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Study of the bioavailability in the rabbit of the TCDD present in powdered soil from Seveso Zone A (Milan)*

A.Bonaccorsi[1], A.di Domenico[2], R.Fanelli[1], F.Merli[2], R.Motta[1], R.Vanzati[2], G.Zapponi[2]

1 Istituto di Ricerche Farmacologiche "Mario Negri", Milan

2 Laboratory of Comparative Toxicology and Ecotoxicology, Supreme Health Authority, Rome

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1 INTRODUCTION

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the most toxic of the polychlorodibenzodioxins and is, as a rule, an unwanted micro-contaminant of 2,4,5-trichlorophenol, produced on a large scale by industry. TCDD has also been formed accidentally in relatively high quantities in a few accidents at chemical plants, including the one at the ICMESA chemical plant (Seveso, Milan) in July 1976. This event led to diffuse TCDD contamination of the environment whose gravity is still visible 5 years later (1).

Free TCDD has been the subject of numerous toxicological studies, mostly designed to characterise its toxic properties rather than to assess its absorption by the organism (2). However, exposure to the poison in the environment is often due to the presence of environmental substrates to which TCDD is in some way bound; more rarely is the compound found in the free form. Thus the present study arose from the need to establish the bioavailability of TCDD when the substance is administered to the rabbit on powdered soil and not in the free form. Since previous work had shown that the liver is the target organ in that species (3), accumulation of TCDD in the liver was taken as index of absorption. The various formulations containing TCDD were administered by gastric tube. The extent of bioavailability may be of importance in an assessment of the toxicological risk.

2 METHODS OF ANALYSIS

The determination of TCDD in particulate soil was done by means of a known GC-MS method (4) adapted for small samples (~2 g) and using the following steps: extraction in a Soxhlet apparatus,

chromatography on a multilayer column (described later) and chromatography on a column of activated alumina.

The determination of TCDD in liver tissue was done by a GC-MS method described in the literature (5), using the following steps: alkaline digestion, extraction with a solvent, chromatography on an Extrelut column and chromatography on a column of activated alumina.

As shown in Table 1, recovery by the method as a whole and by two purification steps is on average always more than 90% in the case of powdered soil samples. In the case of liver samples recovery calculated with the addition of a tracer is 80%. For further details see Appendix.

3. PREPARATION OF THE FORMULATIONS

10 kg of dry agricultural soil was removed from an area in Zone A with a high level of contamination (mean TCDD >3000 $\mu g/m^2$). The stone-free material was spread on plastic sheets for drying in the air (t = 25-30°C) to constant weight (weight variations <2% over 24 h), crushed vigorously, remixed and sieved for collecting the powder fraction with a particle size of between 200 and 400 mesh. This fraction (~ 0.5 kg) was further mixed in a rotary evaporator at a moderate speed for about 10 h to obtain a product with a high degree of homogeneity. The results of ten analyses on as many portions of powder (on average ~2 g each) taken at random revealed a mean contamination of 81 $\pm$ 8 ppb (Table II). The standard deviation value denotes a satisfactorily homogeneous distribution of the contaminant. Portions of this powder (2.00 $\pm$ 0.05 g each) were administered to rabbits (see Treatment of the rabbits).

TABLE I

Mean recoveries of TCDD for the methods of analysis used and for two specific purification operations.[1]

Subject	Number of data	Mean recovery (%)
Multilayer column	71	96±12
Alumina column	22	94± 5
Soil 1[2]	20	92±15
Soil 2[3]	16	92± 8
Soil 3[4]	31	94± 7
Hepatic tissue[5]	57	80±10

1 For further details and for the individual data on each subject see Table "Recoveries of TCDD" (Appendix).

2 Laboratory-contamined soil analysed within 48 h of addition of TCDD.

3 Laboratory-contaminated soil analysed after "aging" (1 month).

4 Yield of TCDD (Cl^{37}) added to uncontaminated particulate soil and to Seveso particulate soil before extraction, in the range 10–100 ppb.

5 Yield of TCDD (Cl^{37}) added to the sample of hepatic tissue before alkaline digestion, in the range 0.5–5 ppb.

TABLE II

TCDD levels determined in ten samples of powdered soil from Zone A after sieving and homogenisation.

Sample	Sample weight g	Recyclings	Recovery[2] %	TCDD found ng	TCDD found ppb
1	2.15	174	93.0	203	94.4
2	1.80	96	103.5	159	88.3
3	2.06	131	99.5	180	87.4
4	2.11	94	95.7	173	82.0
5	1.92	160	80.0	157	81.8
6	2.32	173	90.0	186	80.2
7	2.00	141	110.0	159	79.5
8	1.89	122	107.5	146	77.3
9	2.00	100	94.5	139	69.5
10	2.00	91	101.5	137	68.5

Mean TCDD[3] concentration and its standard deviation:

81 ± 8 ppb

1 Number of refluxings for the extraction in a Soxhlet apparatus.

2 Calculated on the quantity of tracer (RCDD(Cl^{37})) added to the soil before extraction.

3 No correction was made for losses due to the method of analysis because of the high recoveries ($\bar{r}$>97%).

10 kg of soil presumably free from TCDD but morphologically similar to that of Zone A was treated as described in the preceding section to get ~1 kg of powder (200-400 mesh). Analysis of five samples (~2 g each) revealed no TCDD at an analytical sensitivity of 0.6 ppb. This powdered soil was administered to rabbits as such in the tolerance tests (see Treatment of the rabbits). One group of accurately weighed portions (1.00±0.03 g each) of the same powder was spiked with TCDD in an acetone solution (<3 ml/portion) at a level of 10.0±0.2 or 40±1 ppb); the portions were left to "age" in the dark at 20°C for over 1 month before administration to the rabbits. A second group of portions of powdered soil was contaminated (40±1 ppb) 48 h before administration to the rabbits, sufficient time for the solvent to evaporate. Each portion (or the combination of two) was the single daily dose intended for each animal. Some samples of each group were used for testing the recovery in the critical steps of the analysis method.

The following were prepared: two solutions of TCDD in an acetone-vegetable oil mixture (1:6) containing 10.0±0.2 and 40.0±0.8 ng/ml of poison respectively, and a water-alcohol solution (1:1) containing 40.0±0.8 ng/ml. The single daily doses intended for each animal were 1.00±0.01 and 2.00±0.01 ml according to case.

4 TREATMENT OF THE RABBITS

Before treatment of the animals started, tests were done to find out whether the repeated administration of given quantities of powdered soil could cause digestive tract disturbances that might affect the absorption of TCDD from the gut. The tolerance tests

were done on six rabbits, each of which received a suspension of powdered soil (2.0 g) in water (10 ml) by gastric tube daily for 7 days. The animals presented no ill-effects, apart from a brief interruption of weight gain on the first day of treatment. By the second day the bodyweight growth curve had already resumed its normal trend.

The previously described preparations were dosed by gastric tube for 7 consecutive days to groups of 5-7 male white rabbits of mean starting weight 2.4±0.2 kg (Fig. 1), caged singly. In all, 12 groups of animals were treated with the following preparations (daily doses in brackets): a) powdered soil from Zone A, Soil S (~80-~160 ng daily); b) "aged" laboratory-contaminated soil, Soil 2 (20-40-80 ng daily); c) laboratory-contaminated powder, Soil 1 (40-80 ng daily); d) acetone-oil solution (20-40 ng daily); water-alcohol solution (40-80 ng daily). The daily dose (1.00 or 2.00) of powdered soil was suspended in 10 ml of water. The preparations containing TCDD were given regularly at 09.00. On day 8 the animals were killed and the livers removed and prepared for analysis. Table III gives (a) the weights of the animals at death ($\bar{p}$ = 2.6 ±0.3 kg; see also Fig. 2), (b) the weights of the whole livers (see also Fig. 3) and (c) of the portions of hepatic tissue used for analysis, (d) the individual TCDD levels found in the hepatic tissue: in the case of groups on broadly homogeneous treatments (as will be noted from the lack of significant differences between the specific values of the means) the cumulative mean was calculated on several homogeneous groups. Table III contains three such cases: treatments with TCDD in solution at the dose of 40 ng daily, treatments with laboratory-contaminated powdered soil at the dose of 40 ng daily and treatments with laboratory-contaminated powdered soil at the dose of 80 ng daily. The results are summarised in Table IV.

