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Item ID Number	01466
Author	Brown, Harry D.
Corporate Author	
Report/Article Title	Final Report: Herbicide (2,4-D) Applicator Exposure Measurements
Journal/Book Title	
Year	0000
Month/Day	
Color	☐
Number of Images	48
Description Notes	Supported by USDA Agreement No. USDA-TPSU-RU-0-191 and New Jersey Agricultural Experiment Station Project No. NJ04502.

NE-NJ-H-42

FINAL REPORT

HERBICIDE (2,4-D) APPLICATOR EXPOSURE MEASUREMENTS

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Supported by USDA Agreement No. USDA-TPSU-RU-0-191 and New Jersey Agricultural Experiment Station Project No. NJ04502.

INTRODUCTION

The phenoxy herbicides have been used to control broad-leafed weeds in crops, water sources, forests, pastures, range lands, gardens, lawns, urban and industrial sites. Because of the efficiency, economy and safety, the phenoxy herbicides, especially 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D), besides other herbicides, remain essential for agricultural and other uses (Diaz-Colon and Bovey, 1976). Use of 2,4,5-T and 2,4-D on a large scale to defoliate vegetation has resulted in controversy about the fate of the chemicals. Many of the hazardous properties ascribed to 2,4,5-T are to a lesser extent shared by 2,4-D. As a result, a number of papers dealing with the fate in the environment, toxicological effects on living organisms (Diaz-Colon and Bovey, 1976; Sauerhoff et al., 1977; Piper et al., 1973) have been published. Cooper (1974) compared 2,4,5,-T and 2,4-D. He has reported that commercial samples of 2,4-D in mice were teratogenic and embryotoxic. Increasing controversy about their safe use has raised doubt about their utility in achieving vegetation management objectives.

A Dow Chemical report on Forestry Applicators exposure to 2,4-D has recently been published (Levy, 1980). Nonetheless, in total, only meager information regarding human (e.g. gardeners, small farmers, etc.) exposure to 2,4-D is available. This study reports an investigation conducted under conditions characteristic of actual use pattern to determine the quantity of body surface contact of applicator with the commercial 2,4-D formulation and the quantity of the chemical measurable in serum and urine.

MATERIALS AND METHODS

Applicator

Eleven healthy young male (ages 19 to 31 years) human volunteers were selected and were examined by the University physician prior to the work. Each subject was examined for blood pressure, pulse rate and body temperature on the day of work, both before and after the work period, and similar examinations were made every morning for the next three days (Table 1). Sterile gauze pads (size 0.67 sq. ft.) were attached on the back and chest of each volunteer. A paper cap (area 0.44 sq. ft.) was used to cover the head.

Location

The study was carried out either on the campus of Rutgers University or near the campus in New Brunswick, New Jersey during the period from May, 1981 to August, 1981. The location was covered with weeds and grass. Each area was roughly between one-half to one acre.

Spray Material and Equipment

The spraying material consisted of DMA-4 concentrate (obtained as a gift from Dow Chemical, U.S.A.) which contained 3.8 lb acid equivalent/gal 2,4-D. The herbicide was mixed with tap water prior to use and the resulting mixture was applied at a rate of approximately 1 gal/30 min. by a hand sprayer held at hip level. A DuPont air sampler was attached to the applicator and was kept running during the spraying operation. The areas were covered by spraying six gallons in about three hours as per label instruction.

Weather Conditions

Weather conditions during this study were moderate, temperature ranging between 65° to 78° F with humidity from 50 to 89%. Wind was fairly calm throughout this study except on one occasion, when it gusted to 10 miles per hour.

Sample Collection for Analysis

To ascertain body surface exposure, gauze pads and caps were collected immediately after the work and washed in methanol. Air filter absorbents were also washed in methanol. Residue separated by methanol was evaporated to dryness and then treated with 15% BF_3 in methanol. Finally, it was extracted with n-hexane (Yip, 1975).

Blood samples were drawn before the work and at the end of the day's work. Three more blood samples were drawn on the following three mornings. Overnight pooled urine samples were collected before the work and the day's collections were made during and/or after the treatment. Then the pooled urine samples were collected twice a day for the next 4 to 7 days. Blood sera and urine samples were treated, extracted and analyzed by the method of Sauerhoff et al.* (1977).

Vegetation samples and soil samples from the treated area were collected before the treatment and then once every 24 hours for the next 4 days after the treatment. Samples were treated and analyzed by the methods of Davidonis et al. (1980) and Olson et al. (1978).

Analytical Procedures

2,4-D residue extracted in n-hexane was analyzed on a gas chromatograph (Tracor Model No. 560, equipped with electron capture detector and a six foot glass column packed with 3% OV-101 on 80/100 supelcoport).

Clinical determinations on blood serum and urine were made through the automated procedures of a commercial diagnostic laboratory.

RESULTS AND DISCUSSION

The various data are summarized in tables 1-13 and figures 1-22. At the end of a day's work, an applicator had an average 2,4-D residue of 49.39 µg/sq. ft. on the back, 52.41 µg on the head. The average value of 2,4-D residue on vegetation in the exposed areas was 27.93 µg/g of tissue at the end of the working day (i.e. first day). The residue fell to 8.57 µg/g tissue after four days. On the other hand, the average soil residue value was 2.68 µg/g soil at the end of the first working day, which increased to 5.12 µg/soil (87.6%) on the fourth day.

The average base level for phenolic compounds in serum was 70.7 ng/ml at zero level exposure. Immediately after the day's work, the value went up to 165.3 ng/ml and again went up to 267.7 ng/ml on the second. The difference between the base level and the measured level we take to be a specific measurement of the test material, 2,4-D. The value came down to 123.7 ng/ml on the third day. The average amount of phenolics ranged from 3.45 µg (zero level exposure) to 7.85 µg (fifth day) for the 12 hours of pooled urine.

*Appendix A is a more extensive discussion of methodology.

Some changes were noticed in clinical analysis made on blood serum and urine.

Analysis of 2,4-D retained by the air monitor filter revealed detectable residue. The amount of air drawn through the filter was calculated to be 56.6 to 84.96 liters, and the calculated 2,4-D residue was 43.1 to 60.1 parts per trillion.

Results of this study indicate that there was a considerable amount of 2,4-D in the air, on vegetation and soil surface of the treated areas. An appreciable quantity of 2,4-D also landed on the body surface. All serum and urine samples showed measurable 2,4-D residue.

Statistical Analysis *

The clinical residue and weather data were critically scrutinized by both linear models and regression models. The primary emphasis of the analysis was to identify the parameters that recorded any significant change. This was accomplished by subtracting the baseling value from the observed value and then dividing it by the baseline. Analysis of the resulting values revealed highly significant (α=0.01) changes in the serum creatinine and total protein levels, and significant (α=0.05) change in the urine specific gravity. It was also seen that the total protein and BUN/creatinine were positively correlated with the residue on the chest of the volunteers and with their height. When the peak residue in the body fluids was related with the external factors, it was seen that the serum residue was positively correlated with the 2,4-D concentration in the air and the amount of chemical that had landed on the head and back. Likewise, the urine residue was positively correlated with the air volume and the height of the volunteers. Precipitation (rainfall-high humidity) was negatively correlated with the serum residue, presumably due to decreased chances of vaporization and subsequent inhalation.

*Analysis done by Dr. Robert Trout, Statistician for the NJAES.

CONCLUSION

Our observations, therefore, indicate that during normal working conditions, an appreciable amount of 2,4-D can enter into the applicator's body through inhalation and/or through body surface (dermal) absorption. Lengthy retention times have been observed in some subjects (Table 6). These findings are consonant with the report of Levy et al. (1980) for foresters. Clinical physiology data obtained here are consistent with long retention times for this chemical. We surmise, but have no specific evidence, that the 2,4-D molecule or some portion (phenolic), binds to serum protein and is eliminated as a function of the turnover of the protein molecules. Other than the clinical (chemical) changes noted above, we have seen no pronounced adverse effect of 2,4-D exposure.

REFERENCES

1. Sauerhoff, M.W., Braun, W.H., Blau, G.E. and Gehring, P.J. (1977). The Fate of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Following Oral Administration to Man. Toxicology 8:3-11.

2. Piper, W.N., Rose, J.Q., Lang, M.L. and Gehring, P.J. (1973). The Fate of 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) Following Oral Administration to Rats and Dogs. Toxicol. Appl. Pharmacol. 26:339-351.

3. Levy, T.L. (1980). Determination of 2,4-D Exposure Received by Forestry Applicators. Dow Chemical, U.S.A. Report. October, 1980.

4. Diaz-Colon, J.D. and Bovey, R.W. (1976). Selected bibliography of the phenoxy herbicides. I. Fate in the Environment. MP 1303. Texas Agric. Exp. Stn., College Station.

5. Cooper, P. (1974). Food Cosmet. Toxicol. 12:418-421.

6. Davidonis, G.H., Hamilton, R.H. and Mumma, R.O. (1980). Comparative Metabolism of 2,4-Dichlorophenoxyacetic Acid in Cotyledon and Leaf Callus from Two Varieties of Soybean. Plant Physiol. 65:94-97.

7. Olson, B.A., Sneath, T.C. and Jain, N.C. (1978). Rapid Simple Procedure for the Simultaneous Gas Chromatographic Analysis of Four Chlorophenoxy Herbicides in Water and Soil Samples. J. Agric. Fd. Chem. 26:640.

8. Yip, G. (1975). Analysis for Herbicides and Metabolites. J. Chromatographic Sciences 13:225-230.

CLINICAL XXX DATA

Table 1

PHYSICAL EXAMINATION

Applicators	Temp (°F)					Blood Pressure					Pulse Rate				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	98.0	98.4	97.8	98.4	98.4	128/74	114/70	120/78	120/60	120/78	76	60	80	78	72
No. 2	97.6	98.0	98.0	98.0	97.8	110/78	118/78	110/78	110/72	120/80	60	72	80	68	64
No. 3	97.4	97.6	97.8	97.0	97.8	112/82	120/70	110/74	118/62	120/76	60	72	60	61	64
No. 4	98.2	99.4	97.4	98.0	98.0	90/60	100/68	100/64	96/68	92/60	80	100	76	76	84
No. 5	97.6	98.8	98.0	98.2	98.2	112/74	124/80	120/82	120/80	110/80	72	84	84	80	84
No. 6	97.0	98.6	97.2	97.0	97.4	120/80	110/74	108/78	112/78	112/74	84	88	96	92	100
No. 7	97.4	98.4	97.0	97.0	98.2	104/64	100/60	110/64	120/64	110/70	78	78	80	78	80
No. 8	98.4	98.4	98.6	97.8	98.0	140/76	120/80	112/70	130/68	130/76	80	64	96	80	68
No. 9	98.6	98.4	97.0	98.0	97.2	110/70	110/70	110/80	120/80	120/74	60	60	68	80	64
No. 10	97.8	98.0	97.4	98.2	97.8	104/60	100/70	108/60	112/70	102/60	84	68	84	88	88
No. 11	97.4	98.6	97.2	97.2	97.0	120/68	110/70	110/80	112/70	110/76	80	80	80	68	80

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 2

Detection of 2,4-D Residue
from Gauge Patch (= Body Surface)

Applicator No.	µg/sq. ft. 1		
	Head	Chest	Back
1	8.55	7.81	5.10
2	14.06	11.68	10.60
3	7.06	3.37	3.77
4	222.20	171.50	99.63
5	34.22	94.53	41.16
6	141.80	111.50	74.23
7	76.16	68.39	46.64
8	16.99	13.56	11.86
9	19.12	14.58	10.29
10	18.55	31.46	4.46
11	17.80	14.95	18.63

Table 3

2,4-D Residue on Vegetation

Treated Area	2,4-D µg/g Vegetation					
	0	X	X+1	X+2	X+3	X+4
1	0.59	28.85	16.15	16.67	20.16	-
2	14.74	29.60	22.43	4.32	2.13	-
3	00.00	25.35	7.70	1.92	3.44	2.47

Amount of 2,4-D on Soil Surface

Treated Area	2,4-D µg/g Soil					
	0	X	X+1	X+2	X+3	X+4
1	1.24	3.70	1.90	2.80	7.30	-
2	3.58	4.21	0.72	1.59	7.25	-
3	0.00	0.13	0.15	0.27	0.80	0.93

0 = Pretreatment

X = Posttreatment

X 1 = 1 day after treatment

X 2 = 2 days after treatment

X 3 = 3 days after treatment

X 4 = 4 days after treatment

Table 4

Amount of 2,4-D Residue and Endogenous Phenolic Compounds in Serum

Applicator No.	2,4-D Residue (ng/ml)				
	0	X	X+1	X+2	X+3
1	112.3	75.1	826.6	311.0	112.3
2	54.0	114.4	170.1	250.1	102.3
3	93.5	221.9	131.5	74.6	479.6
4	157.8	443.2	112.8	92.4	84.2
5	56.3	538.3	496.9	169.9	245.1
6	208.3	123.5	862.9	94.3	674.6
7	44.5	224.4	119.3	194.7	178.6
8	3.5	19.1	55.7	43.6	83.4
9	29.0	50.2	41.8	43.0	25.7
10	19.0	7.8	93.3	58.8	36.3
11	00.0	00.0	55.6	27.9	114.8

0 = Pretreatment

X = Posttreatment

X+1 = 1 day after treatment

X+2 = 2 days after treatment

X+3 = 3 days after treatment

Note: Because phenoxy and other phenolic compounds have liquid chromatographic migrations similar to that of 2,4-D, there is a variation in the base for specific measurement of the test compound.

