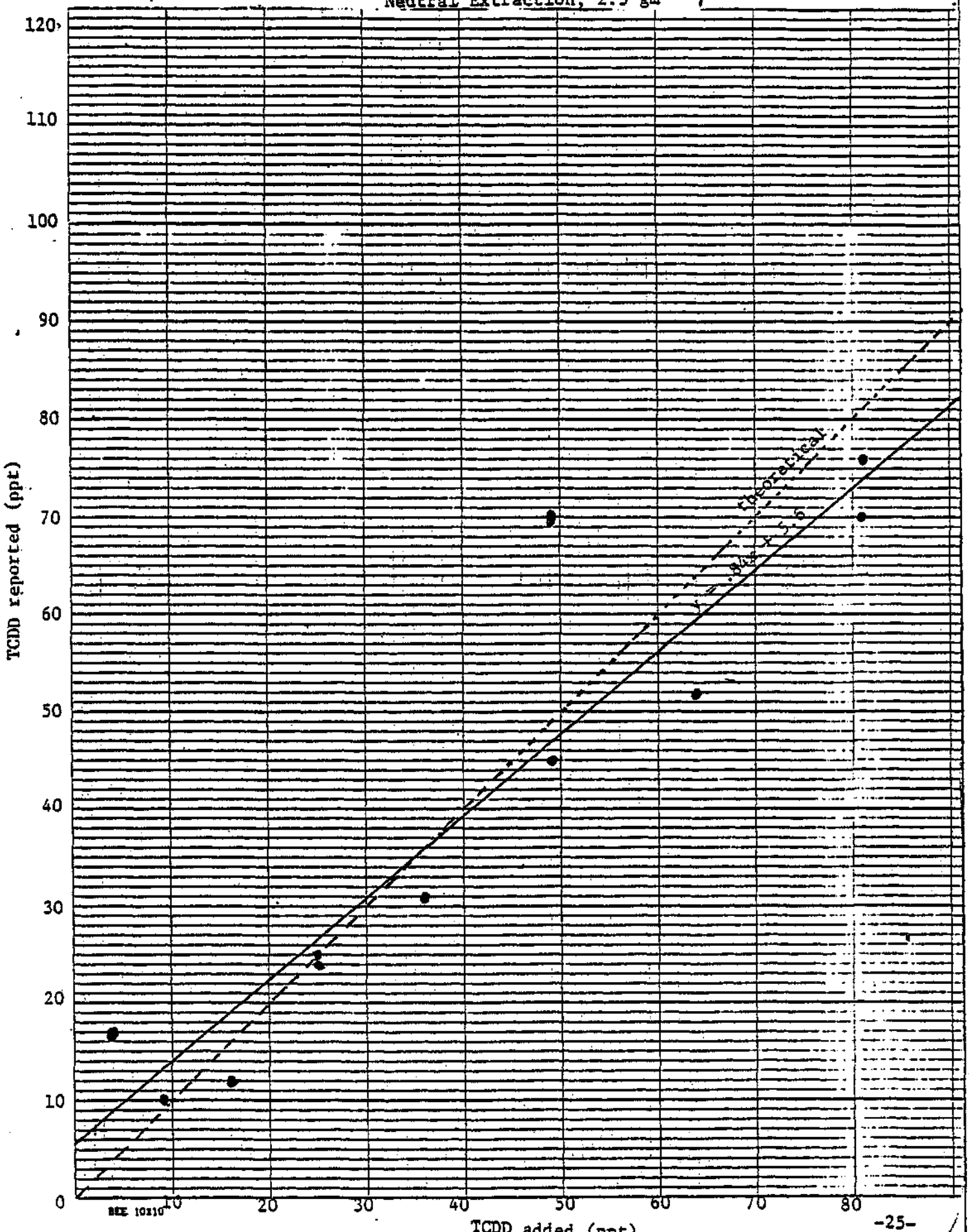


Table C-8.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat
Extraction Lab: Lab D; Method: Neutral extraction
Quantitation Lab: Lab D

Harvard

Sample ID		Ship-	Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	ment	Cl^{37} (%)	Added	320	322	Avg.	320	322
F'-0_1	S-12	41	-	0	nd	nd	nd	23	7.7
G'-0_1	S-1	28	53	0	nd	23	(23)	35	6.9
F'-0.5	S-4	31-1	-	0.5	nd	nd	2.4	16	9.2
		31-2			2.5	2.3		0.8	0.5
F'-1	S-9	37-1		1	nd	-	nd	13	-
		37-2			0.9	nd		0.4	2.2
F'-4	S-2	29	57	4	42	45	44	16	15
F'-9_1	S-10	38	-	9	19	17	18	15	15
G'-9_1	S-13	42-1	81	9	nd	nd	11	16	46
		42-2		9	11	8.5			
F'-16	S-3	30	-	16	25	19	22	9.2	15
F'-25_1	S-6	34	38	25	29	26	28	12	12
G'-25_1	S-11	40	-	25	nd	25	(25)	62	10
F'-36	S-16	45	-	36	32	41	37	8.5	25
F'-49_1	S-15	44	-	49	62	73	68	22	33
G'-49_1	S-8	36	-	49	49	49	49	25	10
F'-64	S-14	43		64	50	54	52	34	8.5
F'-81_1	S-5	33	-	81	-	155	(155)	-	70
G'-81_1	S-7	35		81	89	86	88	24	15

Figure C-8

Beef Fat Samples
Lab D (Avg. of 320, 322 m/e)
Neutral Extraction

Harvard

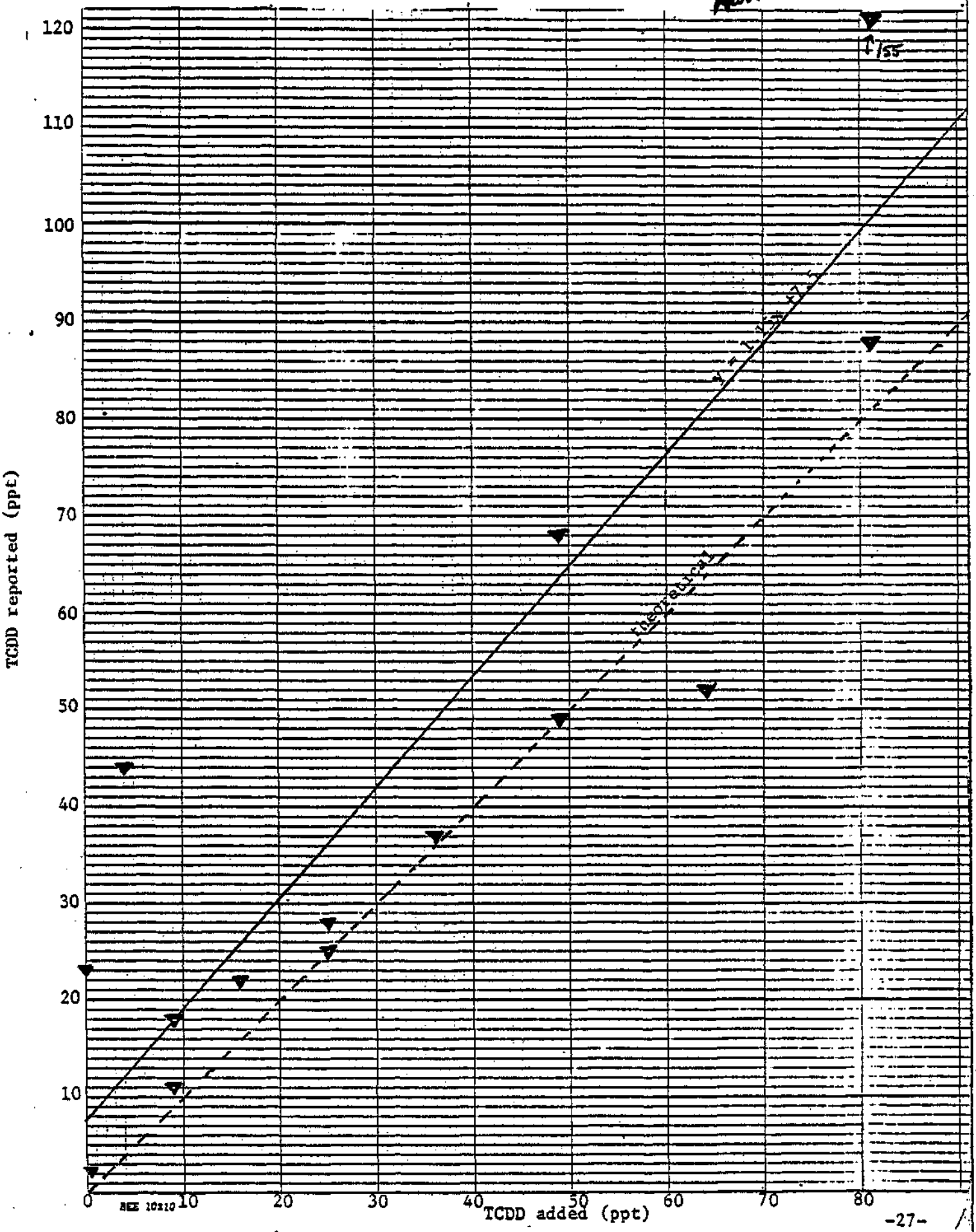


Table C-9.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Beef Fat
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab D Harvard