Table III

Levels of TCDD found in the hepatic tissue of rabbits after seven days treatment

Treatment	Body-weight[1] (kg)	Liver weight (g) Total	Liver weight (g) Sample[2]	TCDD Found[3] (ng)	TCDD Found[3] (ppb)	TCDD found mean value (ppb)
20 ng/die Oil-acetone	1.90	73.9	22.0	7.78	0.353	
	2.43	70.0	21.0	6.04	0.288	
	2.72	107	22.0	5.83	0.265	0.26 ± 0.07
	2.55	84.1	22.0	5.19	0.236	
	2.57	73.0	20.0	3.26	0.163	
20 ng/die Soil 2[4]	2.65	90.1	19.0	6.58	0.347	
	2.57	98.0	20.0	6.46	0.323	
	2.57	86.9	18.0	5.14	0.286	
	2.73	98.0	18.0	5.13	0.285	0.26 ± 0.08
	2.70	100	20.0	4.93	0.246	
	2.70	118	21.0	4.24	0.202	
	2.32	80.0	20.0	2.52	0.126	
40 ng/die Oil-acetone	1.85	72.8	18.9	29.8	1.57	
	2.20	82.9	22.0	29.2	1.32	
	2.35	79.2	23.3	27.7	1.19	1.18 ± 0.27
	2.33	61.5	23.6	27.6	1.17	
	2.47	66.9	24.8	26.7	1.08	
	2.35	96.5	23.5	18.0	0.766	
40 ng/die Alcohol-water	2.25	92.4	22.5	29.3	1.30	
	2.35	96.9	23.6	25.6	1.08	
	2.31	81.4	23.3	23.7	1.02	0.98 ± 0.22
	2.30	77.7	22.8	22.2	0.972	
	2.57	100	25.7	22.4	0.870	
	2.05	62.9	21.0	13.1	0.626	
40 ng/die Alcohol-water	2.67	72.0	18.1	29.3	1.62	
	2.80	88.0	17.0	23.9	1.41	1.31 ± 0.27
	2.75	86.5	21.7	27.1	1.25	
	2.95	108	20.2	19.9	0.984	
		Mean value for the three groups				1.1 ± 0.3
40 ng/die Soil 1[5]	2.57	68.5	28.5	45.6	1.60	
	2.40	79.2	31.7	41.9	1.32	
	2.30	71.4	21.2	16.7	0.786	0.98 ± 0.38
	2.30	91.2	26.8	20.4	0.762	
	2.51	93.0	28.2	19.8	0.701	
	2.99	113	36.4	25.6	0.701	
40 ng/die Soil 2	2.69	80.0	27.6	24.3	0.880	
	2.35	81.4	23.9	19.3	0.807	
	2.65	73.6	19.4	12.6	0.652	
	2.71	110	27.5	16.1	0.586	0.66 ± 0.13
	2.20	87.7	22.0	12.6	0.572	
	2.70	74.4	27.6	15.4	0.560	
	2.35	63.8	25.6	13.2	0.559	

(Continuation of Table: "Levels of TCDD in the liver")

			Mean value for the two groups			0.81 ± 0.31
80 ng/die Alcohol-water	2.80	85.0	14.4	49.1	3.41	
	2.40	83.1	17.2	33.4	3.11	
	2.85	94.9	12.6	32.6	2.59	2.73 ± 0.52
	2.90	119	16.2	38.8	2.40	
	2.80	114	20.1	43.2	2.15	
80 ng/die Soil 1	2.80	93.0	16.0	28.8	1.80	
	2.93	113	18.0	32.2	1.79	
	2.53	109	18.5	30.7	1.66	1.52 ± 0.32
	2.85	111	24.0	28.2	1.17	
	3.15	125	21.8	25.4	1.16	
80 ng/die Soil 2	2.60	97.2	18.7	33.9	1.81	
	2.85	80.0	21.0	33.4	1.59	
	2.65	100	21.0	32.3	1.54	1.56 ± 0.16
	2.72	103	17.0	26.1	1.53	
	2.55	98.0	19.0	25.7	1.35	
			Mean value for the last two groups			1.5 ± 0.2
∿80 ng/die Soil S[6]	2.65	85.0	21.6	27.9	1.29	
	2.90	103	21.8	26.6	1.22	
	2.84	111	19.3	18.2	0.942	
	2.75	115	20.5	16.7	0.814	0.88 ± 0.28
	3.18	128	19.7	13.6	0.692	
	2.90	105	17.8	12.0	0.673	
	2.70	116	21.7	11.7	0.540	
∿160 ng/die Soil S	1.92	75.0	20.0	77.2	3.86	
	2.50	80.1	20.0	58.0	2.90	
	2.58	94.9	20.0	41.6	2.08	
	2.22	88.0	22.0	45.5	2.07	2.18 ± 0.96
	2.43	90.2	21.0	40.5	1.93	
	2.75	100	21.0	31.2	1.48	
	2.27	89.8	25.0	23.0	0.922	

[1] At the end of treatment
[2] Portion of fresh liver used for the determination
[3] Values corrected for losses inherent in the method of analysis used (last column of Table on "Recoveries")

[4] Laboratory-contaminated soil left to "age" (1 month) before administration
[5] Laboratory-contaminated soil administered with 48 h of spiking with TCDD
[6] Powdered soil from Seveso (mean contamination: 81 ppb)

TABLE IV

Mean TCDD levels in the liver of rabbits treated for seven consecutive days with various formulations containing the poison.

TCDD ng/day	Vehicle	Number of specimens	TCDD (ppb) in the liver $\bar{x} \pm \sigma$[2]	CI (99%)[3]
20	Solvent[4]	5	0.26 ± 0.07	0.12–0.40
20	Soil 2[5]	7	0.26 ± 0.08	0.15–0.37
40	Solvent[6]	16	1.1 ± 0.3	0.94–1.3
40	Soil 1-2[7]	13	0.81 ± 0.31	0.54–1.1
80	Solvent[8]	5	2.7 ± 0.5	1.7 –3.8
80	Soil 1-2	10	1.5 ± 0.2	1.3 –1.8
~ 80	Soil S[9]	7	0.88 ± 0.28	0.48–1.3
~160	Soil S	7	2.2 ± 1.0	0.84–3.5

1 For further details and for data on the individual experiments see Table "Levels of TCDD found in hepatic tissue".

2 Mean values and standard deviations.

3 Confidence interval

4 Oil-acetone

5 Laboratory-contaminated soil left to "age" (> 1 month) before administration.

6 Combination of the results for treatments with alcohol-water solution and those of oil-acetone solution.

7 Combination of the results for treatments with the two formulations of soil prepared: Soil 1 and Soil 2, the first of which was dosed within 48 h of spiking with TCDD.

8 Alcohol-water.

9 Powdered soil from Seveso (mean contamination: 31 ppb).

5 CONCLUSIONS

Table V summarises the results of the statistical analysis (ANOVA and Duncan's test) conducted on the data given in Tables III and IV. Regarding the bioavailability of TCDD, our conclusions are as follows:

(a) at the lowest dose tested (20 ng daily) no statistically significant difference in bioavailability emerged between TCDD in solution and TCDD on laboratory-contaminated powder;

(b) at the doses of 40 and 80 ng daily the bioavailability of TCDD distributed over powdered soil appears on average to be 29 and 44% respectively, lower than that of TCDD in solution; the lower limits of the confidence intervals (99%) give a decrease in bioavailability of 5% and 19% respectively;

(c) The bioavailability of TCDD present on the powder of soil taken from Seveso, contaminated after the ICMESA accident, is on average 68% less than that of TCDD administered in solution; here again, however, the lower limit of the corresponding confidence interval (99%) supplies a much smaller variation in bioavailability (-40%);

(d) statistical analysis of the results obtained with treatments at 40 ng daily shows that there are no significant differences in TCDD absorption between recently contaminated powdered soil (Soil 1) and TCDD in solution, in two cases out of three (see Table III);

(e) there is a significant difference (-43% on average) between the bioavailability of TCDD present in the soil from Seveso and that of TCDD on laboratory-contaminated soil; in this case the lower limit of the confidence interval (99%) supplies an estimated decrease in bioavailability of only 5%;

TABLE V

Statistical analysis of the results obtained (previous table) for the evaluation of variations in bioavailability.

TCDD (ng:day)	Formulations compared[2]	Significance level of the variation	Mean reduction (%) of bioavailability $\bar{x}$	CI (99%)[3]
20	Soil 2/Solvent[4]	nonsignificant		
40	Soil 1-2/Solvent[5]	$p < .01$	29	5.0 - 53
80	Soil 1-2/Solvent[6]	$p < .01$	44	19 - 68
80	Soil S/Solvent[7]	$p < .01$	68	40 - 95
80	Soil S/Soil 1-2[8]	$p < .01$	43	5 - 81

1 ANOVA and Duncan test

2 In each pair the TCDD of the second formulation (Solvent) was assigned maximum bioavailability (100%). This was arbitrarily attributed also to the TCDD of the second formulation of the pair Soil S/Soil 1-2.