Table 5

Amount of 2,4-D in Serum

Applicator No.	2,4-D (ng/ml) X	X+1	X+2	X+3
1	--	714.3	198.7	0
2	60.4	116.1	196.1	48.3
3	128.4	38.0	--	386.1
4	285.4	--	--	--
5	482.0	440.6	113.6	188.8
6	--	654.6	--	466.3
7	179.9	74.8	150.2	134.1
8	15.6	52.2	40.1	79.9
9	21.2	12.8	14.0	--
10	--	74.3	39.8	17.3
11	--	55.6	27.9	114.8

Legend

X = Posttreatment

X+1 = 1 day after treatment

X+2 = 2 days after treatment

X+3 = 3 days after treatment

Table 6 Total Amount of 2,4-D and Endogenous Phenolic Compounds Excreted in the Urine

µg 2,4-D Excreted per 12 hours

Applicator No.	0	1	12	24	36	48	60	72	84	96	108	120	132	144	156
1	3.73	7.12	2.38	2.58	6.49	5.70	2.93	21.20	28.96	-	-	-	-	-	-
2	3.22	7.17	1.22	2.85	6.00	5.75	4.46	6.44	1.09	-	-	-	-	-	-
3	2.85	4.25	10.00	8.87	1.75	0.60	1.33	10.79	4.07	-	-	-	-	-	-
4	8.11	15.44	21.38	7.66	9.56	30.55	24.87	13.09	6.23	-	-	-	-	-	-
5	0.32	1.31	1.79	1.60	1.31	2.62	3.32	2.27	7.39	-	-	-	-	-	-
6	9.95	4.56	15.62	5.04	23.39	13.87	6.28	8.46	13.16						
7	9.91	10.81	15.25	2.73	3.12	2.89	0.91	5.00	8.84	-	-	-	-	-	-
8	0.00	0.11	0.57	2.77	1.34	3.46	2.84	3.08	5.30	1.97	1.49	2.68	0.00	0.00	0.00
9	0.00	0.75	0.00	0.85	2.46	8.28	6.57	1.46	4.09	1.42	2.10	3.73	2.21	4.31	4.47
10	0.00	1.94	1.73	1.63	2.83	2.21	2.73	8.48	4.35	-	-	-	-	-	-

0 = Pretreatment

1 = Posttreatment

Table 7

HEMATOLOGY

›pli- ıtors	WBC x 10^3					RBC x 10^6					HGB (g/dl)					HCT (%)				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
›. 1	6.0	7.1	6.3	7.0	5.5	4.78	4.↓68	4.↓61	4.↓50	4.↓43	15.5	15.1	14.8	14.6	14.6	44.4	43.7	44.8	41.↓6	41.↓5
›. 2	5.7	6.8	5.9	5.2	5.2	4.99	5.06	5.15	4.85	4.89	15.9	15.6	16.2	15.0	15.3	43.2	43.8	44.7	42.0	42.4
›. 3	6.7	6.1	5.2	5.6	5.0	4.↓69	4.↓64	4.↓63	4.71	4.↓65	14.9	14.8	14.5	14.6	14.9	41.↓2	41.↓2	41.↓2	40.↓9	40.↓6
›. 4	6.5	7.6	6.6	6.7	6.5	5.72	5.76	5.75	5.85	5.50	15.5	15.6	15.3	16.8	14.8	43.9	44.0	43.5	44.6	41.↓8
›. 5	6.3	6.5	5.9	7.0	6.2	5.34	5.22	5.28	5.56	5.27	15.9	15.7	15.7	16.8	15.6	44.6	44.1	44.7	46.5	43.6
›. 6	6.7	7.8	6.7	8.4	6.9	5.39	5.23	5.46	5.43	5.03	16.8	16.9	17.1	17.8	16.2	47.6	46.3	48.5	47.8	44.9
›. 7	6.6	8.1	9.8	8.2	7.9	5.45	5.20	5.42	5.33	5.29	16.5	15.9	16.3	17.0	16.3	48.0	44.9	47.3	46.9	46.0
o. 8	8.3	8.3	6.2	6.3	6.1	4.97	4.75	4.99	4.99	5.04	15.8	15.2	15.7	15.7	15.8	42.9	41.↓0	42.8	42.6	44.1
o. 9	6.1	6.2	6.0	8.1	7.8	5.21	4.76	5.07	5.42	5.14	13.↓3	12.↓4	13.↓2	13.↓9	13.↓2	38.↓7	35.↓3	37.↓4	40.↓3	38.↓1
o. 10	7.8	7.5	8.1	7.6	7.7	4.97	4.80	5.06	5.33	5.14	16.4	15.8	16.5	17.1	16.3	45.0	43.0	45.0	47.1	45.9
o. 11	5.0	4.8	6.0	6.0	5.9	4.99	4.↓54	4.81	4.95	5.11	14.8	13.↓5	14.4	14.9	14.9	41.↓2	37.↓8	39.↓3	40.↓7	42.0

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 7 (cont.)

HEMATOLOGY

Applicators	MCV (μ^3)					MCH ($\mu\mu g$)					MCHC (%)					Platelet Estimate				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	93	93	97↑	92	94↑	32.4↑	32.0↑	32.0↑	32.3↑	32.9↑	34.7	34.3	32.9	34.9	35.1	N	N	N	N	N
No. 2	87	87	87	87	87	31.7↑	30.8	31.3↑	31.0	31.2↑	36.6	35.5	36.1↑	35.6	36.0	N	N	N	N	N
No. 3	88	89	89	87	87	31.8↑	31.8↑	31.3↑	30.8	32.0↑	36.0	35.9	35.1	35.4	36.6↑	N	N	N	N	N
No. 4	74↓	74↓	73↓	74↓	76↓	27.0	26.9↓	26.6↓	28.5	27.6	35.2	35.2	34.9	37.4↑	35.8	N	N	N	N	N
No. 5	81	81	82	81	83	29.6	30.0	29.7	30.1	30.3	35.4	35.5	35.0	35.8	36.2↑	N	N	N	N	N
No. 6	85	85	86	85	89	31.1↑	32.2↑	31.1	32.6↑	33.0↑	35.2	36.3↑	34.9	36.9↑	36.4↑	Dec	N	N	N	N
No. 7	85	83	84	85	87	30.2	30.4	40.0	31.7↑	31.4↑	34.2	35.2	34.3	35.9	35.7	N	N	N	N	N
No. 8	87	87	87	86	87	30.9	31.1↑	30.4	30.6	30.1	36.3	36.4↑	35.9	36.3↑	35.2	Dec	N	N	N	N
No. 9	75↓	75↓	75↓	75↓	75↓	24.8↓	25.3↓	25.2↓	24.9↓	24.9↓	33.9	34.6	34.6	33.9	34.0	N	N	N	N	N
No. 10	91	90	90	89	90	31.9↑	31.9↑	31.6↑	31.2↑	30.8	35.7	36.1	35.8	35.6	34.9	N	N	N	N	N
No. 11	83	84	83	83	83	28.8	28.9	29.1	29.2	28.3	35.2	35.1	36.0	36.1↑	35.0	N	N	N	N	N

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 8

BLOOD CHEMISTRY

Applicators	Glucose (mg/dl)					BUN (mg/dl)					Creatinine (mg/dl)					Uric Acid (mg/dl)				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	93	88	79	81	76	12	12	13	11	11	0.9	1.0	0.9	0.8	0.8	6.4	6.5	6.1	4.9	5.2
No. 2	78	83	63↓	80	75	18	17	17	19	16	1.2	1.3	1.2	1.2	1.0	6.1	6.3	6.6	6.3	5.5
No. 3	93	94	92	95	87	21	21	22	27↑	26↑	1.0	1.0	1.0	1.1	0.9	3.8	4.5	3.9	4.8	4.1
No. 4	87	112↑	86	92	86	12	14	12	13	15	1.1	1.2	1.2	1.1	1.1	7.6	7.9	7.5	8.4↑	8.1↑
No. 5	197↑	149↑	166↑	163↑	149↑	13	14	12	13	15	1.0	1.0	1.0	0.9	0.9	6.6	7.7	6.6	7.0	6.9
No. 6	111↑	81	95	91	92	18	19	18	16	18	1.2	1.3	1.2	1.1	1.2	6.3	6.6	6.4	5.8	6.8
No. 7	81	137↑	76	88	91	14	15	16	18	15	1.2	1.5↑	1.2	1.1	1.2	7.0	7.1	7.1	7.2	7.3
No. 8	122↑	102	127↑	81	107	15	15	17	13	13	1.1	1.0	1.0	1.0	0.9	5.6	5.3	5.1	5.4	4.4
No. 9	118↑	90	107	79	122↑	17	16	15	17	17	1.1	1.0	1.2	1.0	0.8	5.7	5.8	5.4	6.0	4.9
No. 10	101	84	134↑	99	140↑	15	15	16	15	14	1.0	1.1	1.2	1.1	1.0	6.3	7.0	7.3	6.9	5.9
No. 11	148↑	124↑	112↑	98	111↑	20	19	17	14	12	1.5↑	1.0	1.0	1.0	0.9	7.3	5.1	4.8	5.0	4.7

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 8 (cont.)

BLOOD CHEMISTRY

Applicators	Globulin (g/dl)					A/G Ratio					Bun/Creat					Calcium^{++} (mg/dl)				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	2.7	2.6	2.6	2.3	2.3	1.7	1.8	1.8	1.5	2.0	13.3	12.0	14.6	14.5	14.2	4.3	4.3	4.3	4.5	4.2
No. 2	2.5	2.5	2.4	2.5	2.2	1.9	1.9	1.9	1.8	2.1	15.0	13.1	14.2	15.6	16.2	4.3	4.2	4.1	4.1	4.2
No. 3	2.4	2.4	2.3	2.4	2.2	2.1	2.0	2.2	2.0	2.2	21.0	21.0	22.0	24.5	28.5	4.4	4.5	4.5	4.3	4.5
No. 4	2.5	2.5	2.6	2.3	2.2	1.6	1.7	1.6	1.8	1.9	10.5	11.2	9.7	12.0	14.3	4.4	4.4	4.3	4.4	4.3
No. 5	3.2	3.5	3.4	3.2	2.8	1.4	1.3	1.3	1.4	1.7	12.4	13.5	12.8	13.6	16.2	3.8	3.8	3.9	4.0	4.0
No. 6	3.0	2.8	2.9	2.6	2.2	1.6	1.7	1.6	1.8	2.1	14.7	14.1	14.2	14.2	14.5	4.2	4.5	4.3	4.4	4.4
No. 7	3.1	3.1	2.9	2.7	2.5	1.6	1.4	1.5	1.6	1.8	11.7	10.1	13.6	15.7	12.7	4.2	4.2	4.3	4.4	4.3
No. 8	2.5	2.6	2.4	2.4	2.6	1.9	1.4	1.9	2.0	1.7	14.7	15.2	16.9	13.9	15.4	4.3	4.3	4.2	4.4	4.3
No. 9	2.8	2.7	2.5	2.7	2.5	1.6	1.7	1.8	1.7	1.7	15.9	15.6	12.6	17.1	20.4	4.2	4.1	4.3	4.3	4.2
No. 10	2.8	2.7	2.8	2.5	2.6	1.6	1.8	1.8	2.0	1.8	16.0	13.3	13.4	13.3	14.1	4.2	4.2	4.2	4.4	4.2
No. 11	2.5	2.7	2.8	2.7	3.0	1.9	1.7	1.6	1.7	1.5	13.3	18.3	17.3	14.2	13.7	4.3	4.2	4.1	4.3	4.3

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 8 (cont.)

BLOOD CHEMISTRY

Applicators	Alk. Phos. (IU/1)					SGOT (IU/1)					LDH (IU/1)					GGT (IU/1)					T. Bili. (mg/dl)				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	42	40	43	45	39	22	22	20	22	21	160	175	201	140	164	23	23	22	20	20	0.6	0.7	0.4	0.3	0.8
No. 2	79	79	80	82	79	25	26	24	22	23	209	224	↑227	↑231	↑235	16	16	15	15	15	0.7	0.7	0.8	0.7	0.6
No. 3	69	61	71	79	79	22	24	25	24	26	151	161	157	177	172	10	10	11	12	12	0.4	0.6	0.5	0.7	0.4
No. 4	64	62	63	63	63	11	11	16	16	15	136	152	157	144	164	24	24	24	23	21	0.5	0.5	0.7	0.6	0.4
No. 5	90	81	88	87	87	20	23	20	29	28	154	167	153	150	148	36	36	36	39	38	0.2	0.2	0.5	0.5	0.5
No. 6	75	68	76	75	70	26	23	23	36	23	166	153	176	146	145	16	15	16	14	14	0.9	0.8	1.0	↑1.3	↑1.1
No. 7	116	111	115	114	117	28	33	23	26	24	160	148	152	136	129	15	17	14	14	14	0.6	0.9	0.3	0.2	0.2
No. 8	↓46	↓51	↓44	46	45	31	32	31	20	20	152	154	157	151	154	13	14	13	17	15	0.9	0.7	0.9	0.8	0.5
No. 9	↓52	↓64	80	67	68	32	40	34	33	40	183	201	191	↑228	197	10	14	21	18	17	↑1.1	0.8	0.9	0.7	0.9
No. 10	↓69	96	111	103	99	41	32	34	23	26	195	179	187	169	165	16	10	11	14	12	↑1.1	0.8	0.9	0.9	0.8
No. 11	116	↓58	↓61	58	58	31	32	32	22	24	213	189	202	191	159	10	10	10	13	13	0.5	0.6	0.6	↑1.1	↑1.3

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 8 (cont.)

BLOOD CHEMISTRY

Applicators	Cholesterol (mg/dl)					Calcium (mg/dl)					T. Protein (g/dl)					Albumin (g/dl)				
	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3	0	X	1	2	3
No. 1	179	173	177	108↓	169	9.9	9.8	9.7	9.1	9.4	7.3	7.2	7.1	5.6↓	6.7	4.6	4.6	4.6	3.3↓	4.5
No. 2	223	221	215	219	175	9.8	9.6	9.3	9.5	9.3	7.2	7.2	7.0	7.2	6.7	4.7	4.7	4.6	4.7	4.6
No. 3	148↓	143↓	148↓	151	147↓	10.2	10.4	10.3	10.0	10.2	7.4	7.3	7.3	7.4	7.1	5.0	4.9	5.0	4.9	4.9
No. 4	170	180	170	172	172	9.6	9.7	9.4	9.7	9.2	6.7	6.8	6.7	6.6	6.4	4.1	4.3	4.1	4.3	4.2
No. 5	202	222	218	218	208	9.0	9.4	9.5	9.5	9.4	7.7	8.2↑	7.9	7.8	7.6	4.4	4.7	4.5	4.6	4.7
No. 6	210	208	209	208	192	10.0	10.6	10.2	10.1	9.7	7.7	7.6	7.5	7.3	6.9	4.7	4.8	4.6	4.7	4.7
No. 7	154	152	148↓	147↓	150	10.3	9.9	9.8	9.9	9.6	8.1↑	7.4	7.2	7.0	6.8	5.0↑	4.4	4.3	4.3	4.4
No. 8	165	169	170	180	172	9.7	9.9	9.7	10.1	9.7	7.1	7.3	7.1	7.2	7.1	4.6	4.8	4.7	4.8	4.5
No. 9	153	160	163	163	149↓	9.7	9.5	9.6	10.0	9.4	7.3	7.3	7.0	7.5	6.4	4.5	4.6	4.4	4.8	4.4
No. 10	158	168	170	168	155	9.6	10.0	10.0	10.5	9.7	7.3	7.6	7.6	7.5	7.3	4.5	4.9	4.9	4.9	4.7
No. 11	161	149↓	160	160	157	9.9	9.6	9.5	9.9	9.9	7.3	7.1	7.3	7.3	7.4	4.8	4.5	4.5	4.6	4.4

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 9

ACID PHOSPHATASE AND HAPTOGLOBIN

Applicators	ACID PHOSPHATASE (IU/1)					HAPTOGLOBIN (mg/dl)				
	0	X	1	2	3	1	2	3	4	5
No. 1	0.2	0.3	0.0	0.0	0.0	22	17	20	21	25
No. 2	0.3	0.3	0.4	0.0	0.0	24	26	24	25	18
No. 3	0.2	0.1	0.1	0.0	0.0	71	89	87	82	85
No. 4	0.0	0.0	0.0	0.0	0.04	92	113	111	76	108
No. 5	0.0	0.0	0.0	0.0	Missing	109	105	108	99	122
No. 6	0.0	0.0	0.0	0.02	0.12	189	197	204	174	238
No. 7	0.0	0.0	0.0	0.0	0.16	87	95	85	118	134
No. 8	0.2	0.0	0.0	Missing	Missing	79	157	81	91	72
No. 9	0.0	0.0	0.0	Missing	0.2	90	90	91	165	80
No. 10	0.0	0.0	0.0	Missing	0.1	95	154	173	86	161
No. 11	0.0	0.0	0.0	Missing	0.6	159	114	118	118	128