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37} (%)	Added	320	322	Avg.	320	322
$F\text{-}0_1$	FE	S0-5	-	0	10	nd	nd	3	66
$F\text{-}0_2$	FE	-		0	(nd)	(nd)		(11)	(15)
$G\text{-}0_1$	GB	-		0		nd			6
$G\text{-}0_2$	GB	-		0		nd			6
F-0.5	FG	-		0.5		nd			14
F-1	FC	-		1		nd			10
F-4	FI	S0-4	-	4	13	nd,nd	nd	4	6.1 (3.1)
					(8.3)	nd,36		8.3	5.0 (7.0)
$F\text{-}9_1$	FK	S0-1	83	9	29	16	24	10	4.4
$F\text{-}9_2$	FK	-		9	35	(14)		9.8	(8)
$G\text{-}9_1$	GC	-		9					
$G\text{-}9_2$	GC	-		9					
F-16	FL	-		16					
$F\text{-}25_1$	FD	S0-3	-	25	32	44	37	3.0	5.0
$F\text{-}25_2$	FD	-		25	(34)	(37)		(8.0)	(7.0)
$G\text{-}25_1$	GE	-		25					12
$G\text{-}25_2$	GE	-		25					4
F-36	FA	-		36					6
$F\text{-}49_1$	FH	-		49					
$F\text{-}49_2$	FH	-		49					
$G\text{-}49_1$	GA	-		49					
$G\text{-}49_2$	GA	-		49					
F-64	FB	S0-6	-	64	71	68	74	10	10
					(83)	(73)		(14)	(13)
$F\text{-}81_1$	FJ	S0-2		81	114	143			6
$F\text{-}81_2$	FJ	-		81	(145)	(154)	127	(17)	
$G\text{-}81_1$	GD	-		81	107			10	
$G\text{-}81_2$	GD	-		81	(100)			(17)	

Figure C-9

Beef Fat Samples
Lab D
Acid/Base Cleanup (2.5 gm) Harvard

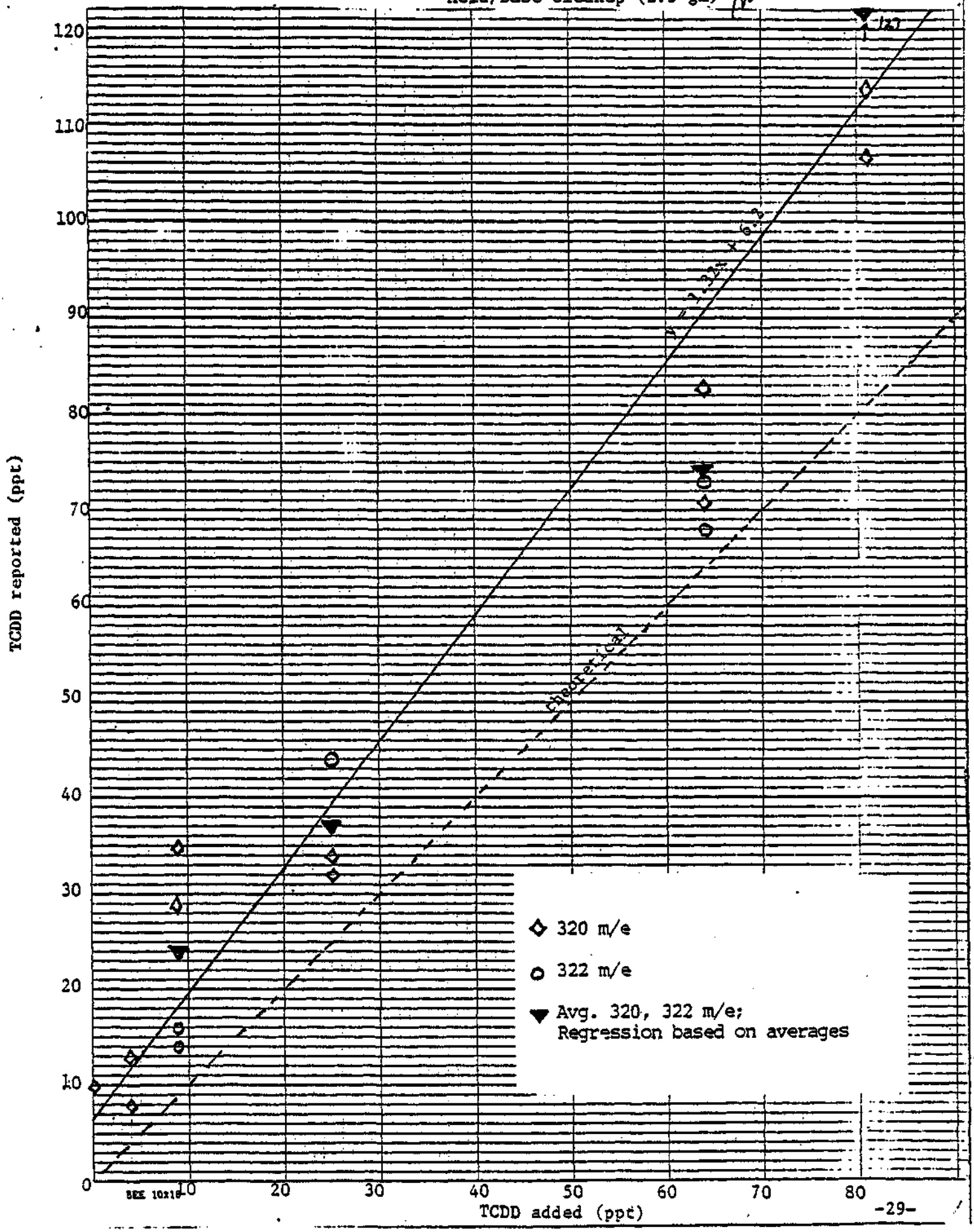


Table C-10.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Human Milk (10 g sample)
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab A W.S.

Sample ID		Ship-	Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	ment	Cl^{37} (%)	Added	320	322	Avg.	320	322
$M\text{-}0_1$	AB	71	103	0		1			1
$M\text{-}0_2$	AB	77	92	0		4			2
$N\text{-}0_1$	BB	58	80	0		nd			2
$N\text{-}0_2$	BB	60	103	0		2			2
M-0.5	AD	54	99	0.5		nd			1
M-1	AE	59	91	1		nd			1
M-4	AF	63	92	4		1			1
$M\text{-}9_1$	AC	53	93	9		3			1
$M\text{-}9_2$	AC	55	89	9		5			1
$N\text{-}9_1$	BC	61	69	9		5			4
$N\text{-}9_2$	BC	64	107	9		4			1
M-16	AG	56	79	16		5			1
$M\text{-}25_1$	AH	70	99	25		15			2
$M\text{-}25_2$	AH	76	90	25		11			2
$N\text{-}25_1$	BD	66	81	25		15			1
$N\text{-}25_2$	BD	74	104	25		7			1
M-36	AI	69	96	36		28			1
M-49	AA	72	110	49		29			1
M-49	AA	78	119	49		29			3
N-49	BA	57	100	49		36			1
N-49	BA	62	98	49		39			1
M-64	AJ	68	85	64		36			2
M-81	AK	67	89	81		31			1
M-81	AK	75	104	81		21			2
N-81	BE	73	136	81		114			2
N-81	BE	79	121	81		24			1

Figure C-10

Human Milk Samples
Lab A (322 m/e) W.S.