3 Confidence interval

4 Laboratory-contaminated soil after "aging" versus solution in oil-acetone.

5 Laboratory-contaminated soil ("aged" and "not aged") versus solution (in alcohol-water and oil-acetone).

6 Laboratory-contamined soil ("aged" and not "aged") versus solution in alcohol-water.

7 Powdered soil from Seveso versus solution in alcohol-water.

8 Powdered soil from Seveso versus laboratory-contaminated soil ("aged" and not "aged").

(f) two highly significant linear regressions may be derived from the data obtained, viz. from the treatments with TCDD in solution and with TCDD vehicled by the laboratory-contaminated powder (Fig. 4); the two regression lines permit an estimate of the mean levels that might have been present in the liver following treatment with the above formulations at the dose of 160 ng daily: in contrast to what happens in the case of the laboratory-contaminated soil, the value extrapolated for treatments with TCDD in solution is significantly higher than the value determined experimentally for the group of animals treated with powdered soil from Seveso, Soil S, at the same daily dose (~160 ng daily).

In sum, the data obtained indicate that in the rabbit the absorption of TCDD from orally dosed preparations is clearly less when the poison is in the soil than in solution. This phenomenon becomes apparent at the highest doses.

Acknowledgment

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APPENDIX

Methods of analysis

(a) For the powdered soils:

The procedure of analysis for the samples of powdered soil was one that has already been widely used for TCDD determinations in various environmental substrates at Seveso (4), adapted for the type of samples under study as reported later on. For a detailed discussion of the procedure see References. The three steps used (extraction, purification on a multilayer column and purification on an alumina column) were subjected to numerous preliminary tests in order to ensure practically quantitative recoveries and a high degree of reproducibility.

Extraction. All the samples of powdered soil were extracted in a Soxhlet apparatus for ~24 h (refluxing 4-6 times/h) with dichloromethane (~75 ml, HPLC grade) as solvent. The sample was prepared for extraction by mixing intimately ~2 g of dry powder with 8 g of anhydrous sodium sulfate. The extract was left to evaporate slowly under a stream of nitrogen in order to obtain a moist residue.

Purification on multilayer column. This column (I.D. 10 mm: total length 250 mm) consisted (from bottom to top): glass wool; anhydrous sodium sulfate 0.5 cm; activated silica gel 1.5 cm; mixture of anhydrous sodium sulfate and sodium bicarbonate (4:1 w/w) 2 cm; celite 545 1 cm; mixture of concentrated sulfuric acid and celite 545 (1:1 w/w) 5 cm; anhydrous sodium sulfate 1 cm. The sequence of the layers is shown in Fig. 1. Before use, the multilayer column (one for each extraction) was washed with three portions (10 ml each) of dichloromethane, followed by three portions (10 ml) of n-pentane. The residue of extraction was taken up with 4 ml of n-pentane

(HPLC grade) and transferred to the top of the column before the pentane washings had eluted completely; the operation was repeated another seven times in the same way to ensure quantitative transfer. Percolation was by gravity. The eluate collected (~32 ml) was allowed to evaporate to dryness spontaneously.

Purification on column of activated alumina. Alumina Merck (neutral aluminium oxide 90, 170-230 mesh) was kept in an oven at 130°C±5°C for 14 h and then stored in a desiccator on silica gel ready for subsequent use. The columns (I.D. 10 mm, total length ~250 mm) were prepared by inserting first a piece of glass wool, followed by 5 cm of alumina (~4 g) and 0.5 cm of anhydrous sodium sulfate. The residue from the multilayer column was taken up quantitatively with five successive portions (2 ml each) of n-hexane and transferred in succession on to the top of the column. There followed three washings (10 ml each) with a mixture of n-hexane and dichloromethane (1%) and four elutions with a total of 20 ml (5 ml per elution) of a mixture of n-hexan and dichloromethane 20%. This eluate (~20 ml) was carefully collected in a beaker and left to evaporate to a moist residue; this was then transferred quantitatively fn a suitable container evaporated to dryness and diluted to a known volume for the assa by means of 1rGC/1rMS according to the procedure described fn th reference cited.

(b) For the samples of hepatic tissue:

The determinations on the liver samples were done by means of a procedure already used for the determination of TCDD in rabbit liver (5), as reported later. For a detailed discussion of the procedure see references.

Alkaline digestion. About 20 g of liver was hydrolysed with 10 ml NaOH 10 N plus 20 ml ethanol at 90°C and refluxed for 1 h.

Extraction. After cooling, the sample in the alkaline medium was extracted twice with n-hexane (20 ml each time).

Purification on column of Extrelut. A column of Extrelut was pretreated with 20 ml concentrated sulfuric acid and left to equilibrate for 4-14 h. The total hexane solution (~40 ml) was left to percolate through the column, which was subsequently washed with 20 ml n-hexane. The eluate was evaporated to dryness in a stream of nitrogen at room temperature.

Purification on column of activated alumina. The columns of neutral alumina (45 x 5 mm), with a layer of anhydrous sodium sulfate at the top (5 mm), were washed with 3 ml dichloromethane and activated at 270°C for 12 h or at 400°C for 4 h. The residue from the column of Extrelut was taken up with n-hexane (total volume ~3 ml) and transferred to the top of the column. This was followed by an elution with 6 ml carbon tetrachloride followed by 4 ml dichloromethane. The latter fraction, containing TCDD, was evaporated to dryness. The residue was taken up with dioxane (0.100 ml) and analysed by GC and mass fragmentography.

In some cases samples from groups on like treatment were pooled and analysed by high resolution GC (25000 theoretical plates) and MS according to a method reported in the literature (6).

All the reagents and standards used in this study were of a high degree of purity and were grade HPLC or RS, where possible. The TCDD used was of the same purity as the environmental standards. Table VI and Figs. 6-11 summarise all the results and information appropriate for defining the quality of the analysis methods used.

Table VI

Recoveries (%) of TCDD for the methods of analysis used and for two specific purification operations.

Multilayer column		Alumina column	Soil 1 [1]	Soil 2 [2]	Soil 3 [3]	Hepatic tissue [3]	
141.0[4]	95.0	102.0	135.8[4]	106.0	110.0	108.5[4]	80.0
123.5	95.0	101.5	116.7	102.6	107.5	99.0	80.0
120.0	94.5	101.0	109.4	98.0	105.5	98.2	78.5
115.5	94.0	100.0	104.6	97.5	103.5	96.6	78.2
114.6	94.0	99.4	102.0	97.0	101.5	95.2	77.5
114.5	93.5	98.0	102.0	95.5	99.5	94.8	77.3
114.3	91.5	96.5	99.1	94.0	99.5	91.0	77.0
110.5	91.0	95.6	99.0	93.5	99.5	89.3	77.0
110.5	91.0	95.0	98.0	93.0	99.0	89.0	77.0
109.0	90.0	94.0	97.6	92.5	98.0	88.6	76.0
108.5	90.0	93.5	97.6	92.0	97.5	88.3	76.0
108.0	89.0	93.5	95.5	91.0	97.5	88.0	75.5
107.5	89.0	92.5	95.0	86.0	97.0	88.0	75.2
105.0	88.9	92.0	94.0	81.9	95.7	88.0	75.0
104.5	88.0	91.8	92.0	81.4	94.5	88.0	73.0
104.0	88.0	91.5	87.5	77.9	94.5	87.0	72.8
104.0	88.0	91.0	85.5		93.0	86.2	72.2
103.8	87.0	90.0	69.4		93.0	86.0	71.4
103.5	86.5	89.0	66.6		92.5	86.0	71.0
103.1	85.5	87.5	66.5		90.0	85.0	70.2
103.0	84.0	82.7	60.3		90.0	85.0	67.0
102.6	84.0	82.7			88.5	85.0	65.5
102.0	84.0	74.0[4]			88.5	84.0	64.3
101.5	84.0	47.0[4]			88.0	84.0	63.0
101.5	84.0				88.0	84.0	63.0
100.9	83.5				87.5	83.3	62.0
100.8	83.5				86.5	83.3	62.0
99.9	82.4				86.0	82.4	61.0
99.5	82.0				86.0	82.0	60.4
99.0	81.5				85.0		
98.1	81.0				80.0		
98.0	78.0						
98.0	76.0						
97.5	73.8						
96.0	65.1						
96.0	57.0[4]						
95.0							
Means and standard deviations and number of data used in the calculation:							
96 ± 12		94 ± 5	92 ± 15	92 ± 8	94 ± 7	80 ± 10	
{71}		{22}	{20}	{16}	{31}	{57}	

[1] Laboratory-contaminated soil

[2] Laboratory-contaminated soil left to "age"

[3] Recoveries of TCDD (Cl^{37}) added to samples of powdered soil and of hepatic tissue before extraction

[4] Aberrant (according to Chauvenet)