Legend

0 = Pretreatment

X = Posttreatment

1-3 = Days after treatment

Table 10

URINALYSIS

pH

Applicators	0	X	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a
No. 1	5.0	6.0	5.0	8.0	6.0	8.0	5.0	5.0	6.0	---	---	---	---	---	---
No. 2	6.0	6.0	5.0	6.0	6.0	6.0	6.0	6.0	5.0	---	---	---	---	---	---
No. 3	5.0	8.0	6.0	6.0	8.0	5.0	7.0	5.0	6.0	---	---	---	---	---	---
No. 4	5.0	5.0	5.0	5.0	6.0	5.0	7.0	5.0	6.0	---	---	---	---	---	---
No. 5	7.0	6.0	6.0	6.0	7.0	6.0	6.0	6.0	5.0	---	---	---	---	---	---
No. 6	5.0	6.0	6.0	6.0	6.0	8.0	6.0	6.0	6.0	---	---	---	---	---	---
No. 7	7.0	6.0	6.0	5.0	7.0	5.0	5.0	6.0	7.0	---	---	---	---	---	---
No. 8	6.0	6.0	6.0	5.0	5.0	6.0	6.0	7.0	5.0	7.0	5.0	8.0	5.0	6.0	6.0
No. 9	6.0	6.0	6.0	5.0	5.0	5.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	5.0	6.0
No. 10	6.0	6.0	6.0	5.0	6.0	5.0	5.0	5.0	6.0	---	---	---	---	---	---
No. 11	6.0	6.0	6.0	6.0	5.0	6.0	6.0	6.0	5.0	---	---	---	---	---	---

Legend

0 = Pretreatment

X = Posttreatment

1-7 = Days after treatment

a = 7:00 a.m. collection

b = 7:00 p.m. collection

Table 11

URINALYSIS

COLOR

Appli-cators	0	X	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a
No. 1	Amber Clear	Yellow Clear	Yellow Cloudy	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	---	---	---	---	---	---
No. 2	Amber Clear	Amber Turbid	Amber Turbid	Yellow Cloudy	Yellow Cloudy	Yellow Cloudy	Yellow Clear	Yellow Turbid	Yellow Turbid	---	---	---	---	---	---
No. 3	Amber Clear	Yellow Turbid	Amber Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Turbid	---	---	---	---	---	---
No. 4	Straw Cloudy	Straw Clear	Straw Clear	Straw Cloudy	Straw Clear	Yellow Cloudy	Yellow Clear	Yellow Cloudy	Yellow Cloudy	---	---	---	---	---	---
No. 5	Straw Clear	Straw Cloudy	Amber Clear	Straw Cloudy	Yellow Hazy	Yellow Cloudy	Yellow Clear	Yellow Cloudy	Yellow Cloudy	---	---	---	---	---	---
No. 6	Straw Cloudy	Straw Cloudy	Straw Clear	Straw Cloudy	Straw Hazy	Yellow Cloudy	Yellow Cloudy	Yellow Cloudy	Yellow Cloudy	---	---	---	---	---	---
No. 7	Straw Clear	Straw Clear	Yellow Hazy	Straw Clear	Straw Clear	Yellow Clear	Yellow Clear	Yellow Cloudy	Yellow Cloudy	---	---	---	---	---	---
No. 8	Yellow Sl. cloudy	Yellow Clear	Yellow Clear	Straw Hazy	Straw Hazy	Yellow Cloudy	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Hazy	Yellow Clear	Yellow Hazy	Yellow Cloudy	Yellow Hazy	Yello Clear
No. 9	Yellow Clear	Amber Clear	Yellow Clear	Straw Hazy	Straw Hazy	Yellow Cloudy	Yellow Clear	Yellow Cloudy	Yellow Clear	Yellow Hazy	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yello Clear
No. 10	Yellow Clear	Yellow Clear	Yellow Cloudy	Straw Clear	Straw Clear	Straw Clear	Yellow Clear	Yellow Clear	Yellow Clear	---	---	---	---	---	---
No. 11	Yellow Cloudy	Yellow Clear	Yellow Clear	Straw Clear	Straw Clear	Yellow Clear	Yellow Clear	Yellow Clear	Yellow Turbid	---	---	---	---	---	---

Legend

0 = Pretreatment 1-7 = Days after treatment b = 7:00 p.m. collection

X = Posttreatment a = 7:00 a.m. collection

Table 12

URINALYSIS

SPECIFIC GRAVITY

Applicators	0	X	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a
No. 1	1.022	1.010	1.026	1.007	1.015	1.010	1.023	1.010	1.006	---	---	---	---	---	---
No. 2	1.015	1.028	1.027	1.028	1.028	1.024	1.024	1.026	1.029	---	---	---	---	---	---
No. 3	1.033	1.028	1.033	1.023	1.030	1.026	1.033	1.020	1.030	---	---	---	---	---	---
No. 4	1.024	1.015	---	1.022	1.008	1.020	1.020	1.020	1.016	---	---	---	---	---	---
No. 5	1.018	1.024	1.019	1.022	1.018	1.020	1.022	1.022	1.016	---	---	---	---	---	---
No. 6	1.029	1.028	1.025	1.024	1.026	1.017	1.029	1.024	1.028	---	---	---	---	---	---
No. 7	1.012	1.014	1.016	1.016	1.017	1.014	1.020	1.014	1.012	---	---	---	---	---	---
No. 8	1.026	1.026	1.020	1.022	1.026	1.027	1.027	1.012	1.015	1.017	1.023	1.024	1.030	1.026	1.026
No. 9	1.013	1.027	1.025	1.025	1.031	1.031	1.030	1.032	1.015	1.030	1.025	1.020	1.015	1.023	1.023
No. 10	1.017	1.018	1.026	1.007	1.008	1.006	1.021	1.011	1.018	---	---	---	---	---	---
No. 11	1.025	1.016	1.017	1.020	1.022	1.012	1.016	1.012	1.025	---	---	---	---	---	---

Legend

0 = Pretreatment

X = Posttreatment

1-7 = Days after treatment

a = 7:00 a.m. collection

b = 7:00 p.m. collection

Table 13

URINALYSIS

Appli-cators	0	A	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a
#1	BACTERIA Few CRYSTALS Calcium Oxalate	EPITH: Occas. CRYSTALS Urates	CRYSTALS Uric acid, Urates	EPITH: Rare	EPITH: 1-2	EPITH: 0-1	WBC:0-1 EPITH: Rare CRYSTALS 4-6 Calcium Oxalate	WBC: Occas. EPITH: Occas.	WBC:2-4 RBC: Occas. EPITH: Few	-	-	-	-	-	-
#2	BACTERIA Few	BACTERIA Few EPITH: Few	CRYSTALS Calcium Oxalate	EPITH: Few CRYSTALS Urates	CRYSTALS Urates	WBC:0-1 EPITH: 1-2 CRYSTALS 0-1 Calcium Oxalate	WBC Rare EPITH: 1-2 CRYSTALS 0-1 Calcium Oxalate	EPITH: Occas. CRYSTALS Lg. amt. amorphous urates, few calcium oxalate	CRYSTALS Lg. amt. amorphous urates, few uric acid	-	-	-	-	-	-
#3	BACTERIA Few CRYSTALS Calcium Oxalate	BACTERIA Few PROTEIN: +1	WBC:2-3 CRYSTALS Calcium Oxalate	WBC:0-1 EPITH: Occas. BACTERIA Few CRYSTALS Urates	PROTEIN: +1 WBC:1-2 EPITH: Few CRYSTALS Phosphates	EPITH: 0-1 Few mucus threads	WBC Rare EPITH: 1-2	WBC:4-6 EPITH: Occas. Many mucus threads, spermatozoa present	EPITH: Occas. CRYSTALS Lg. amt. amorphous urates	-	-	-	-	-	-
#4	WBC:0-1 CRYSTALS Full field amorphous urates	WBC:0-1 EPITH: 0-1	WBC:0-1 EPITH: 0-1	WBC:0-1 EPITH: 0-1 CRYSTALS Amorphous urates	WBC:0-1 EPITH: Few	BACTERIA Moderate CRYSTALS Urates	WBC:1-2 CRYSTALS Urates BLOOD 1+ PROTEIN 1+	EPITH: Occas. CRYSTALS Urates	WBC:2-3 EPITH: Few CRYSTALS Urates	-	-	-	-	-	-
#5	WBC:0-1 EPITH: Few CRYSTALS Few amorphous urates	WBC:0-1 CRYSTALS Few Calcium Oxalates, many amorphous urates	WBC:1-2 EPITH: 0-1 CRYSTALS Calcium Oxalate, Few	WBC:0-2 EPITH: Few CRYSTALS Amorphous urates GLUCOSE Trace	WBC:0-1 EPITH: Few CRYSTALS Few Calcium Oxalate, many amorphous urates	EPITH: Few CRYSTALS Calcium Oxalate, Urates	WBC:0-1 CRYSTALS Uric acid, calcium Oxalate GLUCOSE Trace	EPITH: Occas. CRYSTALS Urates	CRYSTALS Urates	-	-	-	-	-	-
#6	WBC:0-1 CRYSTALS Calcium Oxalate, Amorphous urates	WBC:0-1 EPITH: 0-1 CRYSTALS Calcium Oxalate- Few, Amorphous urates	WBC:0-1 CRYSTALS Few calcium Oxalate CASTS: 0-1 hyline casts	WBC:0-1 EPITH: Few	WBC:0-1 EPITH: Few CRYSTALS Few calcium Oxalate. Few mucus threads	EPITH: Occas. CRYSTALS Phosphates BLOOD: T+	EPITH: Occas. CRYSTALS Urates	WBC:1-2 CRYSTALS Urates	CRYSTALS Uric acid, Urates	-	-	-	-	-	-
#7	WBC:0-1 EPITH: 0-1	WBC:0-1 EPITH: 0-1	WBC:0-1 EPITH: 0-1	WBC:0-1 EPITH: Few	WBC:0-1 EPITH: Few	WBC:0-1 EPITH: Few BACTERIA Few CRYSTALS Urates	CRYSTALS Amorphous Sediments BLOOD:1+	WBC:1-2 EPITH: Occas. CRYSTALS Urates	CRYSTALS Amorphous Urates	-	-	-	-	-	-
#8	WBC:1-2 EPITH: Rare CRYSTALS Occas. Calcium Oxalate, 2+ Amorphous Urates, 2+ Mucus Threads	WBC:0-1 EPITH: Rare CRYSTALS Rare Calcium Oxalate	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare	WBC:0-1	CRYSTALS Full Field Amorphous Sediment	CRYSTALS Calcium Oxalate	EPITH: Occas. BACTERIA Mod. amount. Few mucus threads	RBC:Rare EPITH: Occas. Few mucus threads	BACTERIA Many CRYSTALS Calcium Oxalate	WBC:0-1 CRYSTALS Calcium Oxalate	BACTERIA Many CRYSTALS Amorphous Phosphates	PROTEIN 1+ BACTERIA Few CRYSTALS Amorphous Urates, Calcium Oxalate	BACTERIA Moderate	WBC:0-1 RBC:Rare CRYSTALS Calcium Oxalate, 1+ Mucus threads
#9	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare CRYSTALS Rare Calcium Oxalate, 1+ Mucus Threads	WBC:0-1 EPITH: Rare CRYSTALS Frequent Calcium Oxalate, 1+ Mucus Threads	WBC:0-1 EPITH: Frequent	WBC:0-1 EPITH: Freq. CASTS: Calcium Oxalate PROTEIN: +1	CRYSTALS Calcium Oxalate, Amorphous Sediment	CRYSTALS Calcium Oxalate	EPITH: Few BACTERIA Full Field CRYSTALS Moderate amount Calcium Oxalate, Few mucus threads	EPITH: Occasional	EPITH: Moderate	CRYSTALS Calcium Oxalate	EPITH: 0-1 CRYSTALS Calcium Oxalate	EPITH: Occasional	EPITH: Occasional	CRYSTALS Calcium Oxalate
#10	WBC:0-1 EPITH: Rare CRYSTALS Frequent Calcium Oxalate	WBC:0-1 EPITH: Rare CRYSTALS Rare Calcium Oxalate	WBC:0-1 EPITH: Rare BACTERIA Full field	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare	EPITH: Rare BACTERIA Few	EPITH: Rare	EPITH: Rare	EPITH: Occasional	-	-	-	-	-	-
#11	WBC:0-1 EPITH: Rare BACTERIA Full field CRYSTALS Occasional Calcium Oxalate	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare	WBC:0-1 EPITH: Rare	- -	- -	EPITH: Rare	EPITH: Rare BACTERIA Sm. amt. CRYSTALS Lots of amorphous sediment	-	-	-	-	-	-