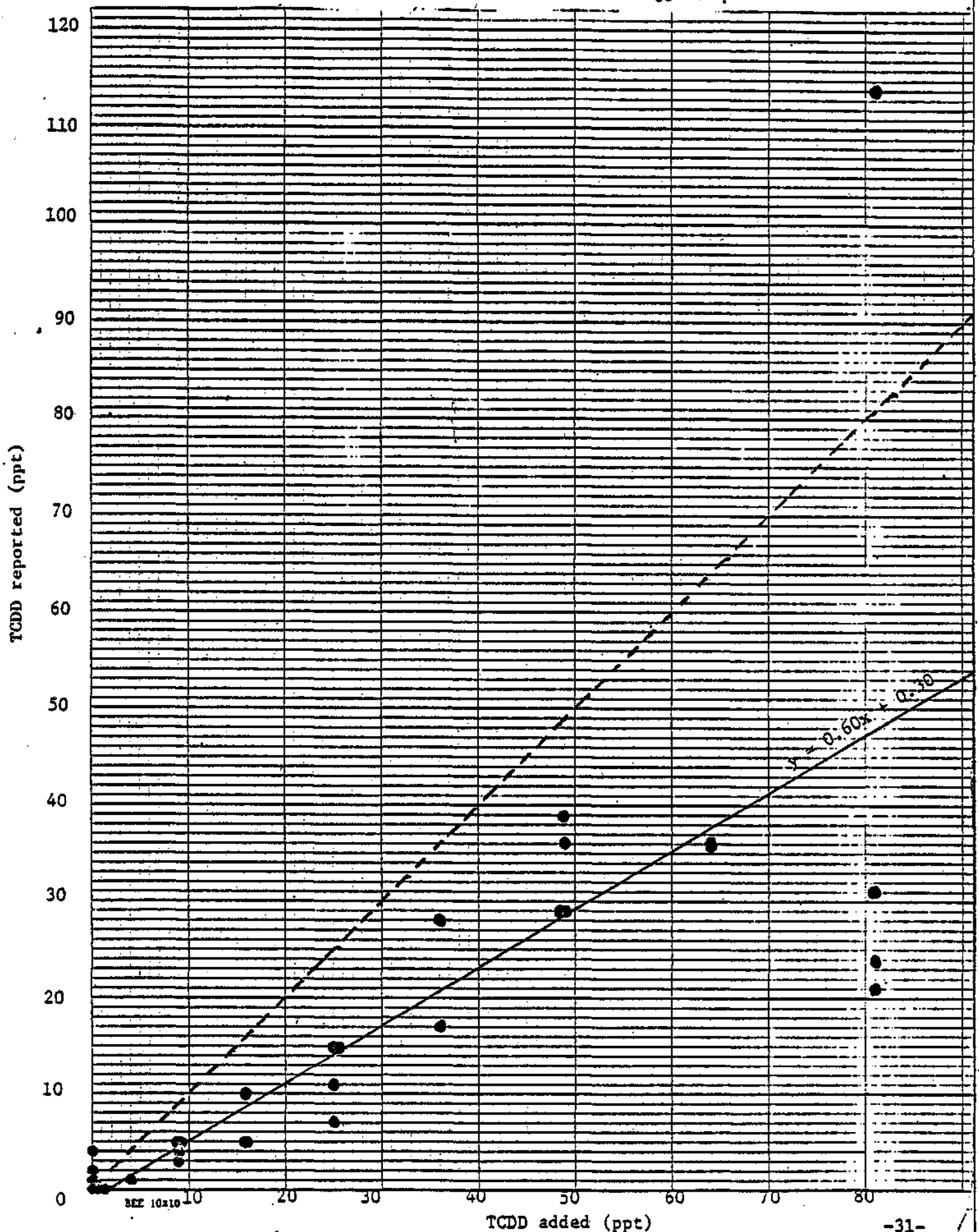


Table C-11.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Human Milk (10 g sample)
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab B

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit	
					Reported				
Study	PML	Shipment	Cl^{37}(%)	Added	320	322	Avg.	320	322
$M\text{-}0_1$	AB	71		0	-	10	(10)	-	2
$M\text{-}0_2$	AB	77		0	-	8	(8)	-	2
$N\text{-}0_1$	BB	58		0	2	17	9.5	2	3
$N\text{-}0_2$	BB	60		0	2	15	8.5	2	3
M-0.5	AD	54		0.5	-	3	(3)	-	1
M-1	AE	59		1	-	7	(7)	-	3
M-4	AF	63		4	nd	4	(4)	2	1
$M\text{-}9_1$	AC	53		9	3	7	5	2	2
$M\text{-}9_2$	AC	55		9	3	7	5	2	2
$N\text{-}9_1$	BC	61		9	2	10	6	2	2
$N\text{-}9_2$	BC	64		9	-	9	(9)	-	2
M-16	AG	56		16	7	9	8	2	2
$M\text{-}25_1$	AH	70		25	7	26	16.5	1	2
$M\text{-}25_2$	AH	76		25	24	35	29.5	4	2
$N\text{-}25_1$	BD	66		25	29	34	31.5	1	2
$N\text{-}25_2$	BD	74		25	-	32	(32)	-	2
M-36	AI	69		36	23	38	30.5	1	2
M-49	AA	72		49	45	69	57	3	2
M-49	AA	78		49	49	53	51	4	2
N-49	BA	57		49	17	21	19	1	2
N-49	BA	62		49	-	10	(10)	-	1
M-64	AJ	68		64	-	82	(82)	-	3
M-81	AK	67		81	-	110	(110)	-	3
M-81	AK	75		81	-	97	(97)	-	2
N-81	BE	73		81	-	110	(110)	-	2
N-81	BE	79		81	-	96	(96)	-	2

Figure C-11a

Human Milk Samples
Lab B (322 m/3) Dow.