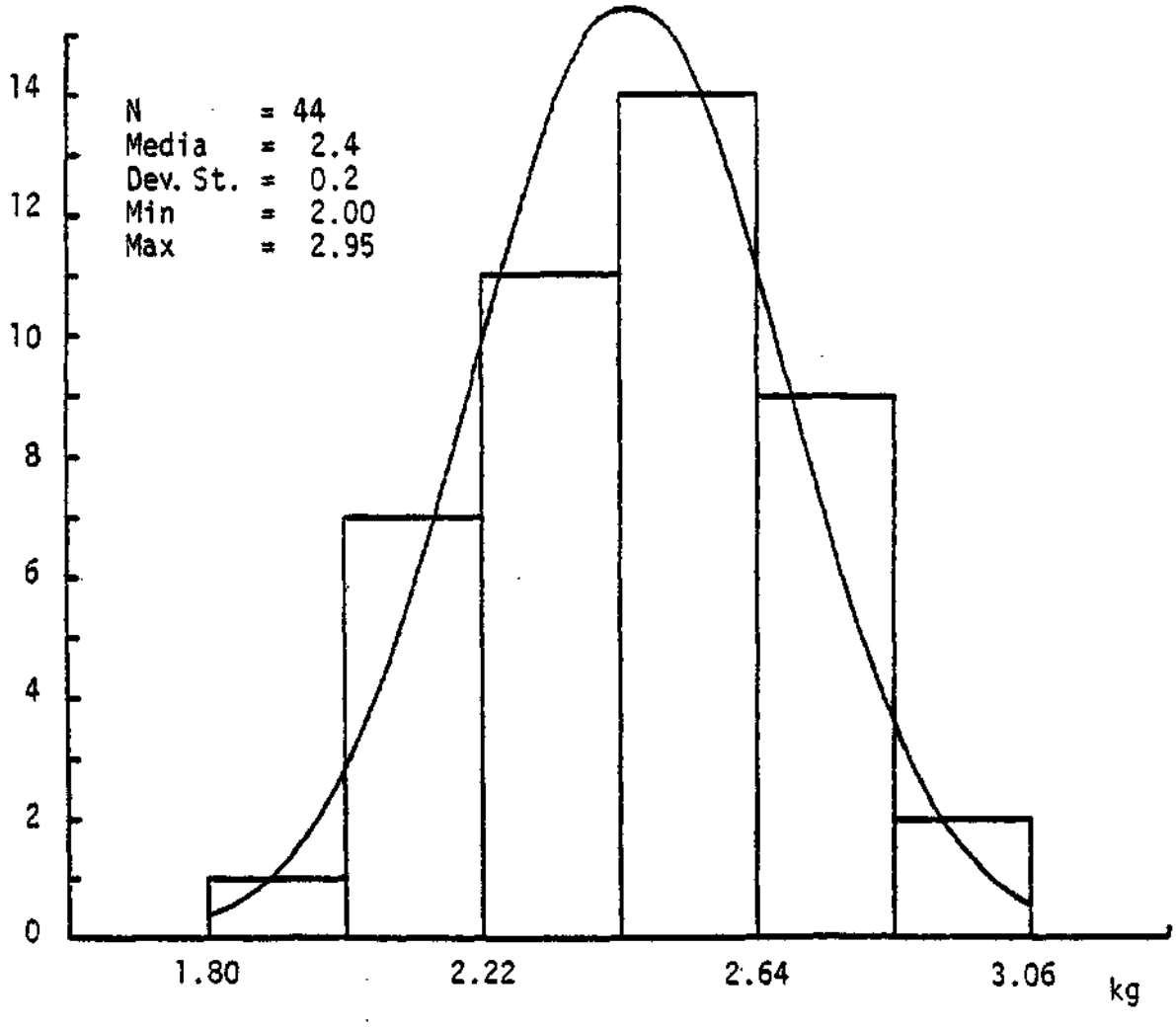

Fig. 1. Distribution by weight group of 44 male white rabbits before treatment

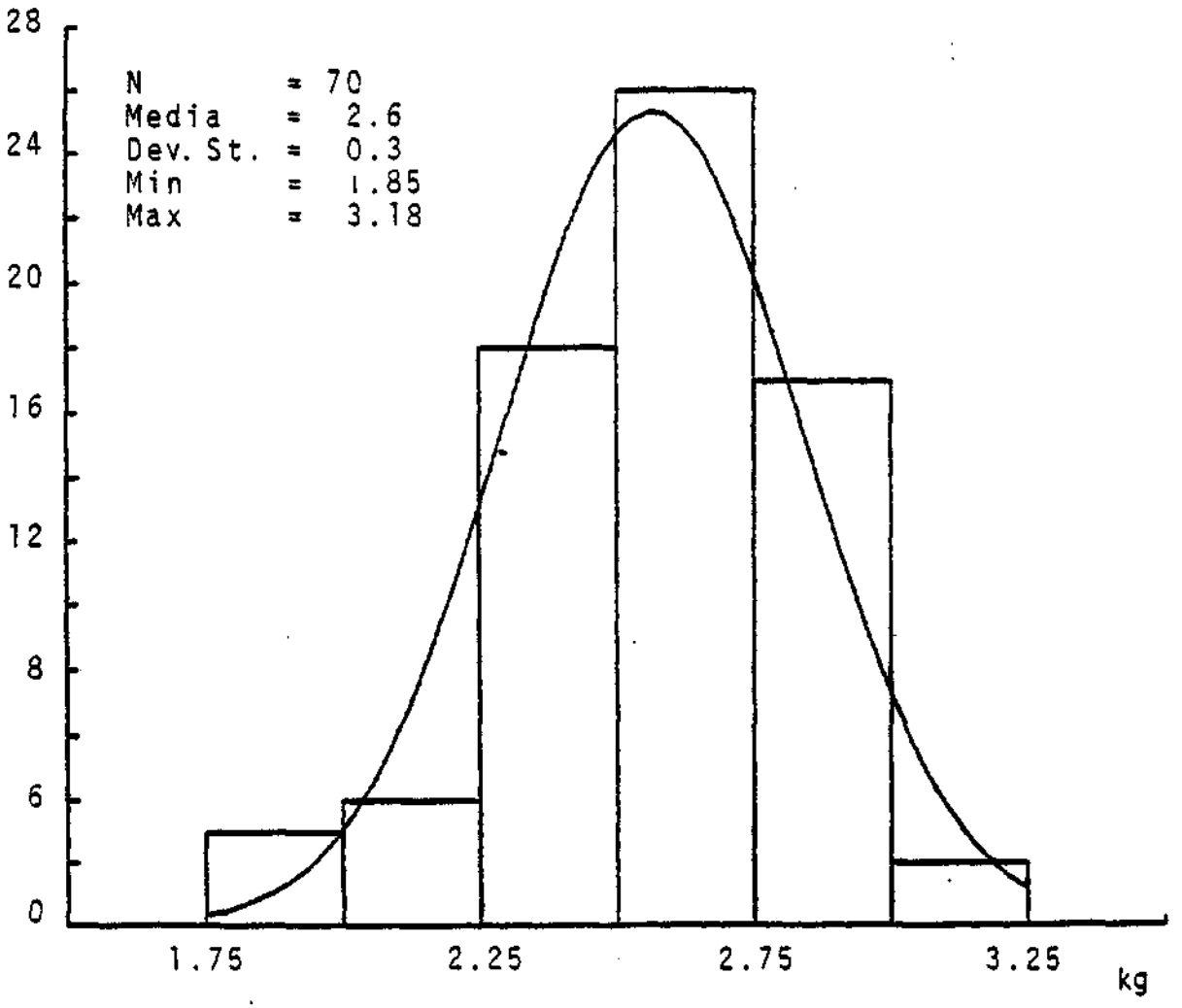

Fig. 2. Distribution by weight group of 70 male white rabbits on day 8 (sacrifice) of treatment. Note (see Fig.1) that the animals presented an average increase in weight in the week of treatment; in some cases there was a slight weight loss unrelated to any specific factor.