Figure 1. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 1

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

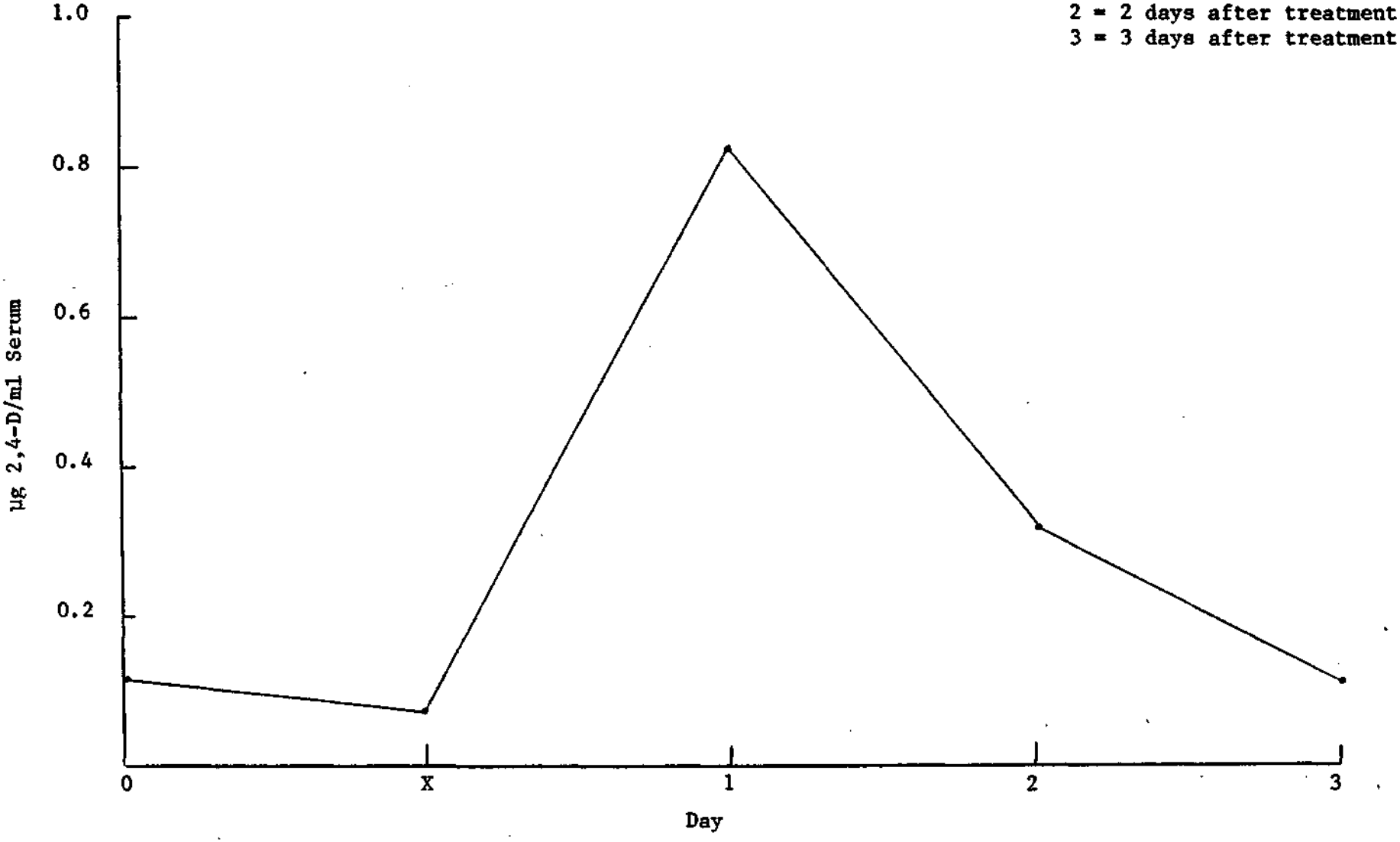


Figure 2. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 2

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

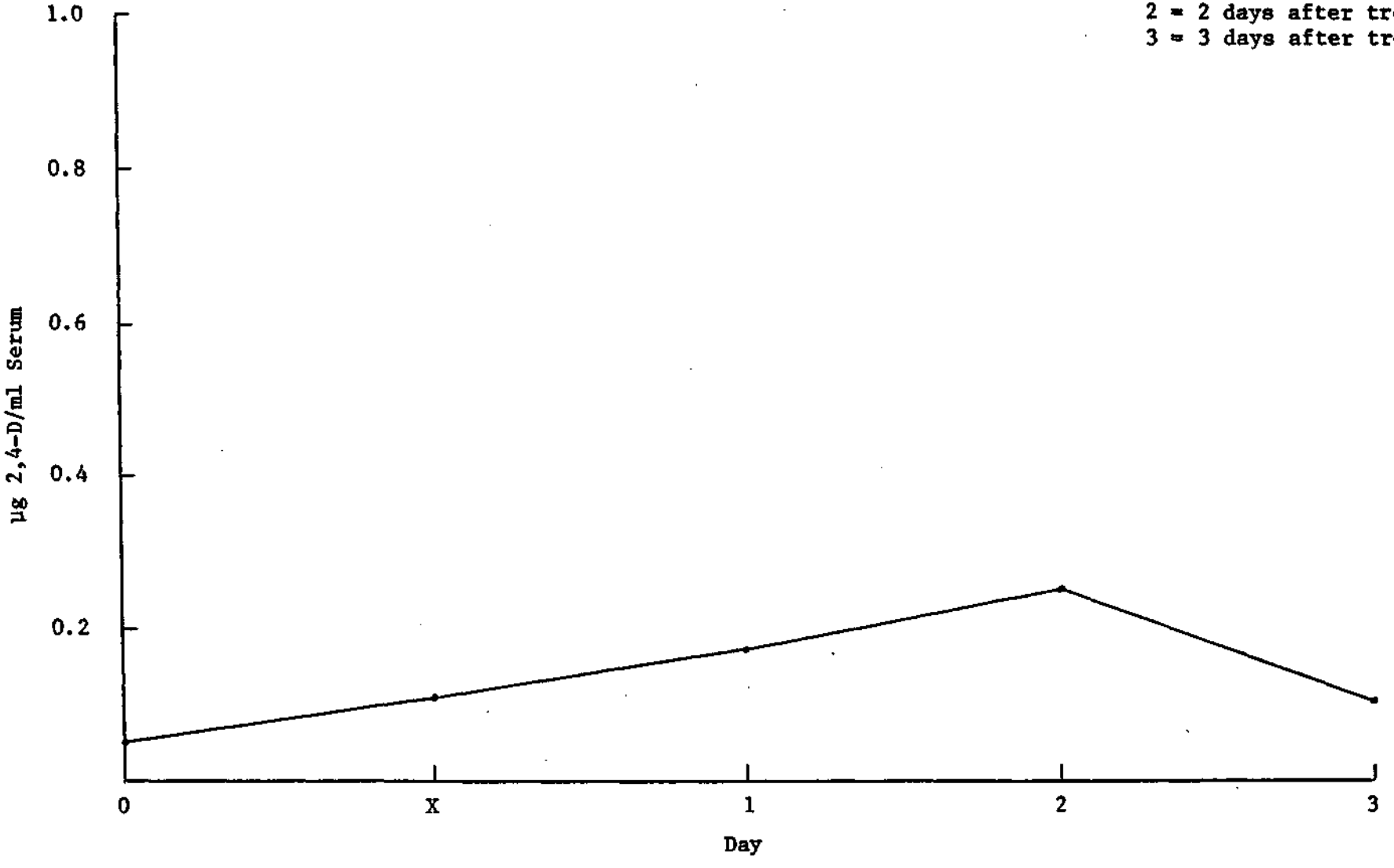


Figure 3. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 3

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

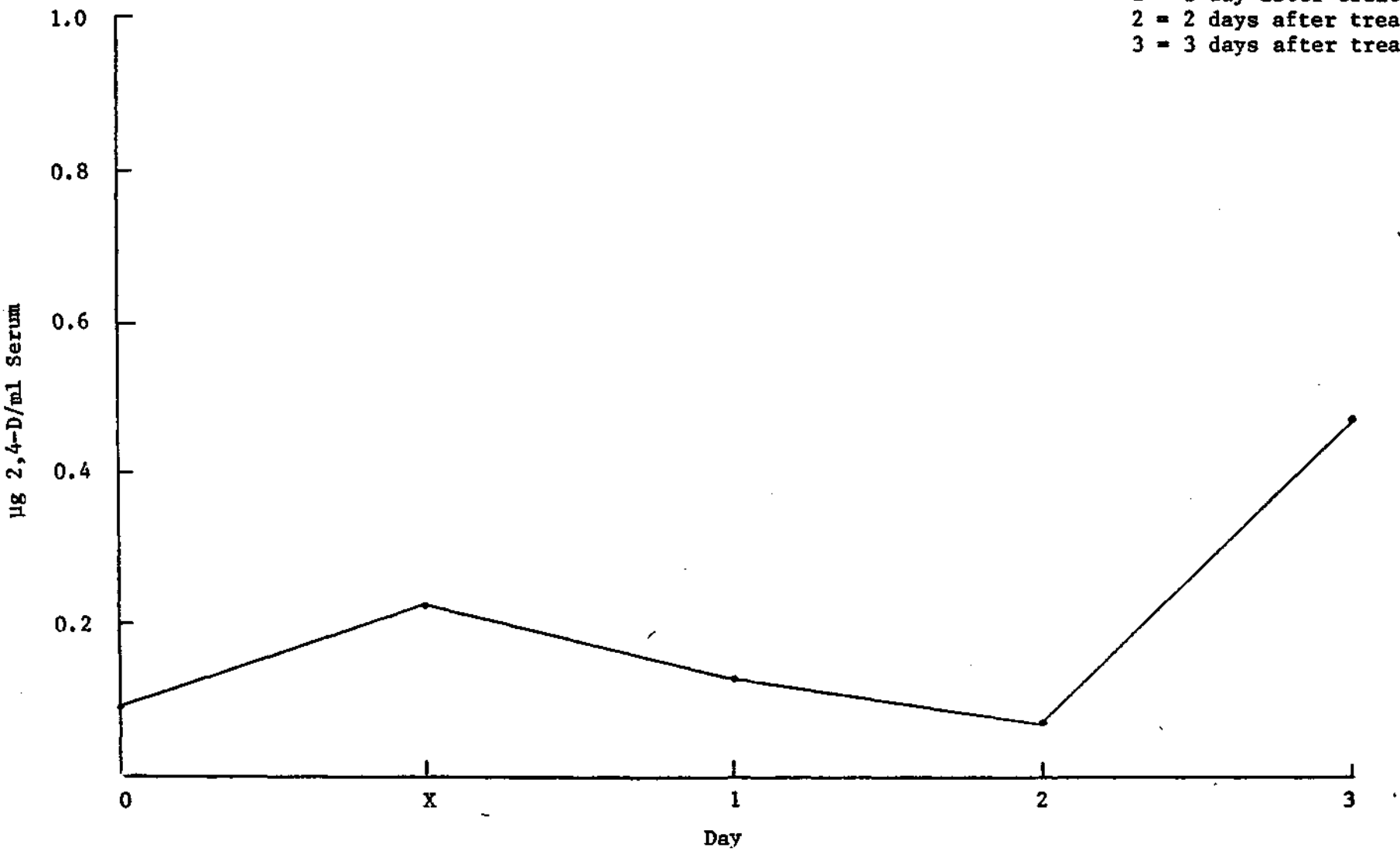


Figure 4. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 4

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

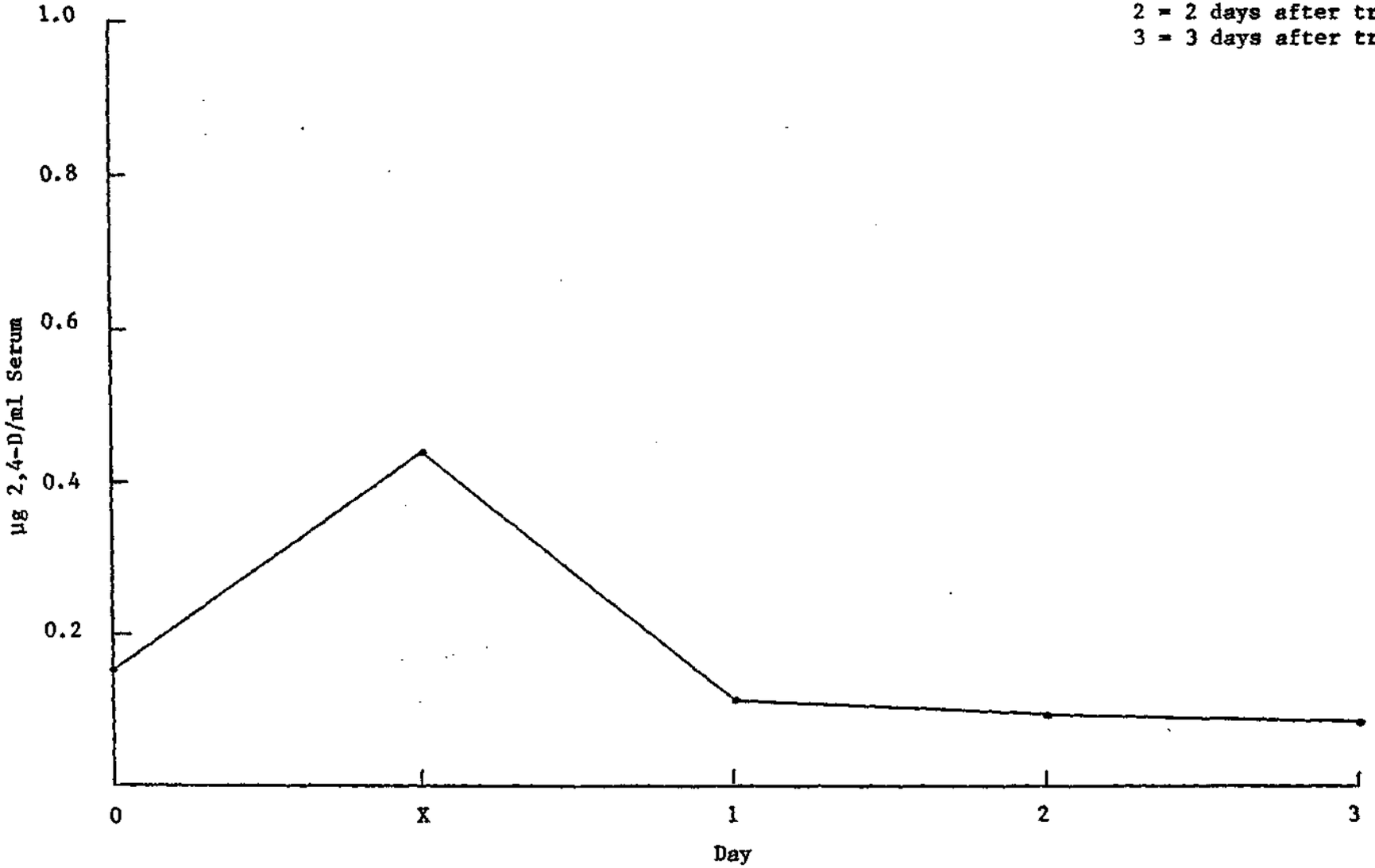


Figure 5. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 5

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

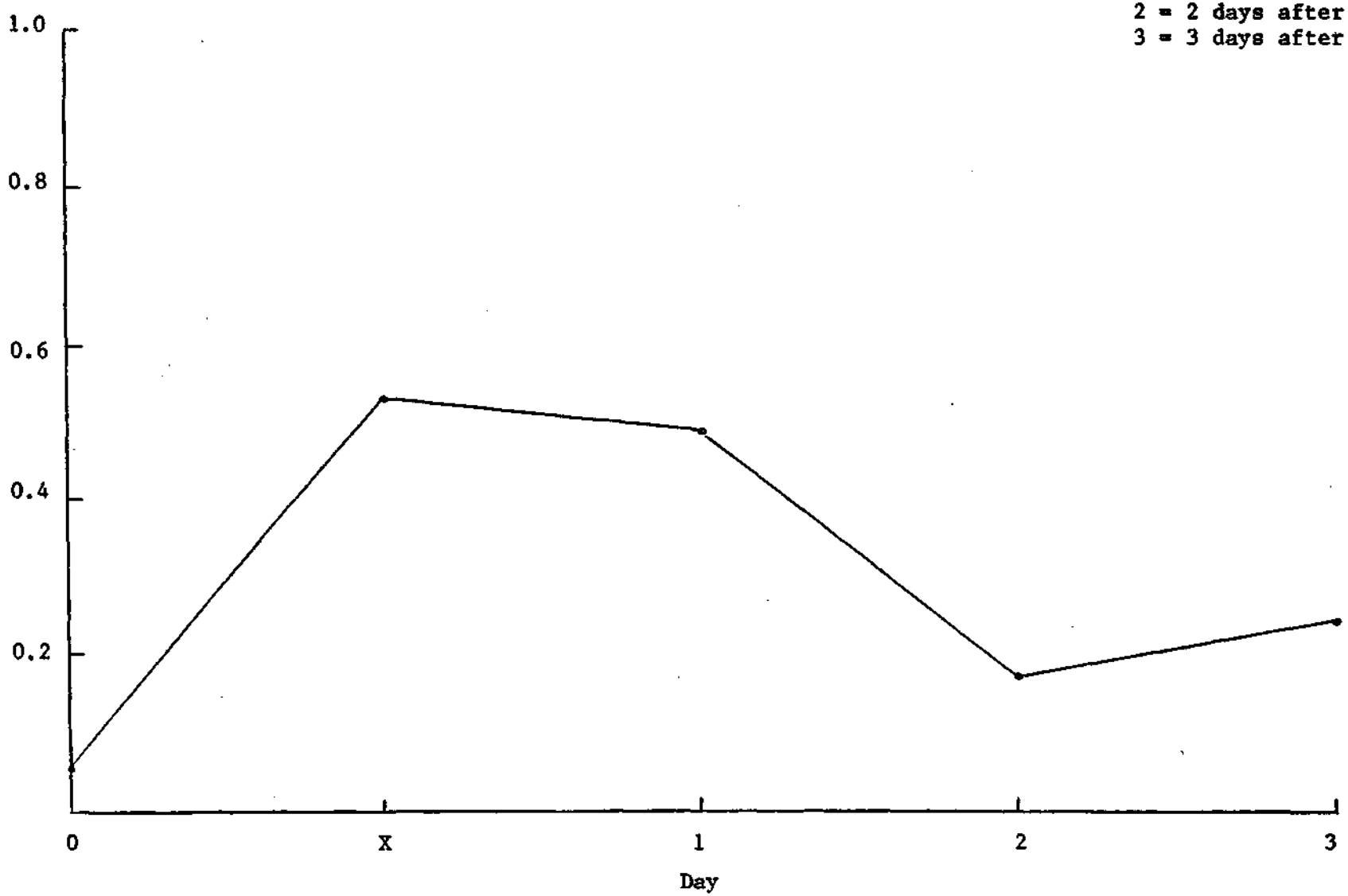


Figure 6. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

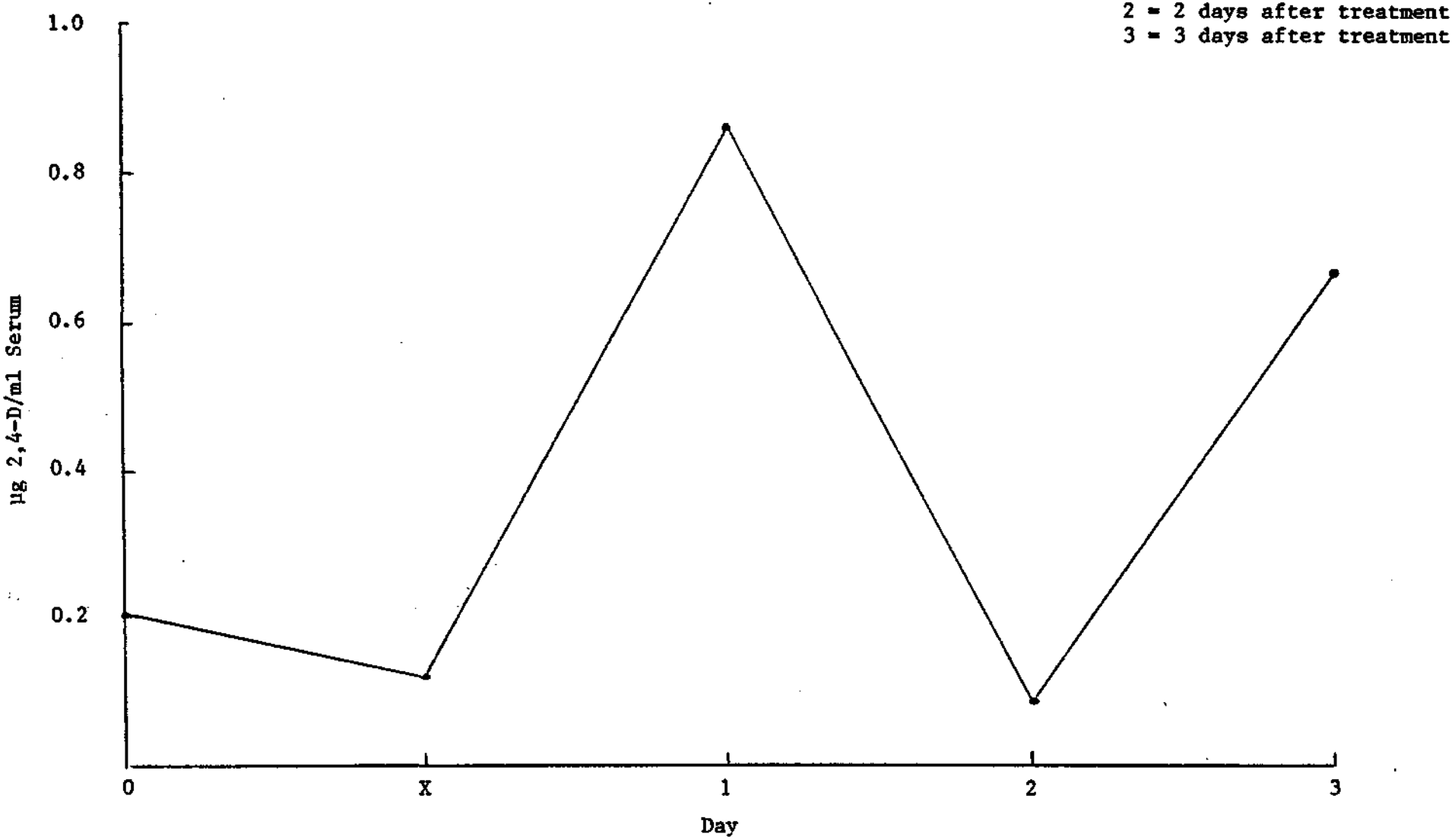


Figure 7. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 7

0 = Pretreatment
X = Posttreatment
1 = a day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