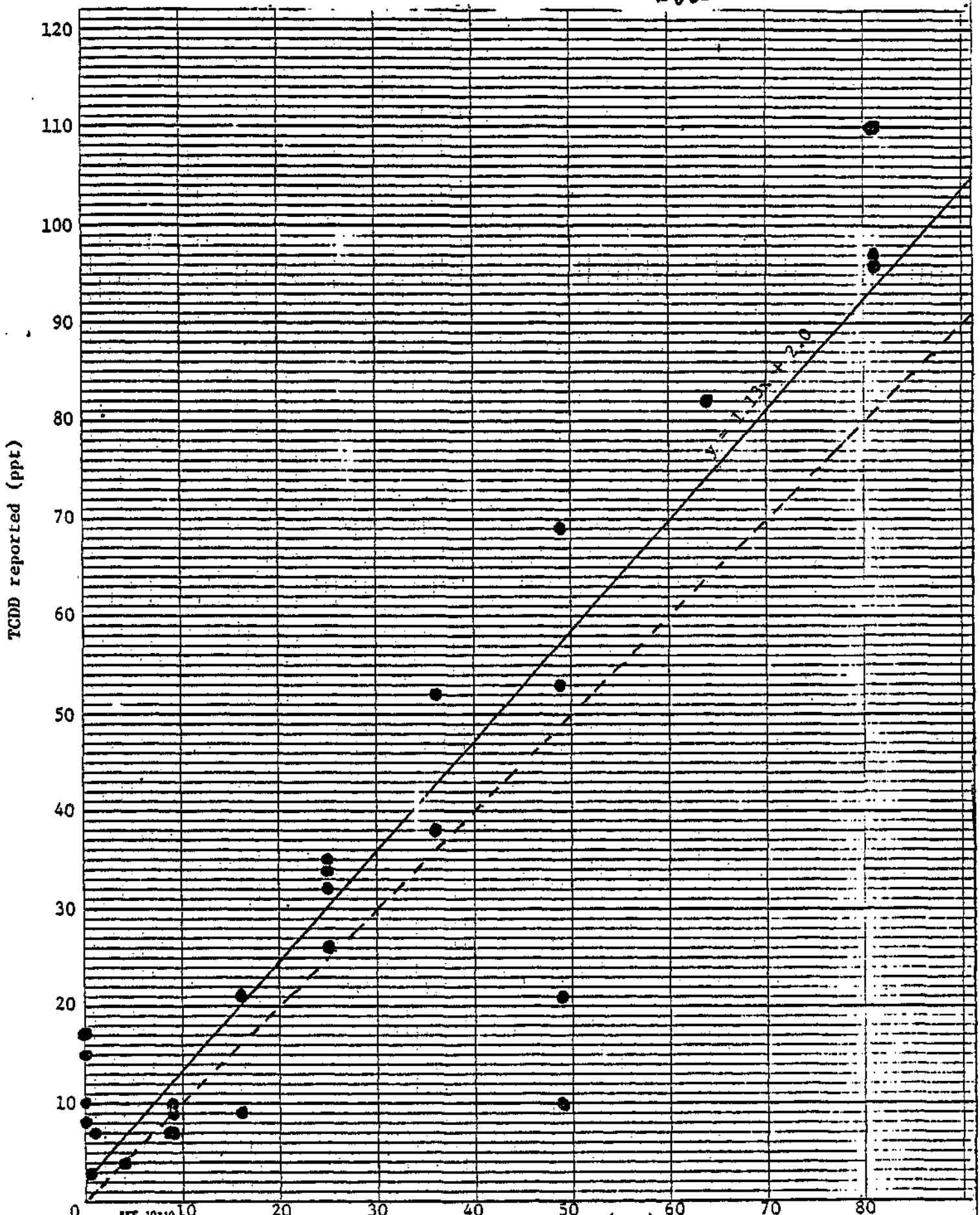


Figure C-11b

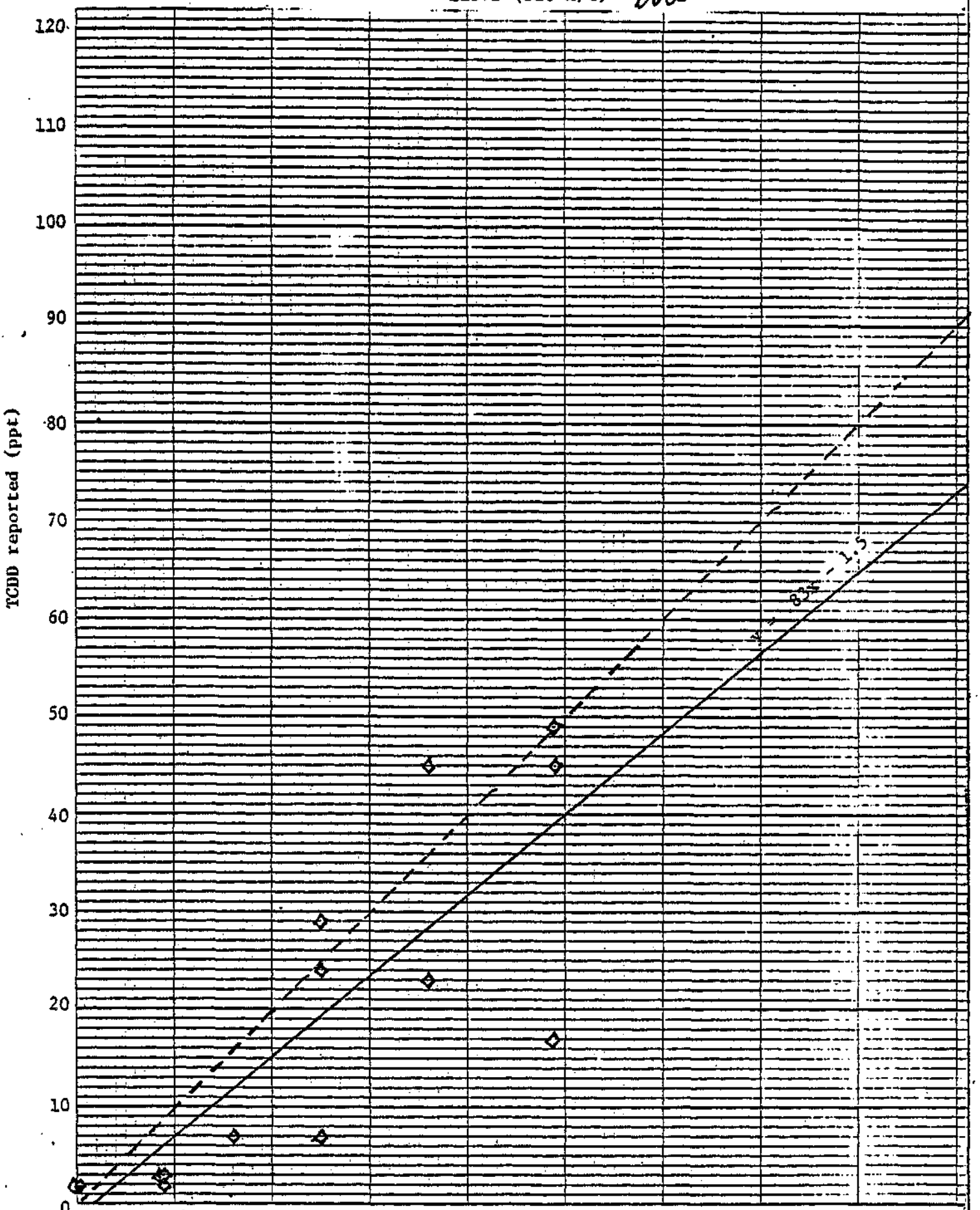

Human Milk Samples
Lab. B (320 m/e) Dow
TCDD reported (ppt)
TCDD added (ppt)
0
10
20
30
40
50
60
70
80
90
100
110
120
10
20
30
40
50
60
70
80
85%

Table C-12.

Dioxin Phase II: Interlaboratory Quantitation Study

Type of Sample: Human Milk + 1 standard
Extraction Lab: PML; Method: Acid/base
Quantitation Lab: Lab E RTP

Sample ID			Recov.	TCDD Levels (ppt)				Detection Limit Avg.	
					Reported				
Study	PML	Shipment	Cl^{37}(%)	Added	320	322	Avg.	320	322
$M\text{-}0_1$	AB	HMT-3	100+	0			1.5		0.3
$M\text{-}0_2$	AB	-10	66	0			0.6		0.1
-	A2	-6	95	0.2			9		1
M-4	AD	-4	64	0.5			0		0.2
-	AI	-9	100	0.8			0.9		0.3
M-1	AE	-11	100	1			1.4		0.4
M-4	AF	-2	77	4			6		0.6
$M\text{-}9_1$	AC	-1	100+	9			14		3
$M\text{-}9_2$	AC	-7	100+	9			5.5		1
M-16	AG	-5	100+	16			29		2.1
M-25	AH	-8	100+	25			34		2
S-1	STD-1	-12	100	1			1.9		0.2

— / BK

Figure C-12

Human Milk Samples

Lab E (Avg. of 320, 322 m/e)