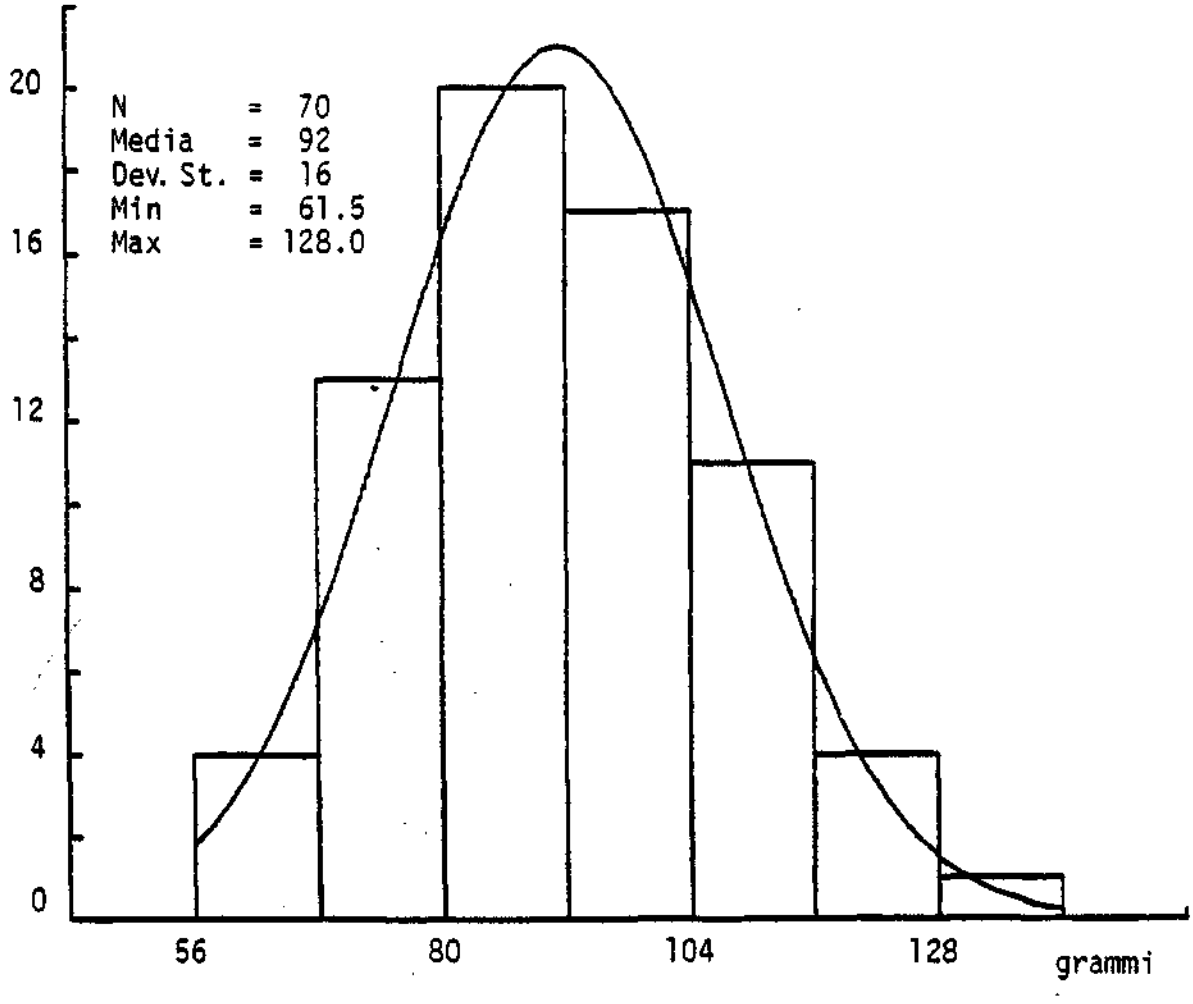

Fig. 3. Distribution by weight group of the livers removed from the rabbits after treatment with various formulations containing TCDD.

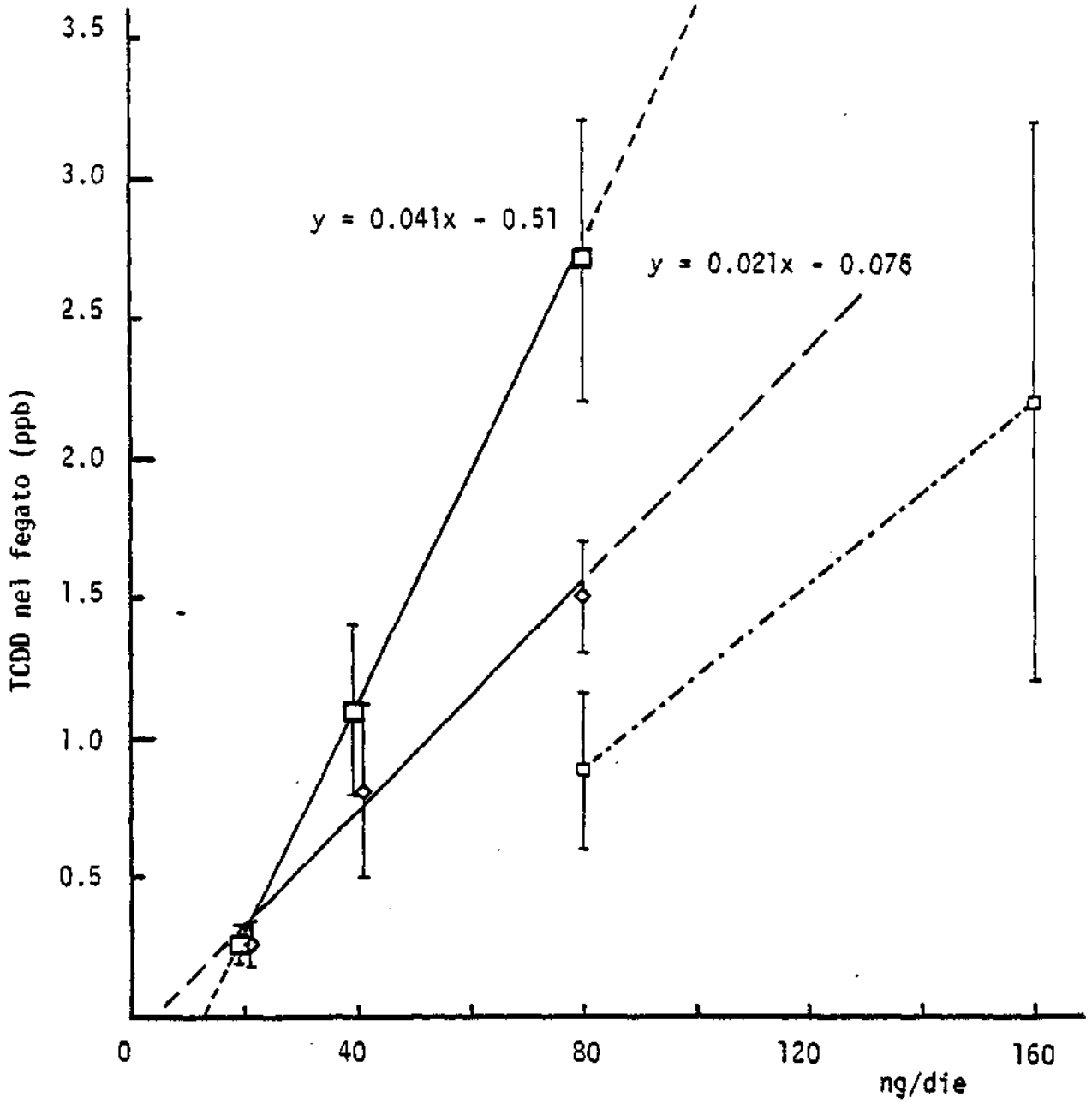

Fig. 4. Regression lines obtained by using the set of data for treatments with TCDD in solution (□) and the set of data for treatments with laboratory-contaminated powdered soil (◇). The significance level of the regressions is $F_{24}^{1} = 172.35$, $P < .001$; $F_{28}^{1} = 106.67$, $P < .001$ respectively. To highlight the results of treatments with Seveso soil, two points have been plotted (□) corresponding to the mean values of the TCDD levels determined analytically in the two groups treated. The standard deviation is shown above and below the value of each mean.