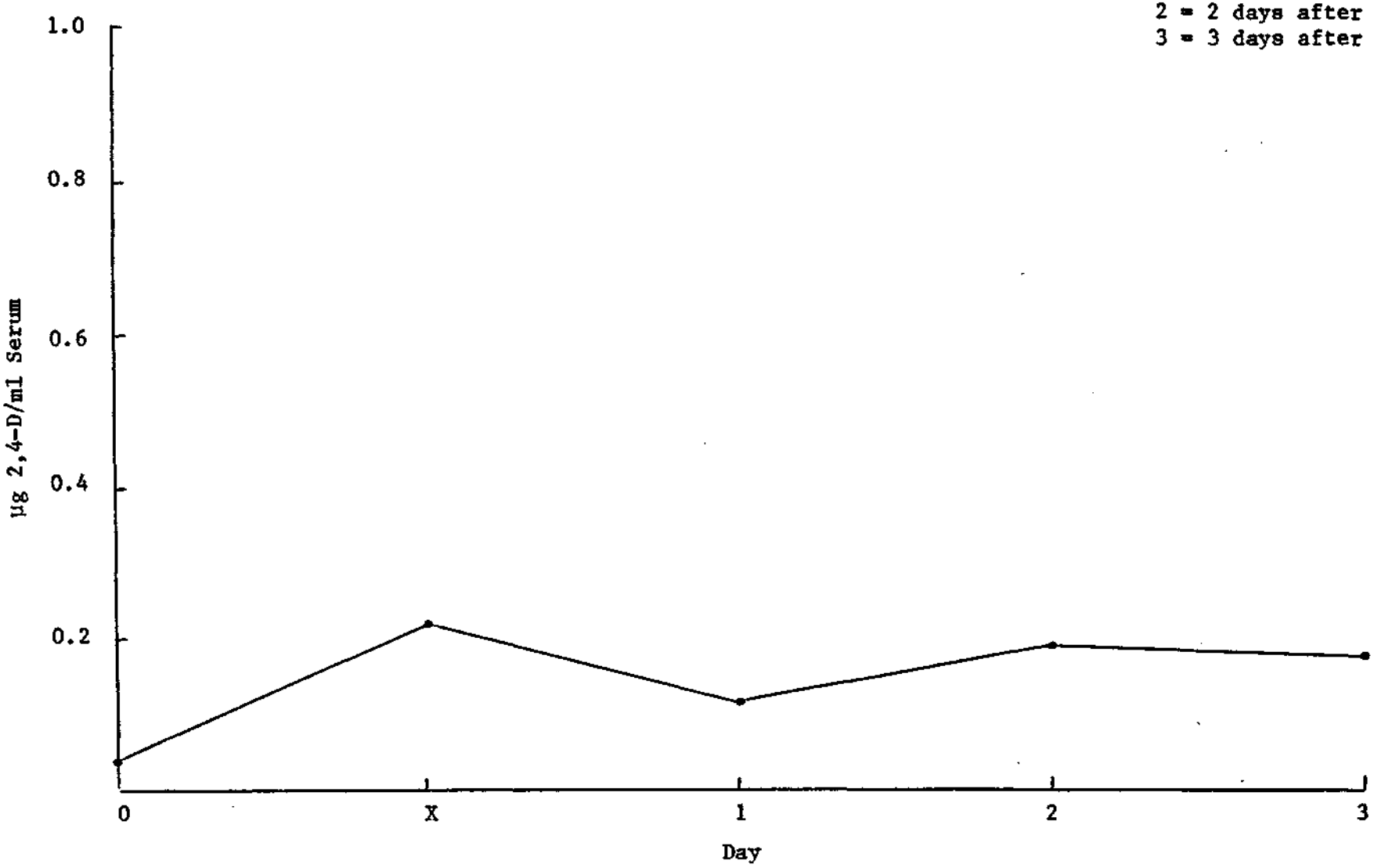


Figure 8. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 8

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

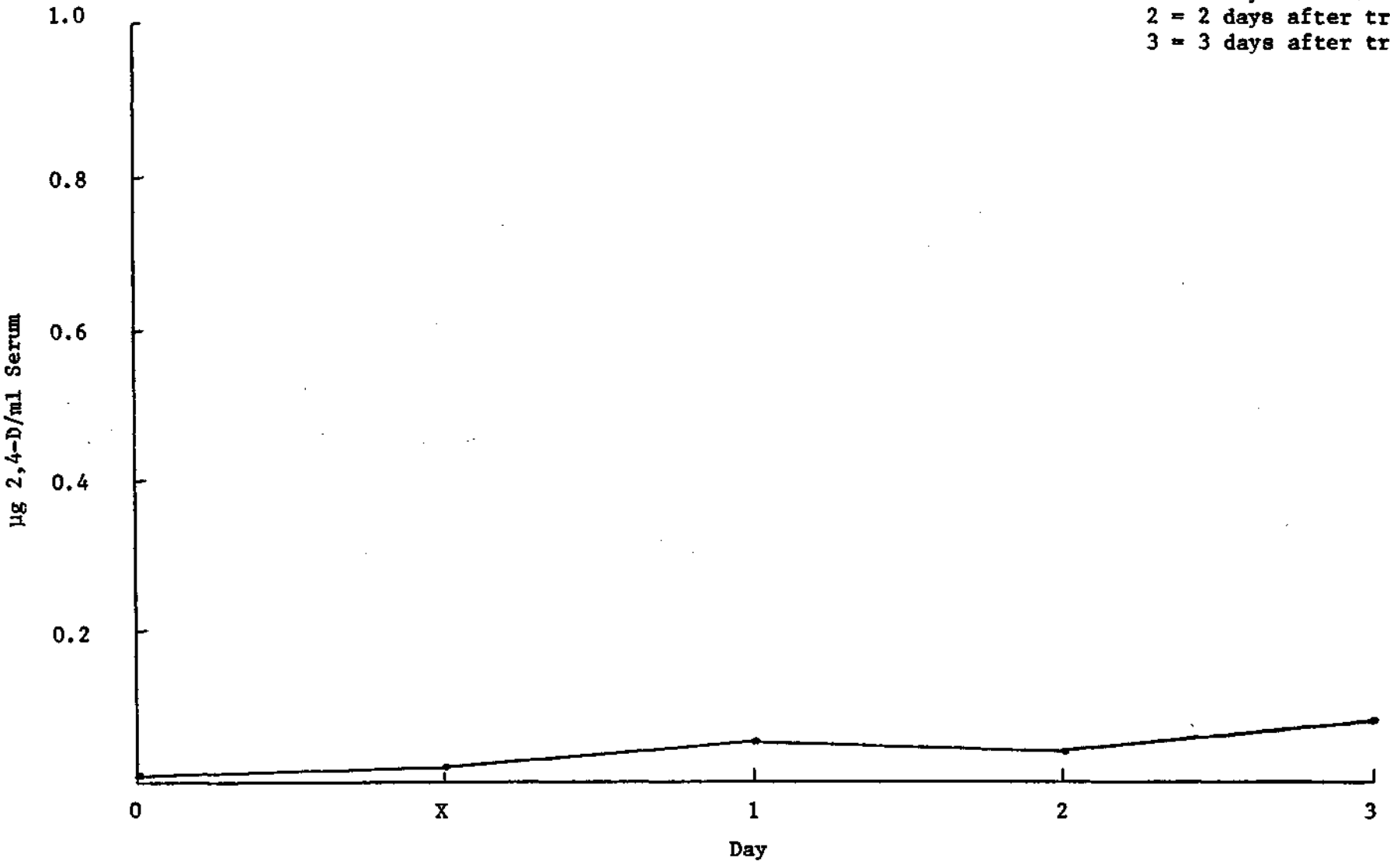


Figure 9. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 9

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

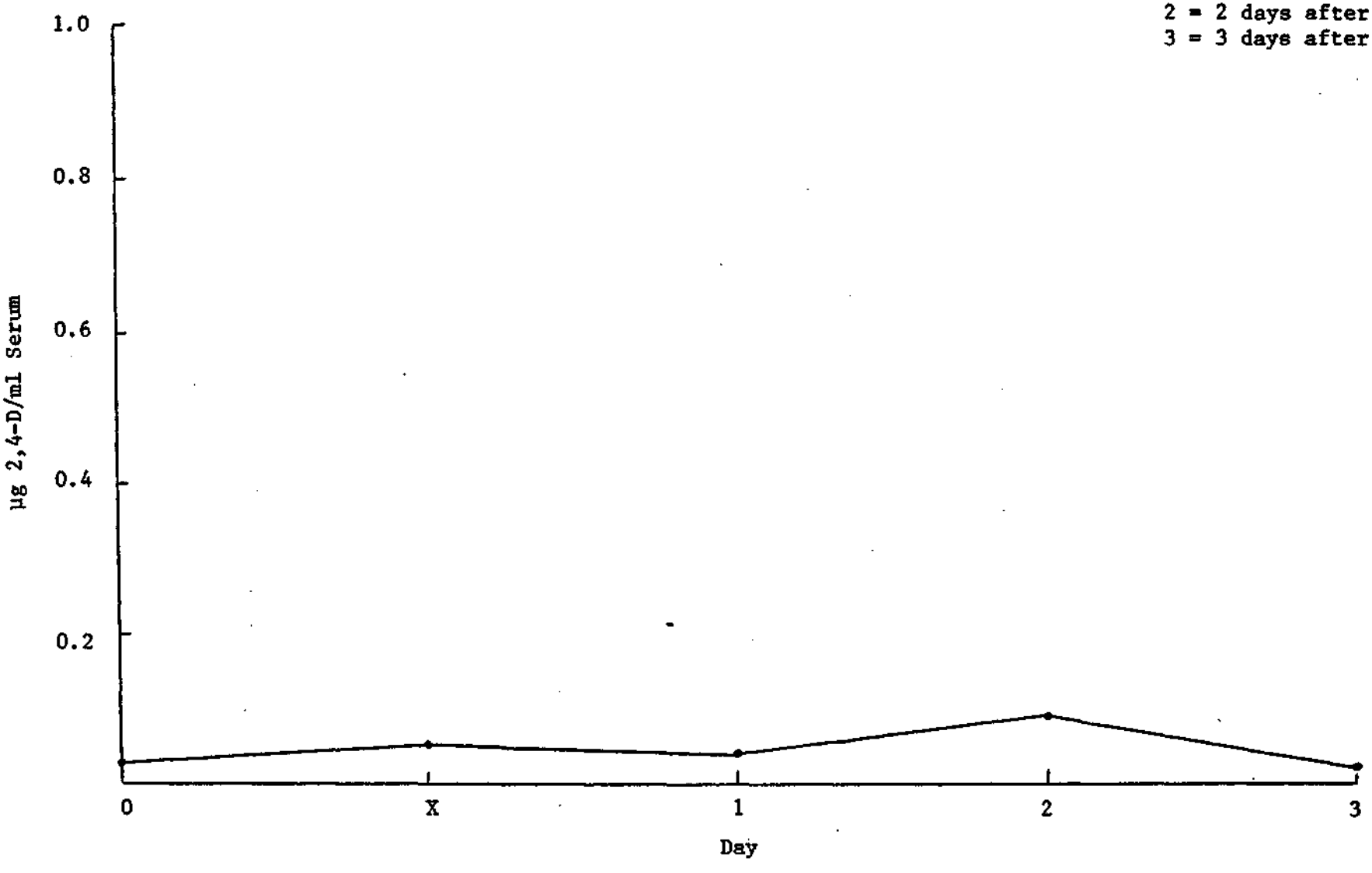


Figure 10. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 10

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

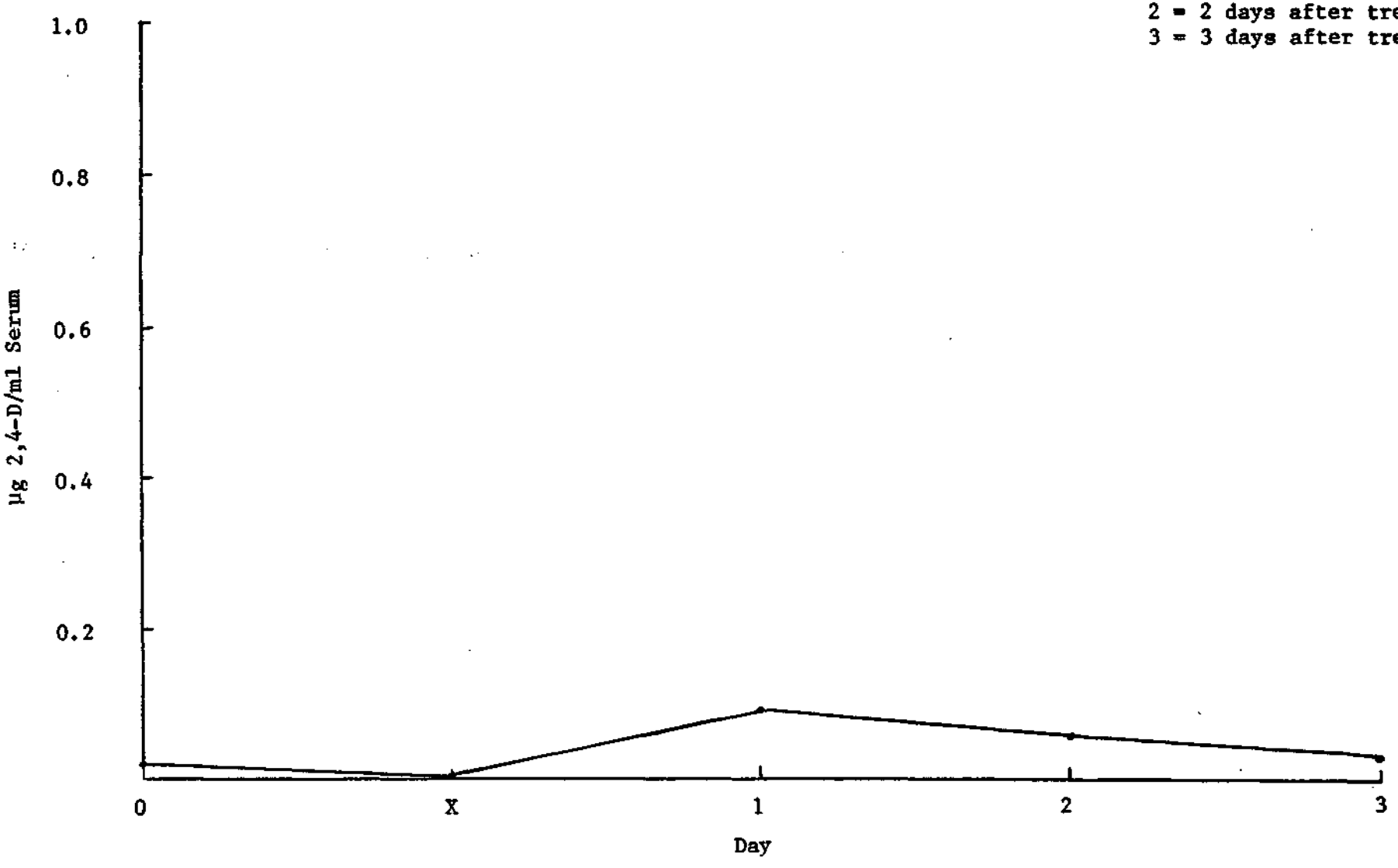


Figure 11. 2,4-D and Endogenous Phenolic Compounds Measured in Serum

Subject No. 11

0 = Pretreatment
X = Posttreatment
1 = 1 day after treatment
2 = 2 days after treatment
3 = 3 days after treatment