RTP

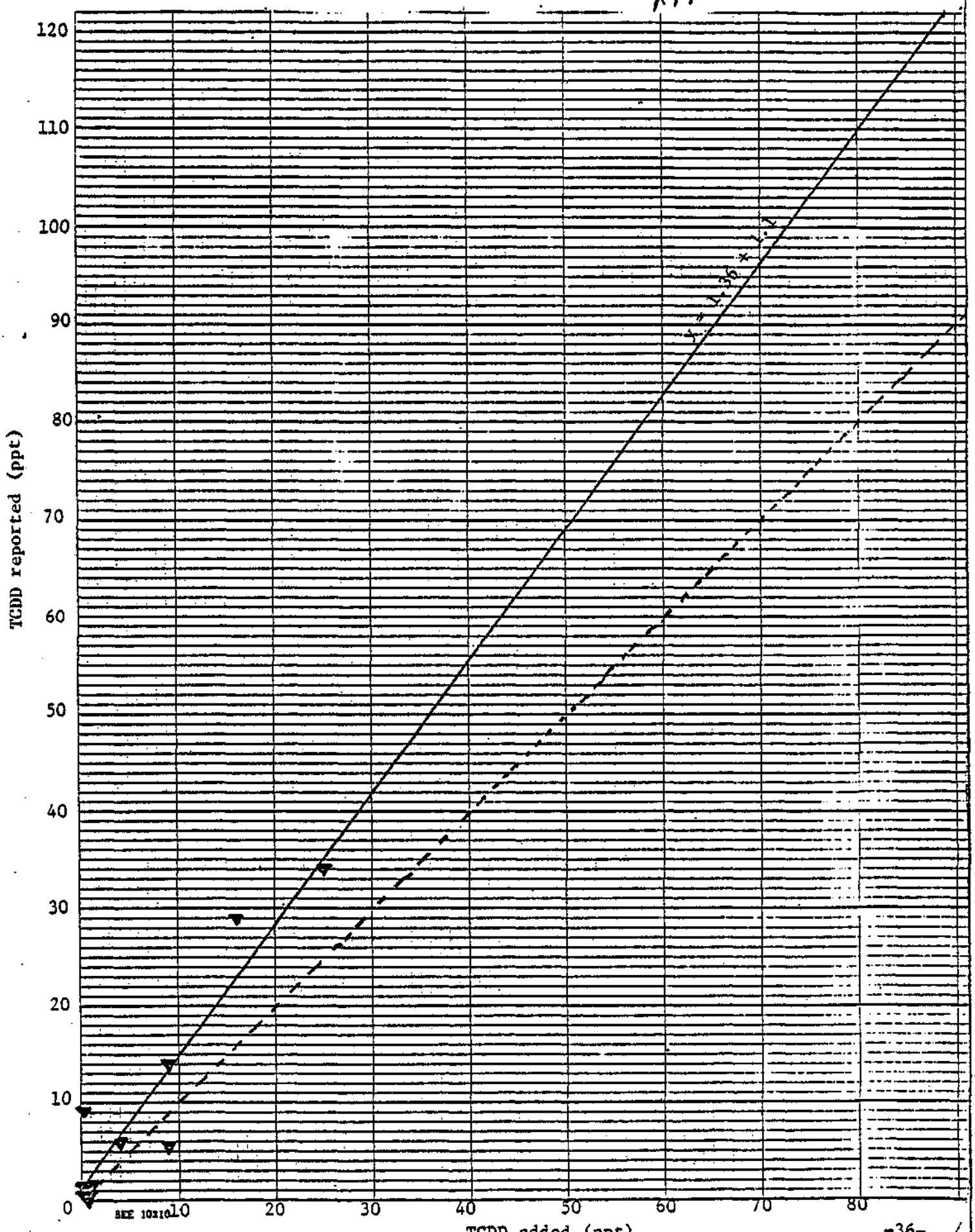


TABLE C-13.

Types of "Valid" and "Erroneous" Values

Reported for Spiked Samples

Laboratory Report	Spiking Category		
	Sample Not Spiked	Sample Spiked	
		S < DL 1/	S ≥ DL 2/
not detected (nd)	valid	valid	false nd (fnd)
positive value	False Positive (FP)	false positive (fp)	valid

1/ Spiking level less than the detection limit.
2/ Spiking level greater than or equal to the detection limit.

TABLE C-14.

Incidence of Reporting Errors[1/] for Standards

Number of Measurements (n) and Errors, by Types

Lab	m/e	Sample Not Spiked (n)	Sample Not Spiked FP	Sample Spiked: Spike < 9 ppt (n)	Spike < 9 ppt fp	Spike < 9 ppt fnd	Sample Spiked: Spike ≥ 9 ppt (n)	Spike ≥ 9 ppt fp	Spike ≥ 9 ppt fnd
A	322	(4)	1	(3)	0	0	(19)	0	2
B	320	(4)	1	(2)	0	0	(19)	0	0
B	322	(4)	1	(2)	0	0	(19)	0	0
C	322	(4)	0	(3)	0	0	(19)	0	0
D	-	(0)	-	(0)	-	-	(0)	-	-
	Avg:								
E	320, 322	(0)	-	(1)	0	0	(0)	-	-
Totals:		(16)	3	(11)	0	0	(76)	0	2

1/ See Table C-13 for error definitions

TABLE C-15.

Incidence of Reporting Errors[1] for Beef Fat Samples[2]

Number of Measurements (n) and Errors, by Type

Lab	m/e	Sample Not Spiked (n)	Sample Not Spiked FP	Sample Spiked: Spike < 9 ppt (n)	Spike < 9 ppt fp	Spike < 9 ppt fnd	Sample Spiked: Spike ≥ 9 ppt (n)	Spike ≥ 9 ppt fp	Spike ≥ 9 ppt fnd
A	322	(4)	2	(3)	2	0	(19)	0	1
B	320	(4)	3	(3)	2	0	(19)	0	0
	322	(4)	4	(3)	2	0	(19)	0	0
C	322	(4)	0	(3)	0	0	(19)	0	0
C	(NE)[2] 322	(2)	0	(3)	1	0	(11)	0	1
D	(NE) 320	(2)	1	(8)	2	0	(14)	1	0
D	(NE) 322	(2)	1	(7)	3	0	(13)	1	0
D	320	(2)	1	(2)	1	0	(10)	2	0
D	322	(2)	0	(4)	0	0	(8)	0	0
E	-	(0)	-	(0)	-	-	(0)	-	-
Totals:									
	Acid/base	(20)	10	(18)	7	0	(94)	2	1
	NE	(6)	1	(18)	6	0	(38)	2	1

1/ See Table C-13 for error definitions
2/ NE denotes neutral extraction; otherwise, acid/base cleanup utilized

TABLE C-16.

Incidence of Reporting Errors[1] for Human Milk Samples[2]

Number of Measurements (n) and Errors, by Types

Lab	m/e	Sample Not Spiked (n)	Sample Not Spiked FP	Sample Spiked: Spike < 9 ppt (n)	Spike < 9 ppt fp	Spike < 9 ppt fnd	Sample Spiked: Spike ≥ 9 ppt (n)	Spike ≥ 9 ppt fp	Spike ≥ 9 ppt fnd
A	322	(4)	3	(3)	0	1	(19)	0	1
B	320	(2)	2	(1)	0	1	(11)	0	0
	322	(4)	4	(3)	2		(19)	0	0
C	-	(0)	-	(0)	-	-	(0)	-	-
D	-	(0)	-	(0)	-	-	(0)	-	-
	Avg:								
E	320, 322	(2)	2	(5)	0	1	(4)	0	0
Totals:		(12)	11	(12)	2	3	(53)	0	0

1/ See Table C-13 for error definitions
2/ All extractions utilized acid/base cleanup

D. Statistical Analysis of Laboratory C Beef Fat and Standard Reports

For practicality, a detailed statistical analysis of analytical results is presented only for Laboratory C in order to determine the optimum known accuracy and precision that can currently be achieved in quantifying low ppt levels of TCDD in samples of the types analysed. (Complete statistical analyses of the results of other laboratories can be conducted if determined advisable.) Laboratory C quantified only standards and beef fat samples; therefore, the exact reliability of the analytical method for human milk is currently speculative.

Two types of upper and lower 95% confidence limits have been calculated for the regressions of reported values (Y) on spiking levels (X), as shown for standards in Figure D-1 and for beef fat in Figures D-2 and D-3. First are the 95% confidence limits for the line itself, as are graphed by the pairs of lines closest to the regression line in the above Figures. These limits are interpreted as follows: The true regression line (as would be determined if the experiment were repeated a countless number of times under the same conditions) lies within the confidence limits unless the test results are sufficiently unusual to be among those expected to occur less than 5% of the time.

The second set of confidence limits, depicted by the pair of lines furthest from the regression line, predict the 95% confidence limits for the result of a single analysis at a particular spiking level. Interpretation is as follows: The result (reported value) of a single analysis of a standard or beef fat sample spiked at a given level can be predicted to fall between the 95% confidence lines unless the analytical result (which includes extraction as well as GC-MS quantitation) is one sufficiently unusual to be expected to occur approximately 5% of the time.