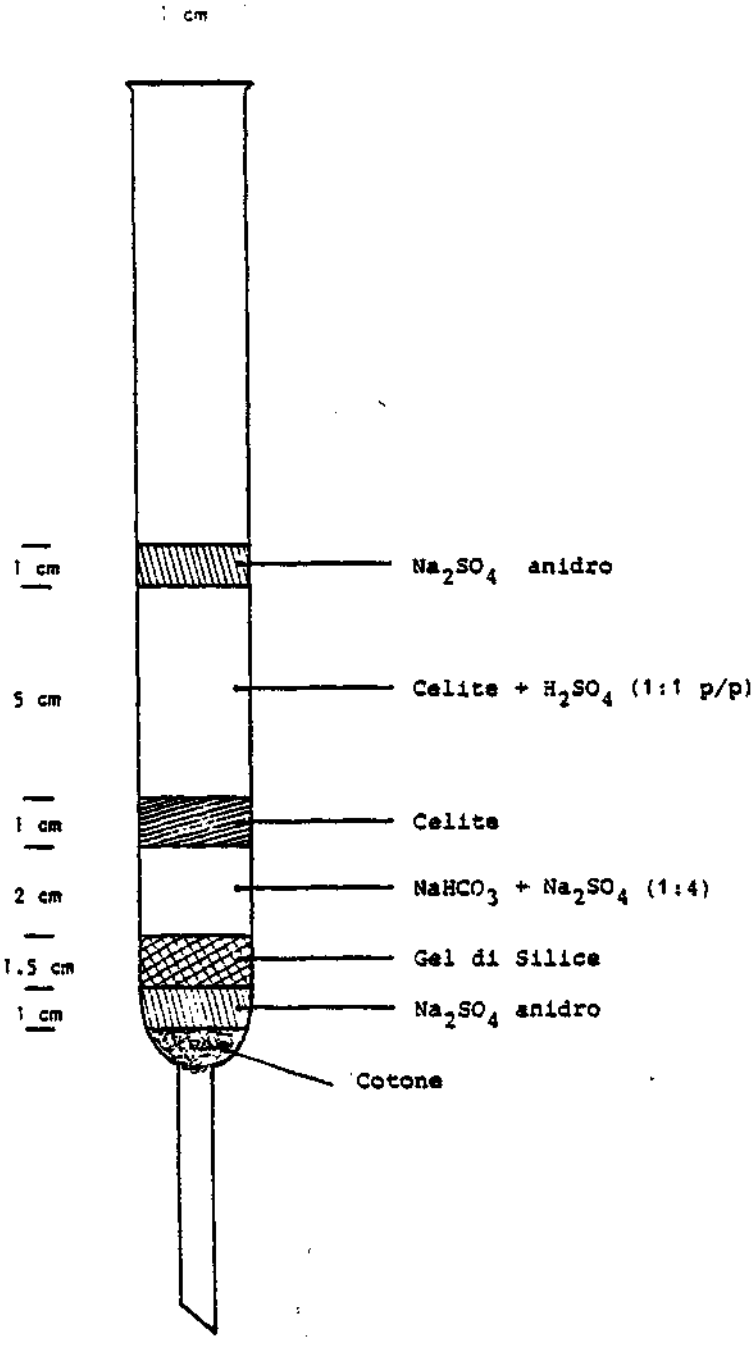

Fig. 5. Multilayer column for the first stage of purification of the extracts. Description in the text.

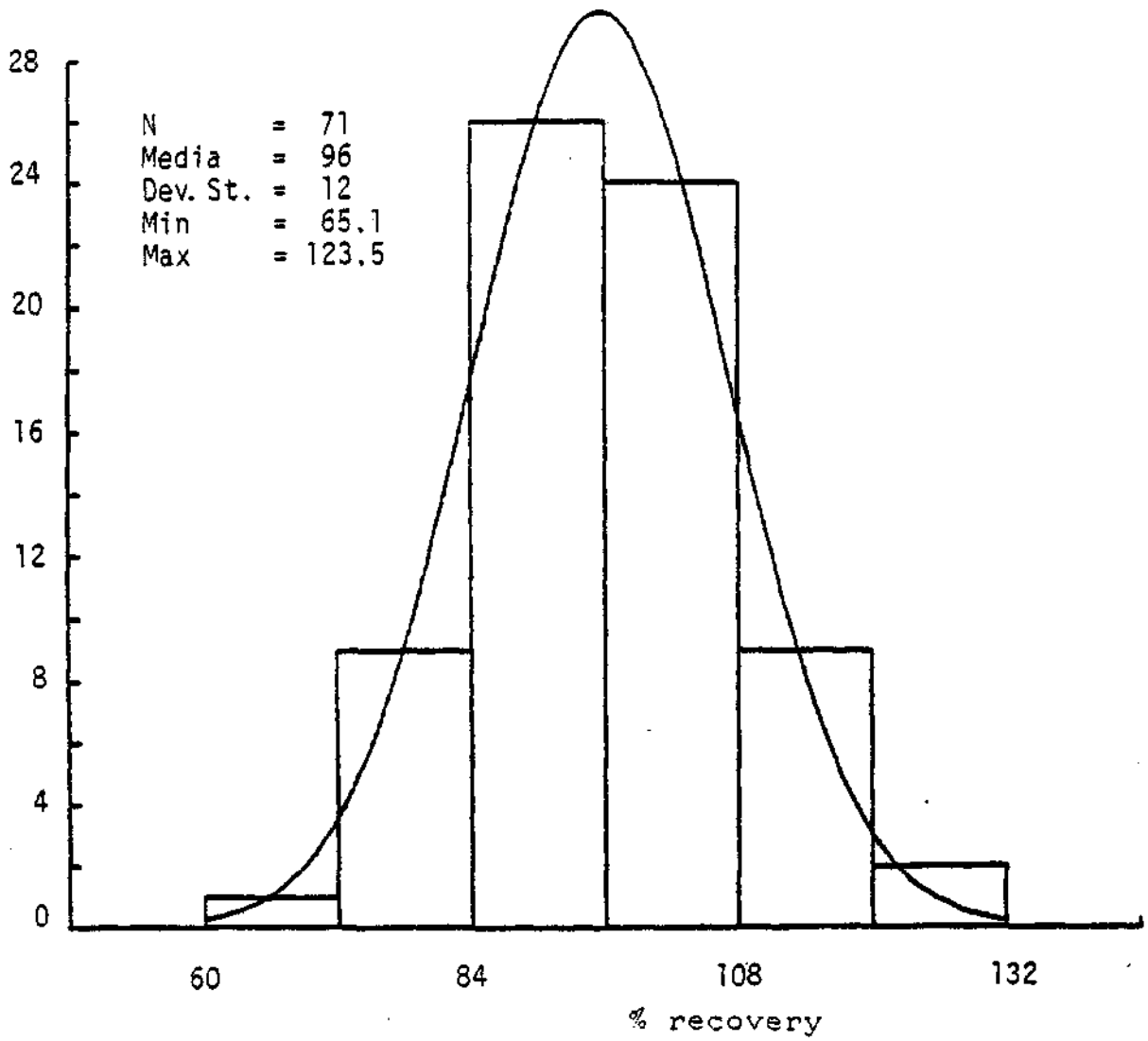

Fig. 6. Block distribution of percentage recoveries obtained from the multilayer columns (see Table VI first column)

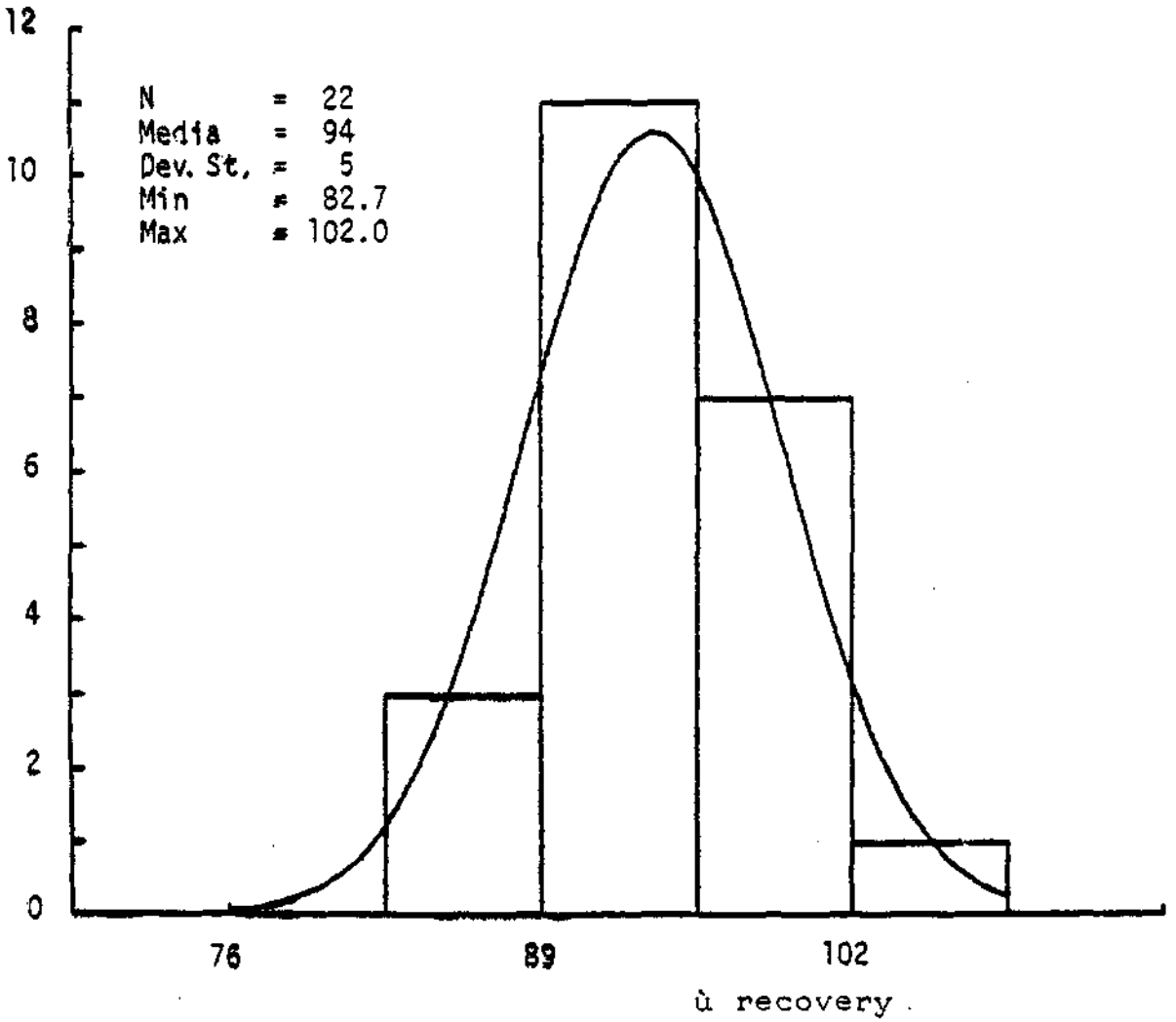

Fig. 7. Block distribution of percentage recoveries obtained from the alumina columns (see Table VI, second column)