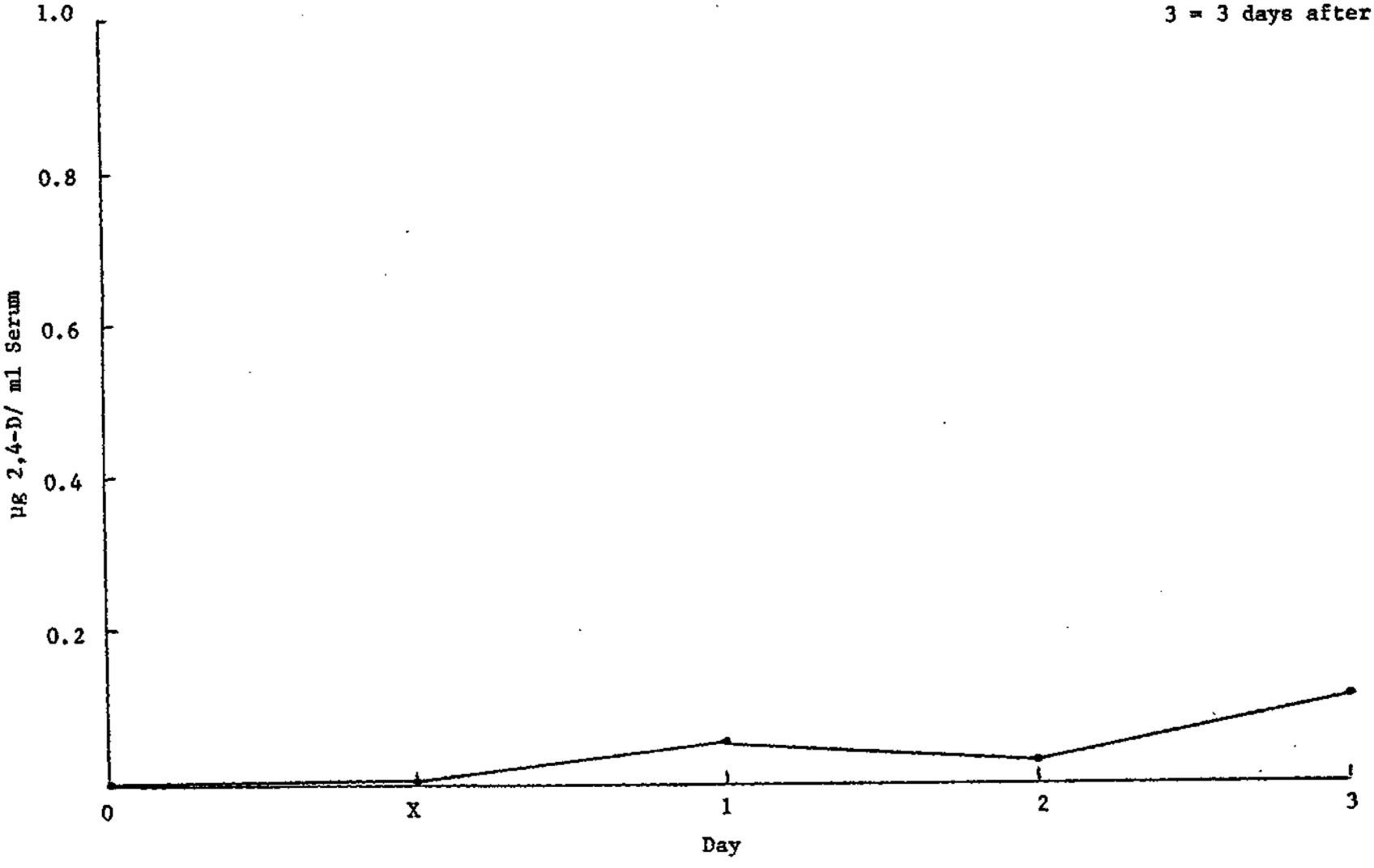


Figure 12. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

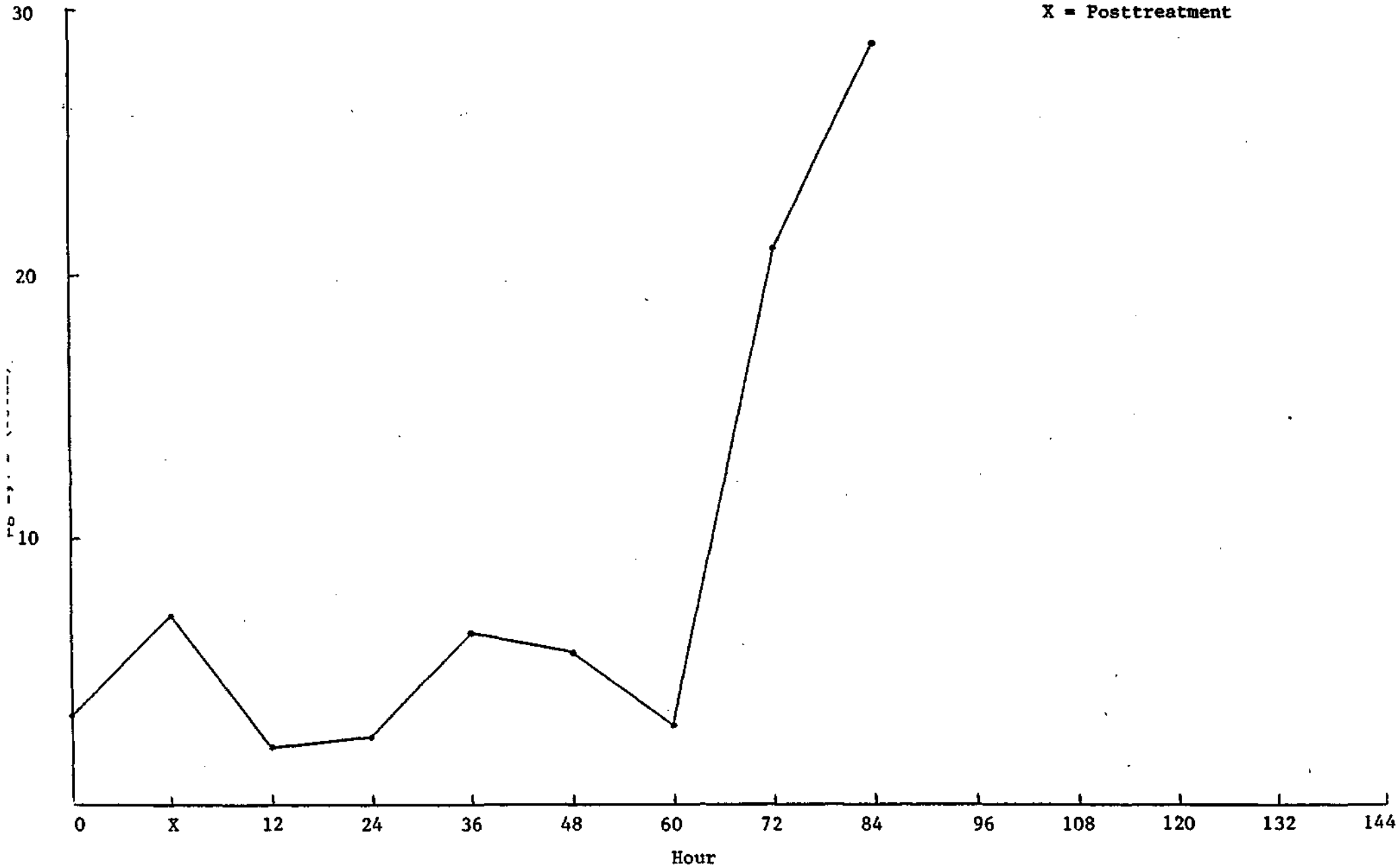


Figure 13. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

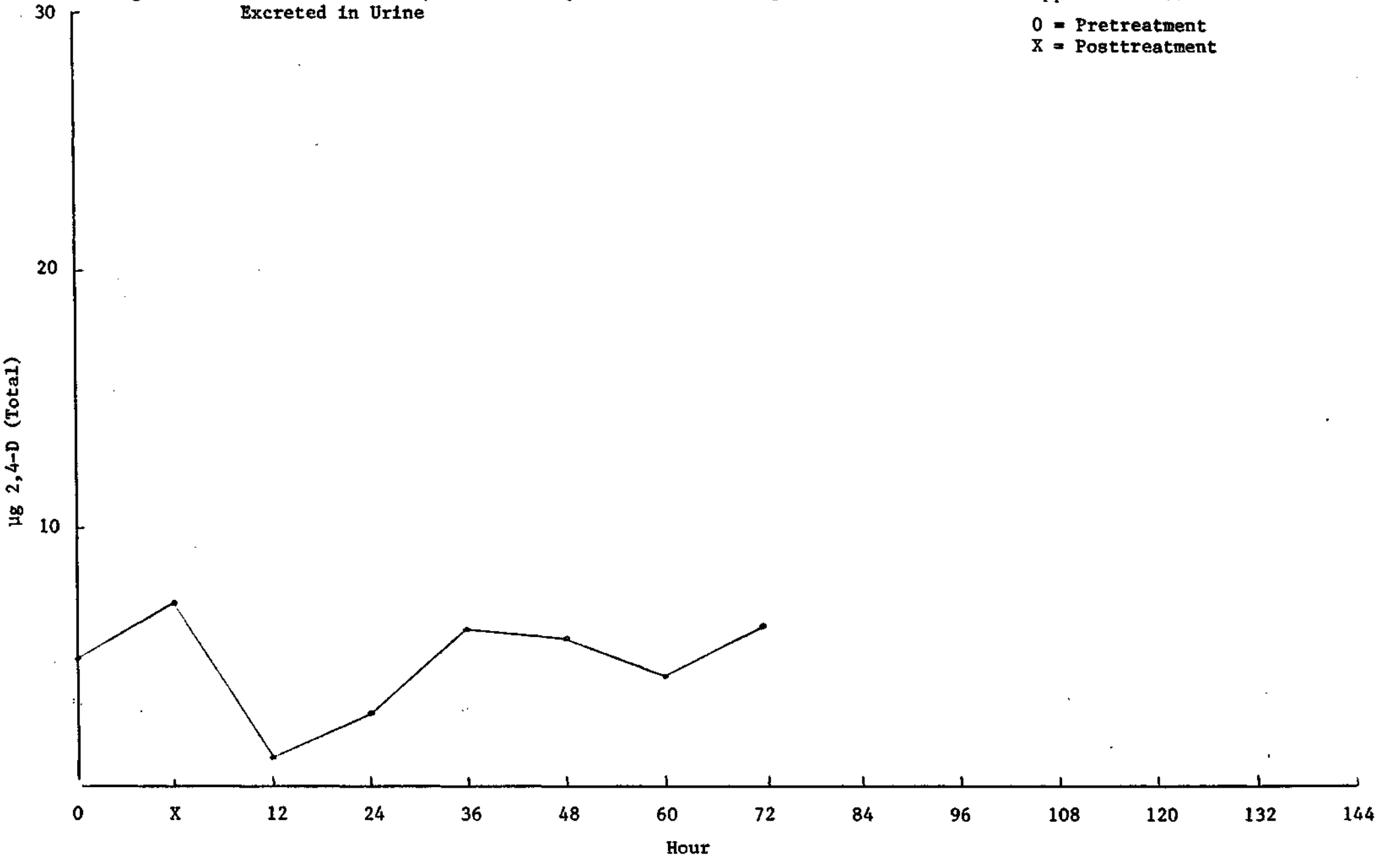


Figure 14. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 3

0 = Pretreatment
X = Posttreatment

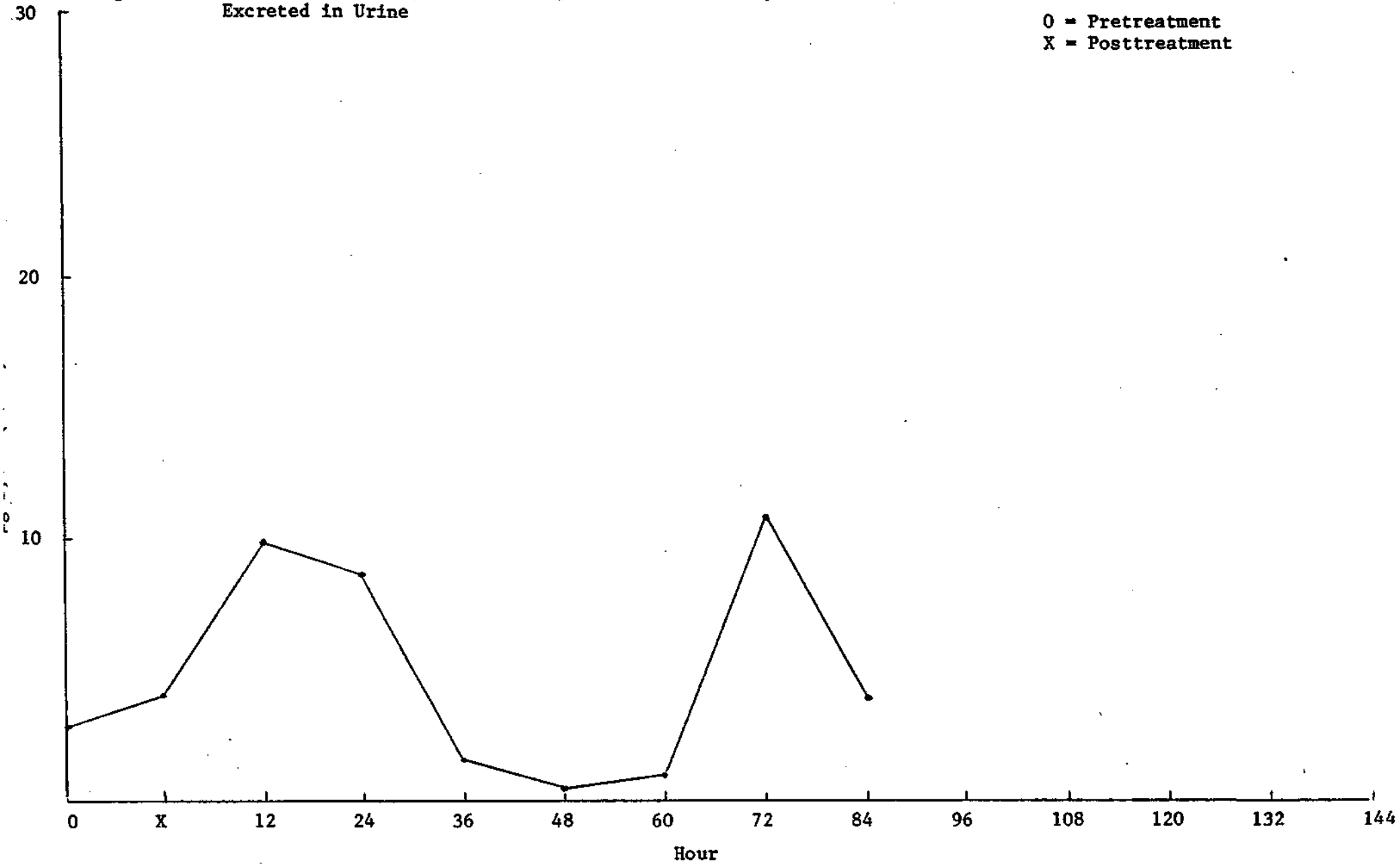


Figure 15. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