In calculating the regression lines and confidence limits, values of "nd" have been excluded. For Lab C all spiking levels below 9 ppt were reported as nd, and therefore, the lower limit of quantitation in this study must be considered to fall somewhere between 5 and 9 ppt. Lab C gave no erroneous reports (i.e., no reports classified as FP, fp or fnd) for standards or for 5g beef fat samples when extraction utilized acid/base cleanup.

The calculated regression line for standards lies very close to the theoretical line, the slope of 0.983 being essentially equal to the theoretical slope of 1 and any point on the line being from 1 to 2 ppt less than the spiking level (Figure D-1). The 95% confidence limits for a predicted result of a single analysis fall only 6 to 7 ppt above and below the regression line. Thus, accuracy can be expressed as a negative

bias averaging about 2 ppt over the range of levels tested, and precision in terms of the confidence limits for the line and for predicted results of individual standards. There was no apparent tendency for increased variability among reported values at higher (or lower) spiking levels, i.e., variance about regression was apparently independent of the spiking level (See Table C-3 to compare values).

The results of the beef fat analyses were slightly more variable than those for standards, as might be expected. In particular, one value was an apparent outlier (reported value 63 ppt; spiking level 25 ppt) and has been both excluded (Figure D-2) and included (Figure D-3) in calculations. The rationale for excluding the value is based on a discussion with the principal investigator at Lab C; he was reasonably certain that on-the-spot calculation of separate measurements of the same GC-MS run would have revealed a discrepancy and the sample would have been rerun. Thus, exclusion of the value assumes a laboratory procedural modification to eliminate the possibility of reoccurance. The reported value of the sample's duplicate was 24 ppt. (A second sample--that spiked at 64 ppt--was originally reported as 32 ppt. Recalculation without knowledge of the spiking level revealed an arithmetic error, resulting in a revised value of 58 ppt, which has been used in calculations.)

Figure D-1

Standards
Lab C (322 m/e)