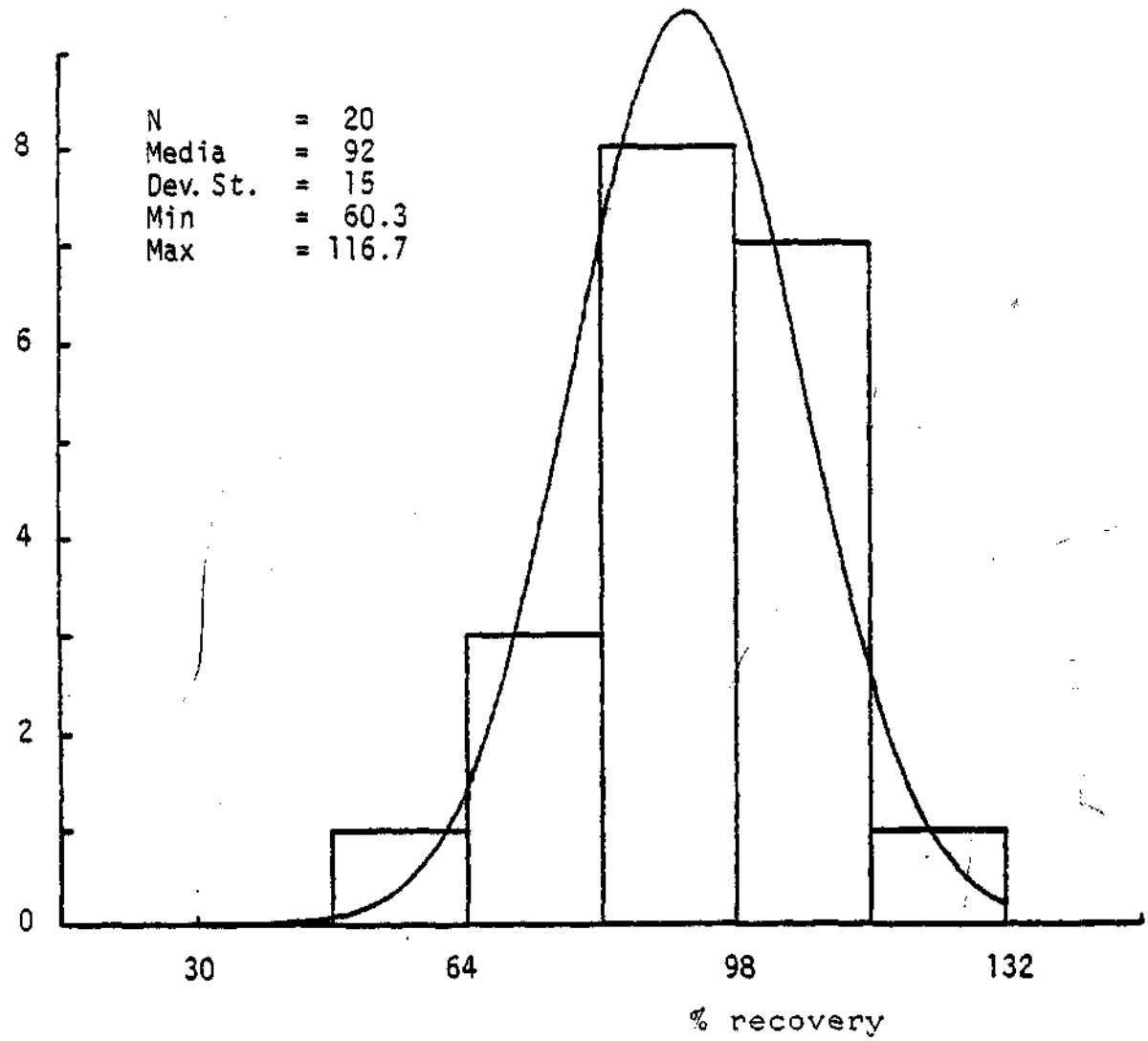

Fig. 8. Block distribution of percentage recoveries obtained on applying the method of analysis to the determination of TCDD added to uncontaminated powdered soil, within 48 h of addition (Soil 1: see Table VI, third column).

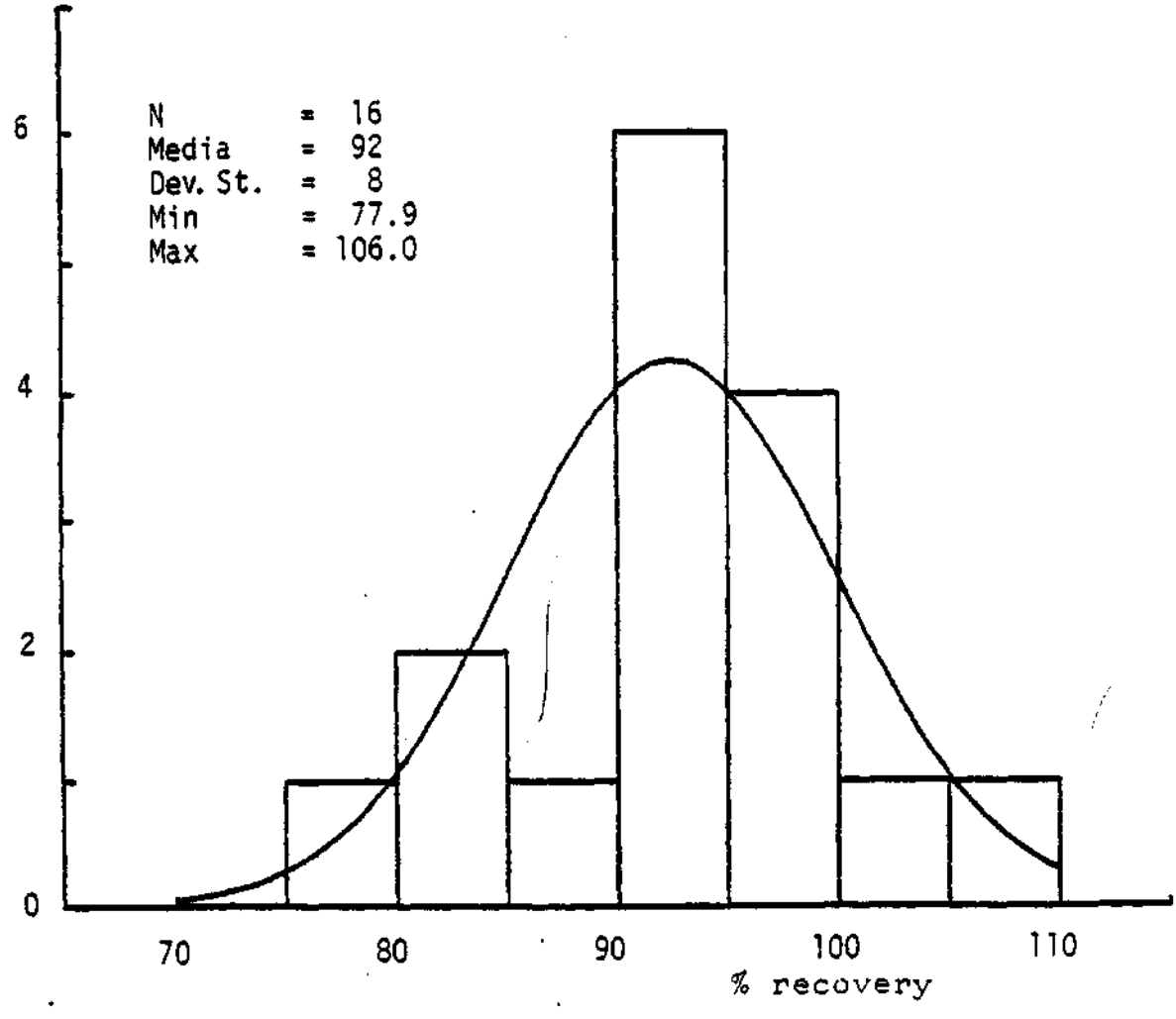

Fig. 9. Block distribution of percentage recoveries obtained on applying the method of analysis to the determination of TCDD added to uncontaminated powdered soil left to "age" (Soil 2: see Table VI, fourth column)