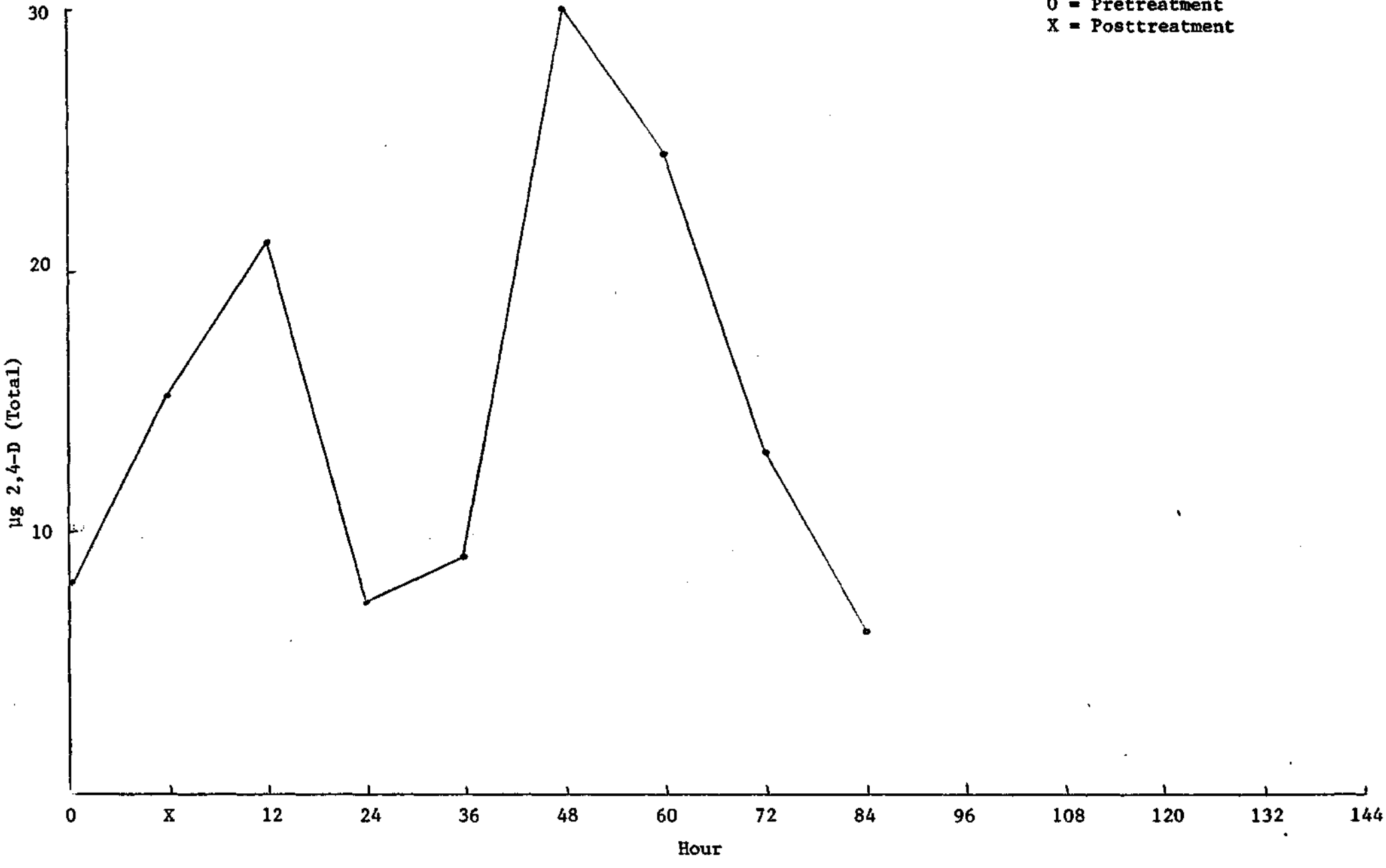


Figure 16. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 5

0 = Pretreatment
X = Posttreatment

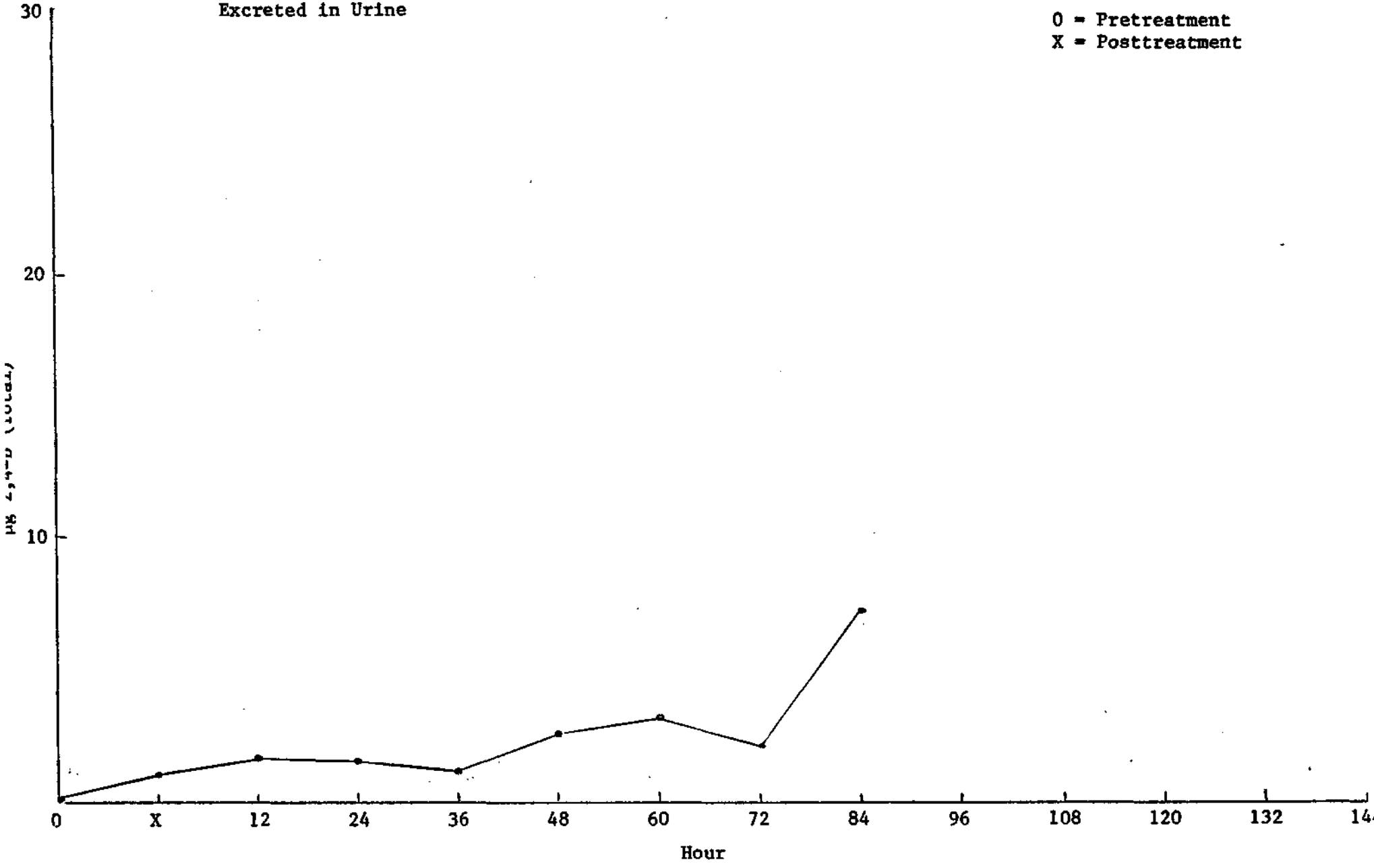


Figure 17. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 6

0 = Pretreatment
X = Posttreatment

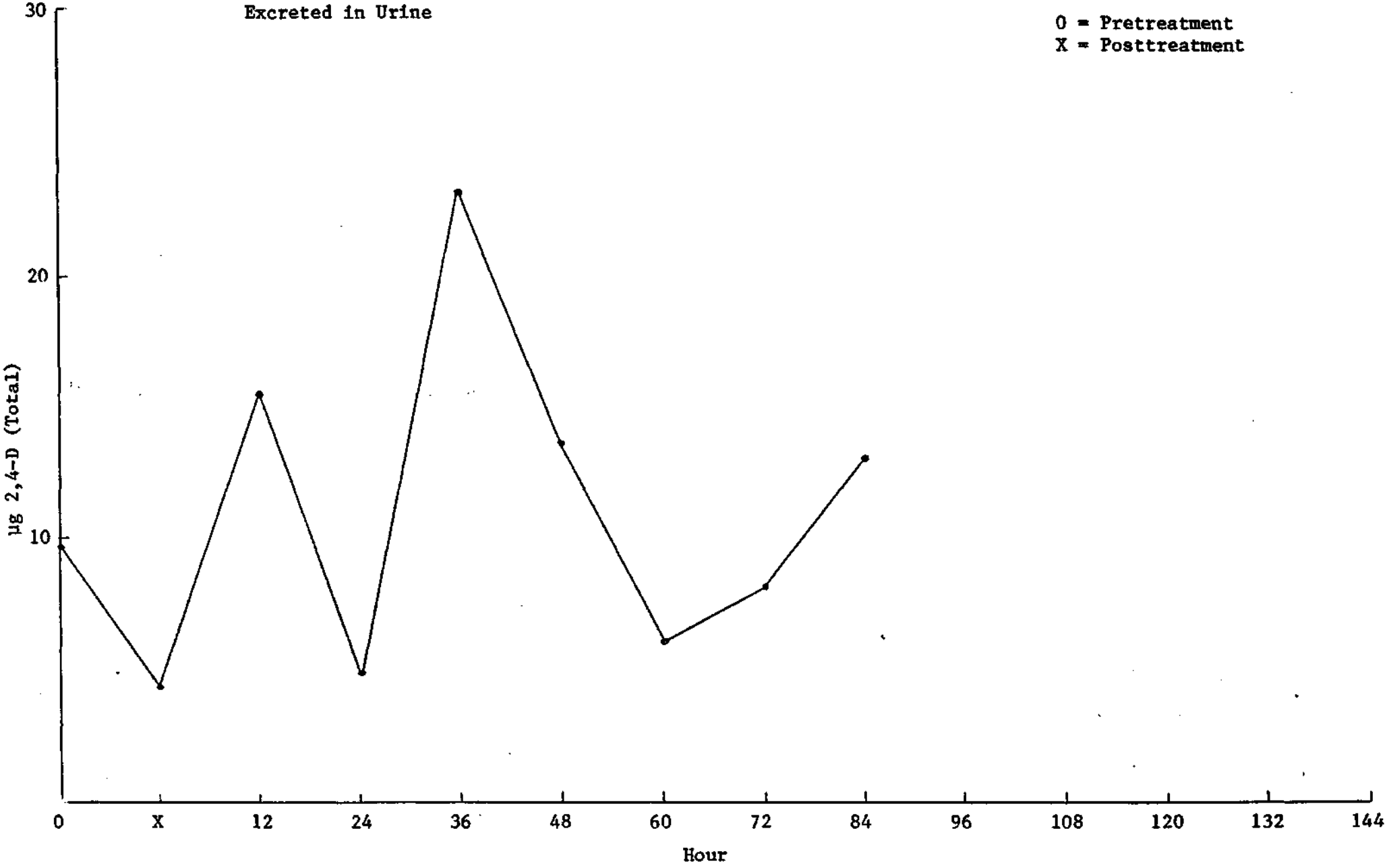


Figure 18. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 7

0 = Pretreatment
X = Posttreatment