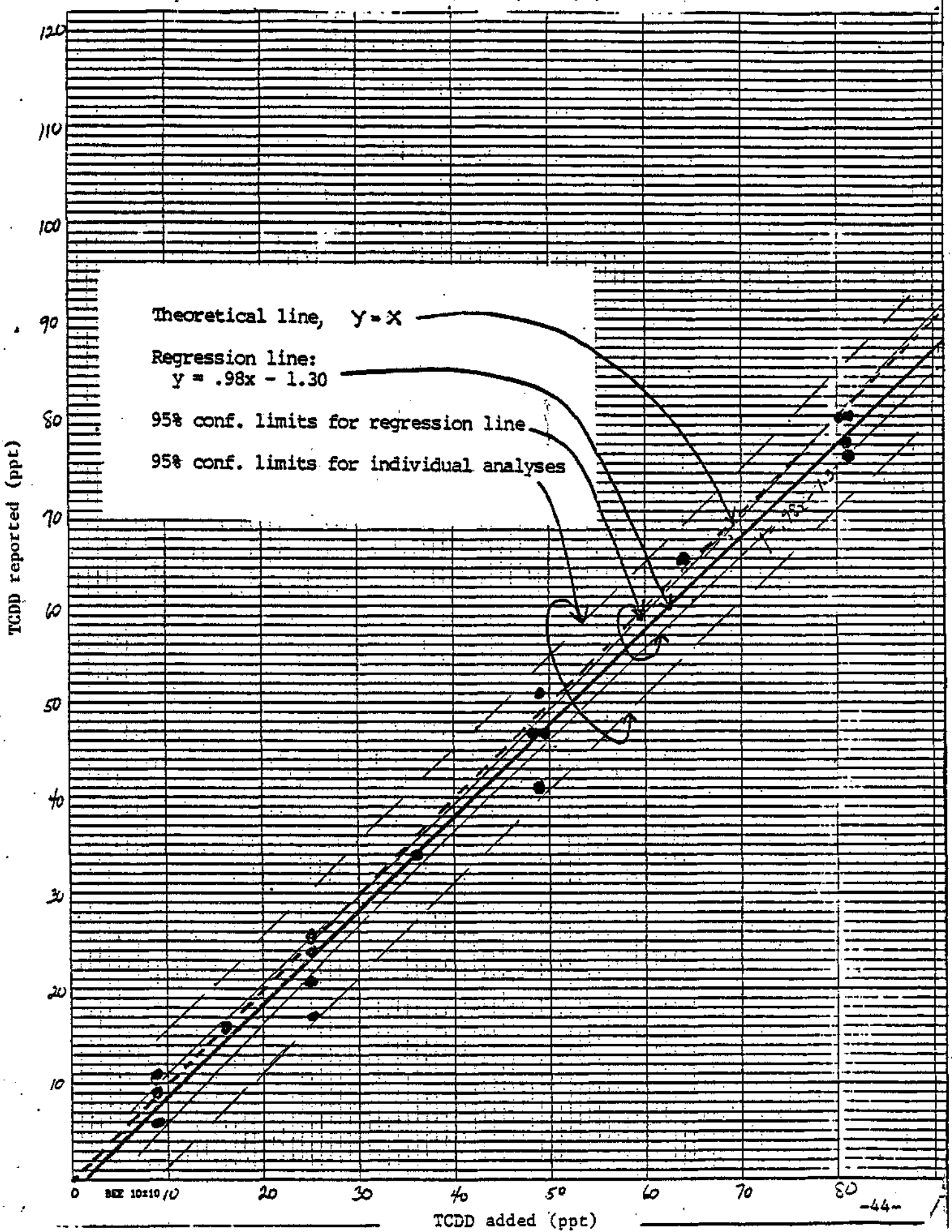


Figure D-2

Beef Fat Samples, Lab C (n = 17): 5g, acid/base, 322 m/e

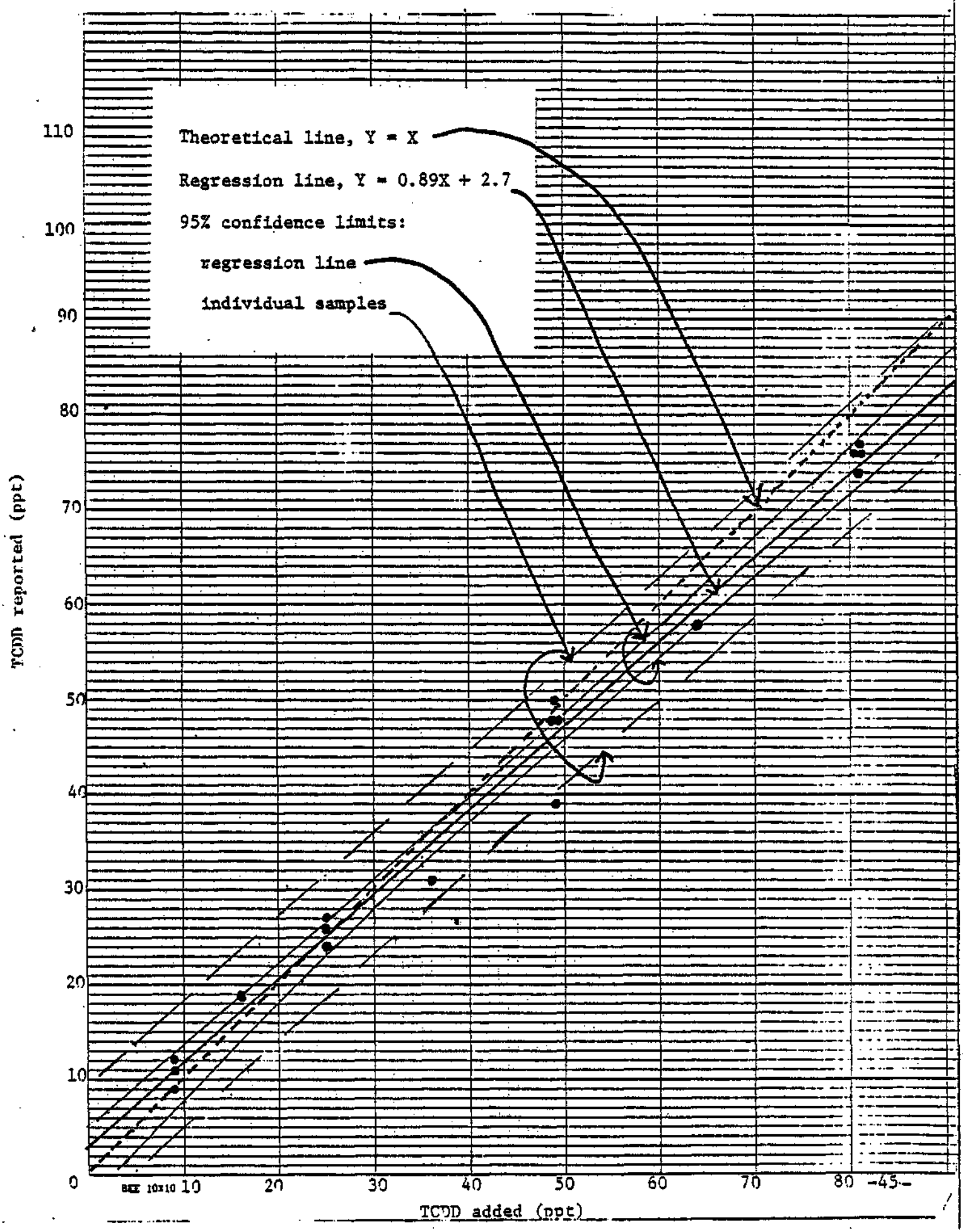
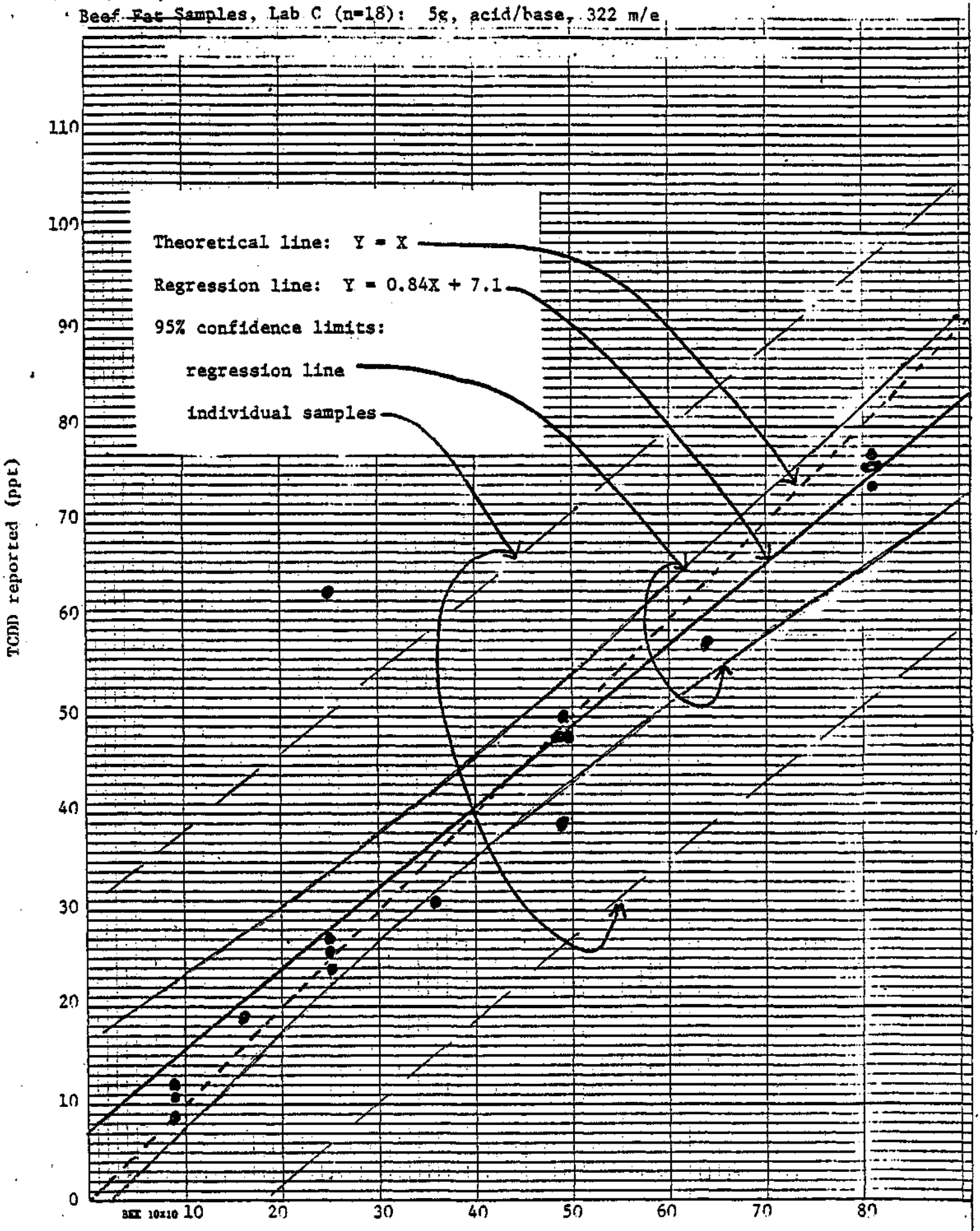


Figure D-3

Beef Fat Samples, Lab C (n=18): 5g, acid/base, 322 m/e



E. Estimation of Actual TCDD Levels, with Confidence Limits, from Spiking Results: Lab C

Using regression statistics developed from spiking study data, a "best estimate" of the "true" level of TCDD in an unspiked sample can be derived from the reported level, as well as statistical confidence limits for that estimate. The procedure is that of estimating the value of the independent variable ($\hat{X}$) for a measurement of the dependent variable (Y), in this case the reported TCDD level in a sample. (The basic approach is used, for example, in estimating LD_{50}'s in dose-response studies.) Confidence limits for such estimates tend to be broad relative to these for the regression line per se.

Figures E-1 and E-2 present estimated "true" values of TCDD ($\hat{X}$) for reported values (Y) ranging from approximately 9 to 80 ppt. In both figures the reported values (Y) now appear on the horizontal axis and the estimated "true" values ($\hat{X}$) on the vertical axis. The slope of the new line is the reciprocal of the slope of the regression of Y on X. The new regression equations appear on the graphs.

The 95% confidence limits for estimates of actual values range from 6 to 7 ppt above and below the predicted value when the aberrant value of 63 ppt is eliminated from the

calculations (Figure E-1) (see part D). When that value is included, the confidence limits are about four times as wide (Figure E-2). For example, the estimated actual value for a reported value of 40 ppt is 42 ppt with 95% confidence limits of 35 to 49 ppt when the aberrant 63 ppt has been excluded from regression calculations. When included, the estimated true level for the same reported value (40 ppt) is 39 ppt with confidence limits of 12 to 66 ppt. Again, the essentiality of developing procedures to detect and correct aberrant results at the onset is emphasized.

The confidence limits presented in the above graph are for a single extraction and GC-MS quantitation of a sample. Confidence limits can be narrowed if 2 or more independent extractions and quantitations of the cause sample are performed and the reported values averaged. This approach may be of value in applied TCDD residue evaluations.