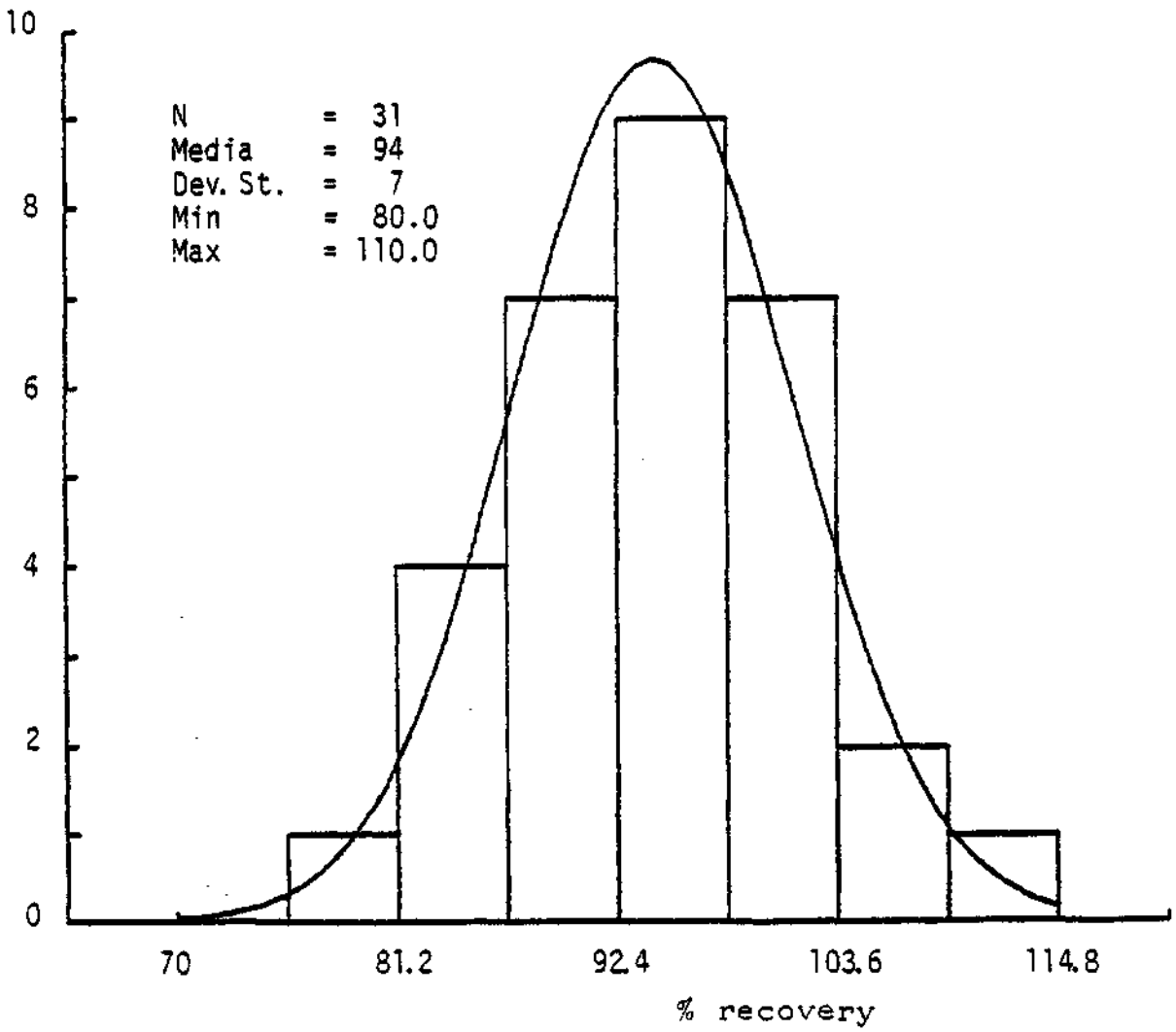

Fig. 10. Block distribution of percentage recoveries obtained on applying the method of analysis to the determination of labeled TCDD added to powdered soil of different types (laboratory-contaminated or contaminated following the ICMESA accident) before extraction (see Table VI, column five)

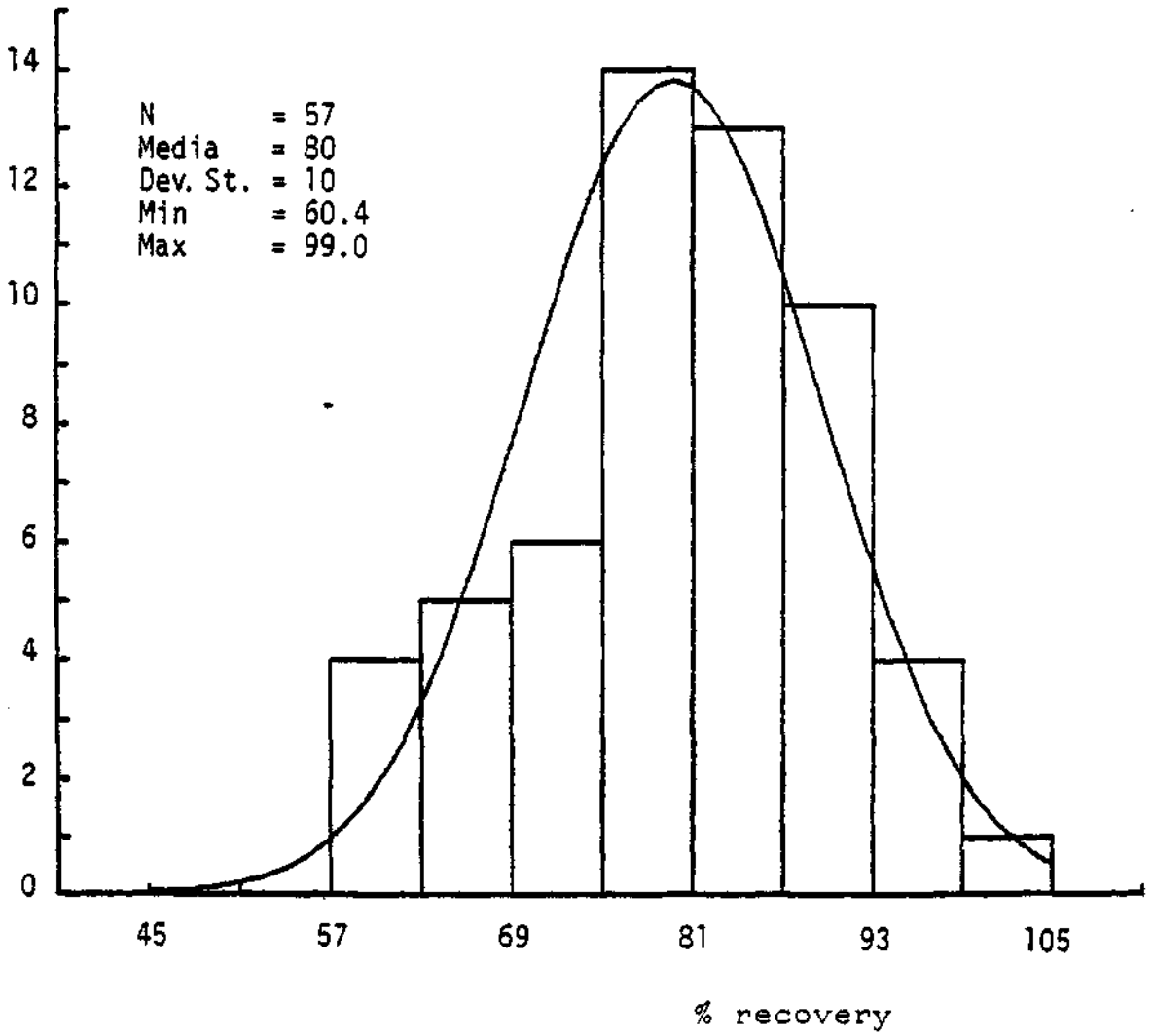

Fig. 11. Block distribution of percentage recoveries obtained on applying the method of analysis to the determination of labeled TCDD added to hepatic tissue before alkaline digestion (see Table VI, sixth column)