Figure E-1

Estimated True TCDD Level in Beef Fat: Lab C
(From regression analysis of 17 spiked samples)

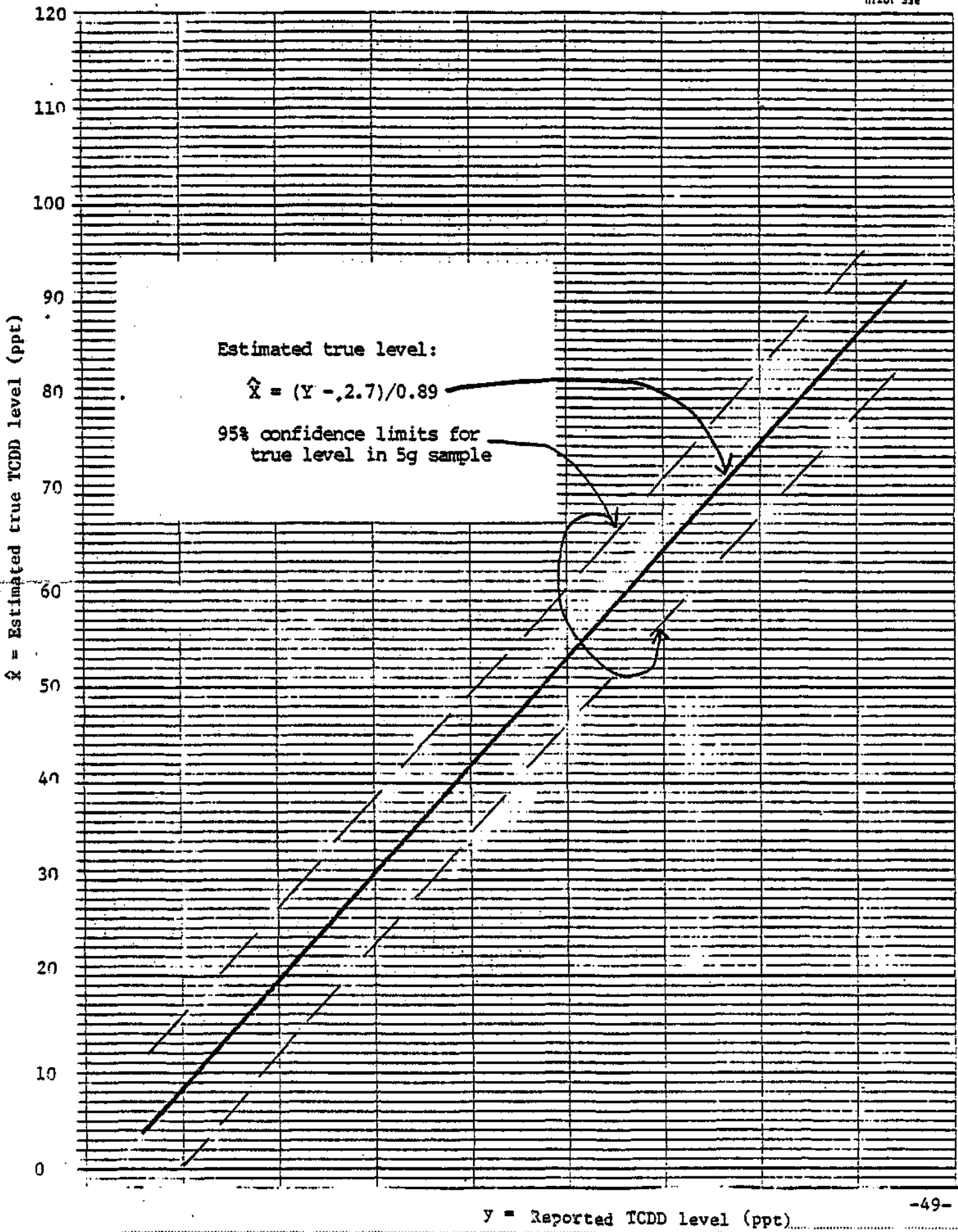
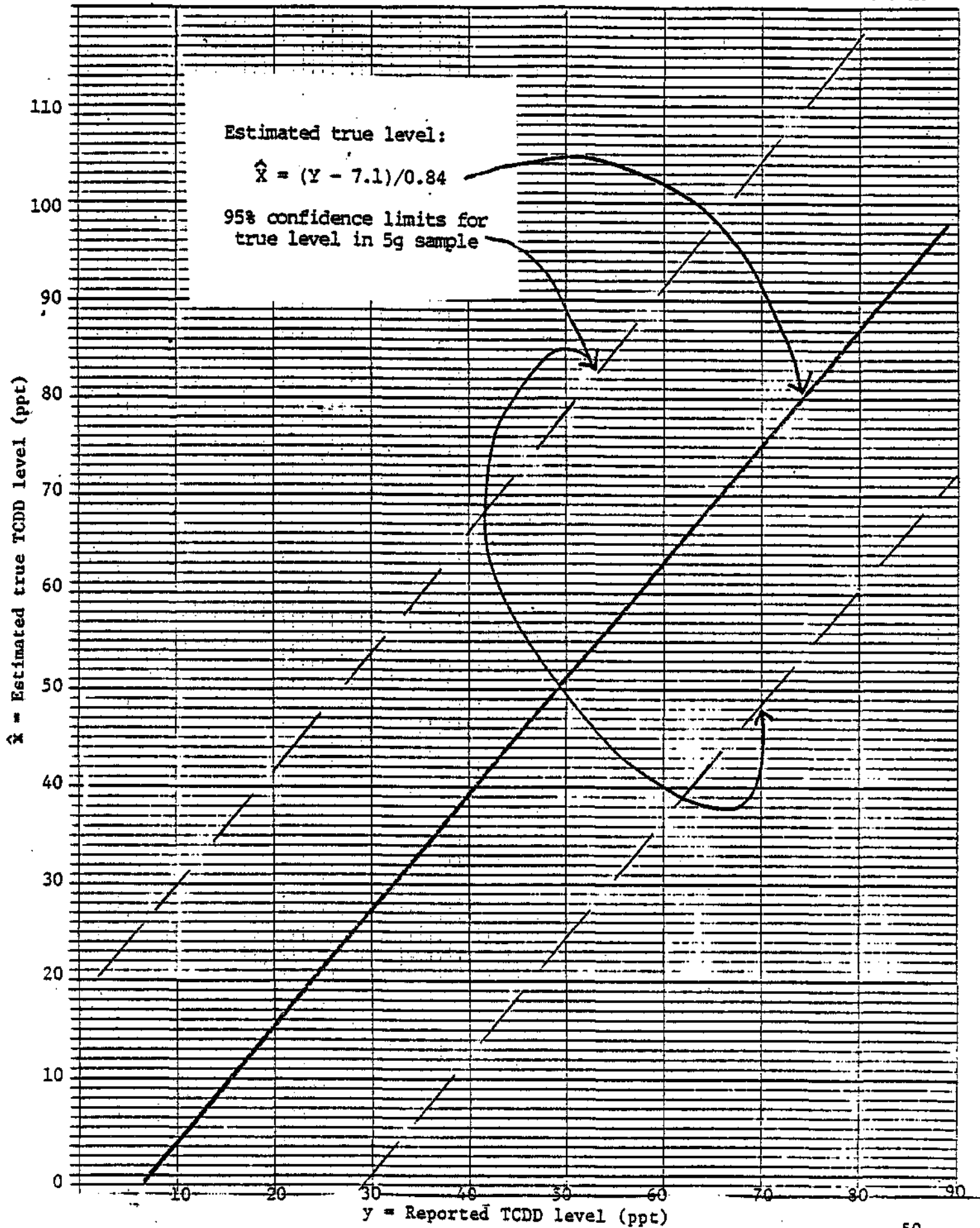


Figure E-2

Estimated True TCDD Level in Beef Fat: Lab C
(From regression analysis of 18 spiked samples)



F. Discussion

This study has demonstrated that for standards and spiked samples of beef fat, the extraction and quantitation methodology exist to quantify TCDD at levels as low as 9 ppt with practicable accuracy and precision. This conclusion assumes that extraction methods are exactly those used at PML and quantitation utilizes procedures and instrumentation indentical or equivalent to that of Lab C. Otherwise, practicable precision has not been fully demonstrated.

The reliability of the above methodology for quantifying TCDD in human milk is yet to be determined. However, based on the fact that milk results are essentially as precise as those for beef fat among laboratories that performed both sets of analyses, a quantitation problem is not anticipated. None-the-less, the procedure needs to be verified with further testing.

Lab C has indicated that their instrumentation may be capable of quantifying TCDD levels below 9 ppt in samples of the type used in the study. Reports of False Positives (positive TCDD values reported for unspiked samples) by laboratories other than Lab C may present a basic problem when attempting to quantify in the range of 0 to possibly 8 ppt. Additional analyses of spiked samples are necessary to determine if a quantification level below 9 ppt can be achieved.