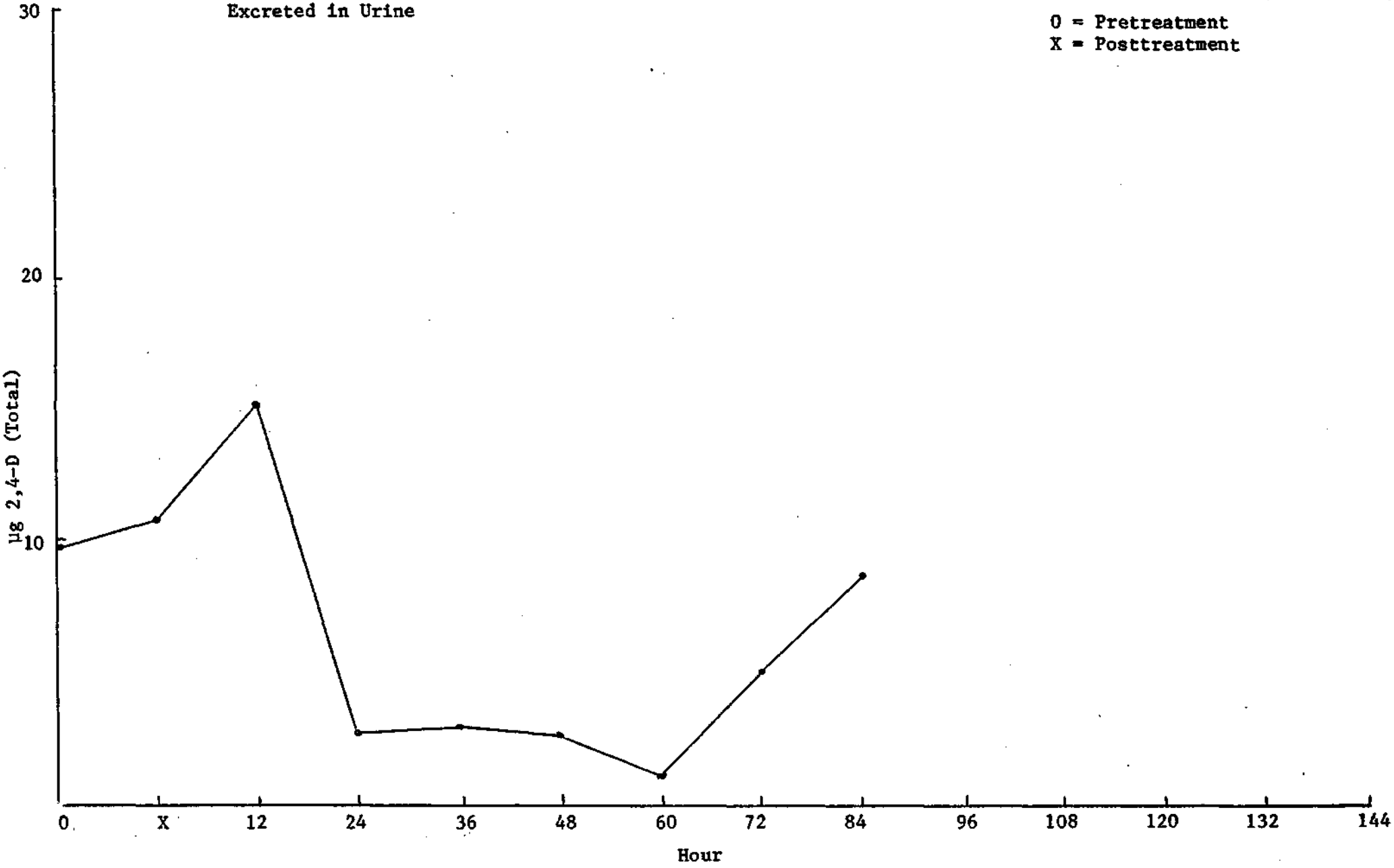


Figure 19. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 8

0 = Pretreatment
X = Posttreatment

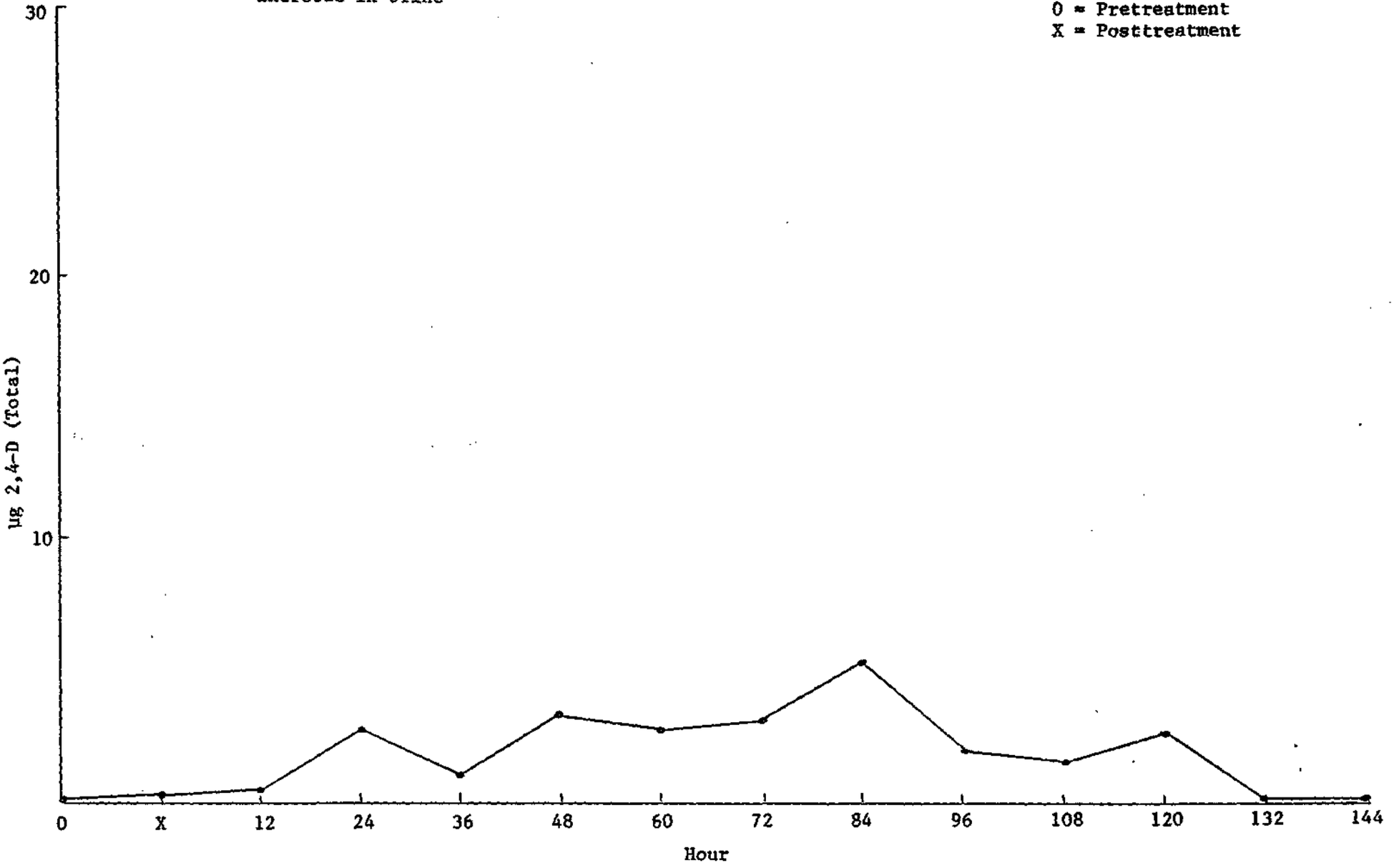


Figure 20. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 9

0 = Pretreatment
X = Posttreatment

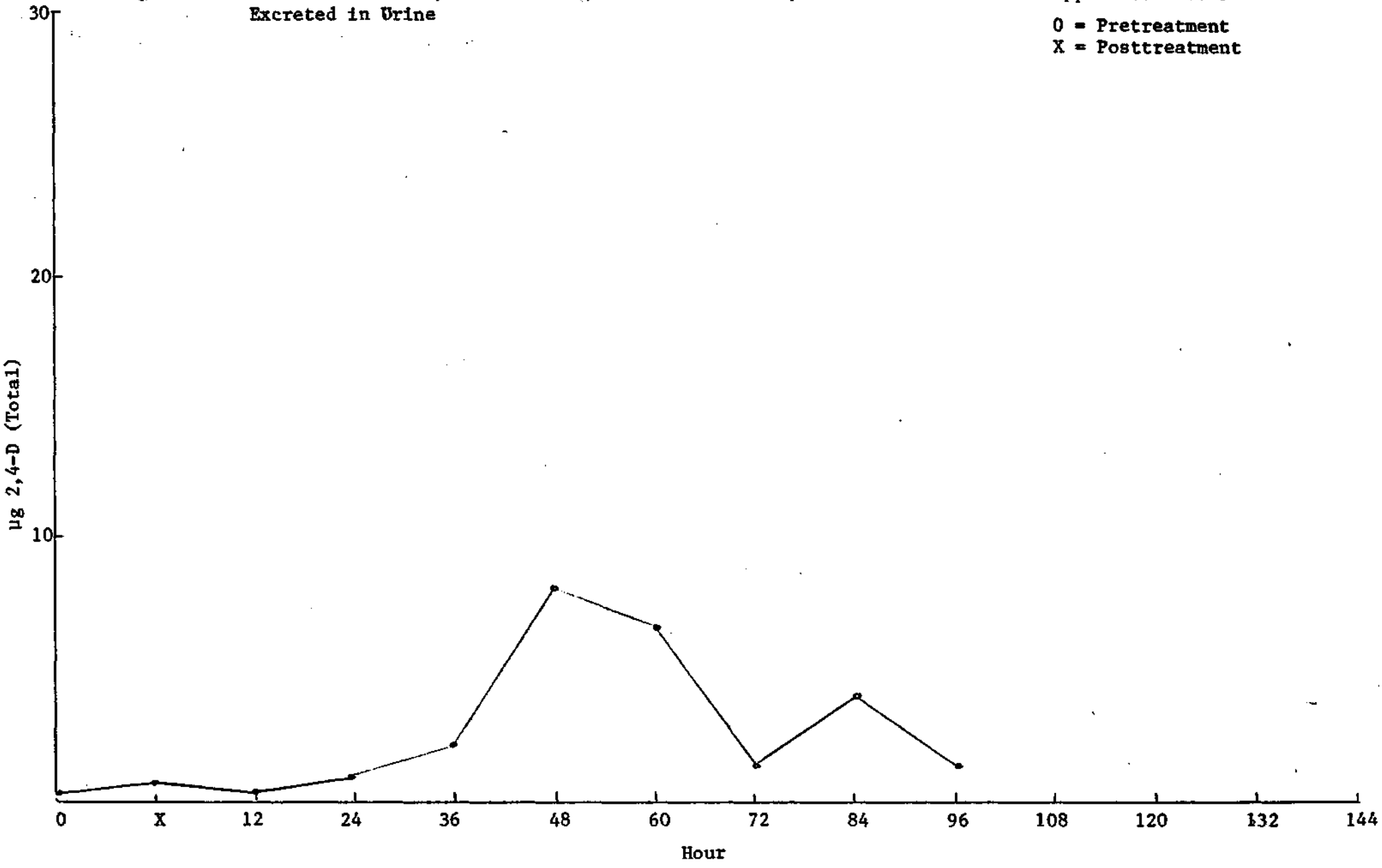


Figure 21. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

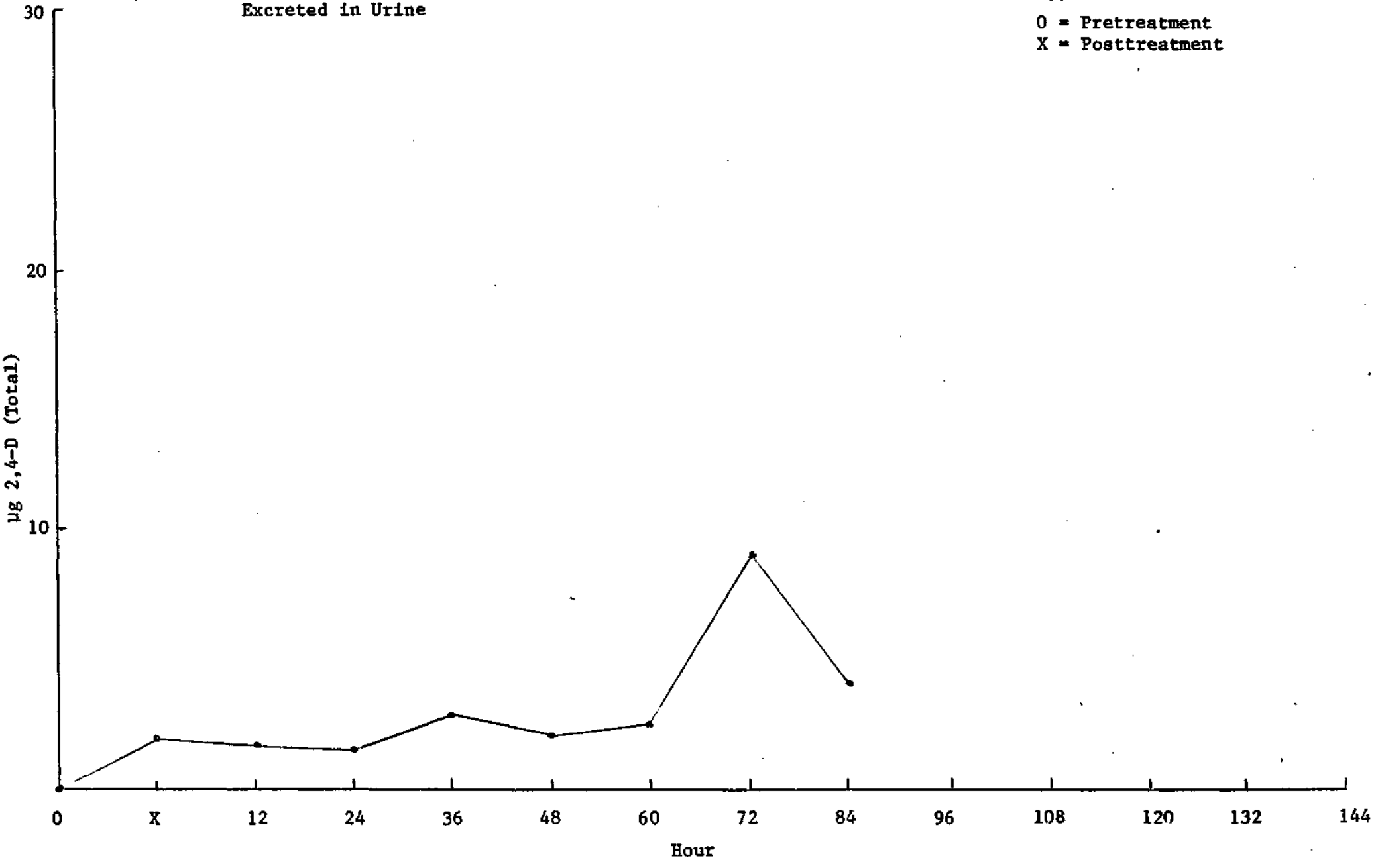
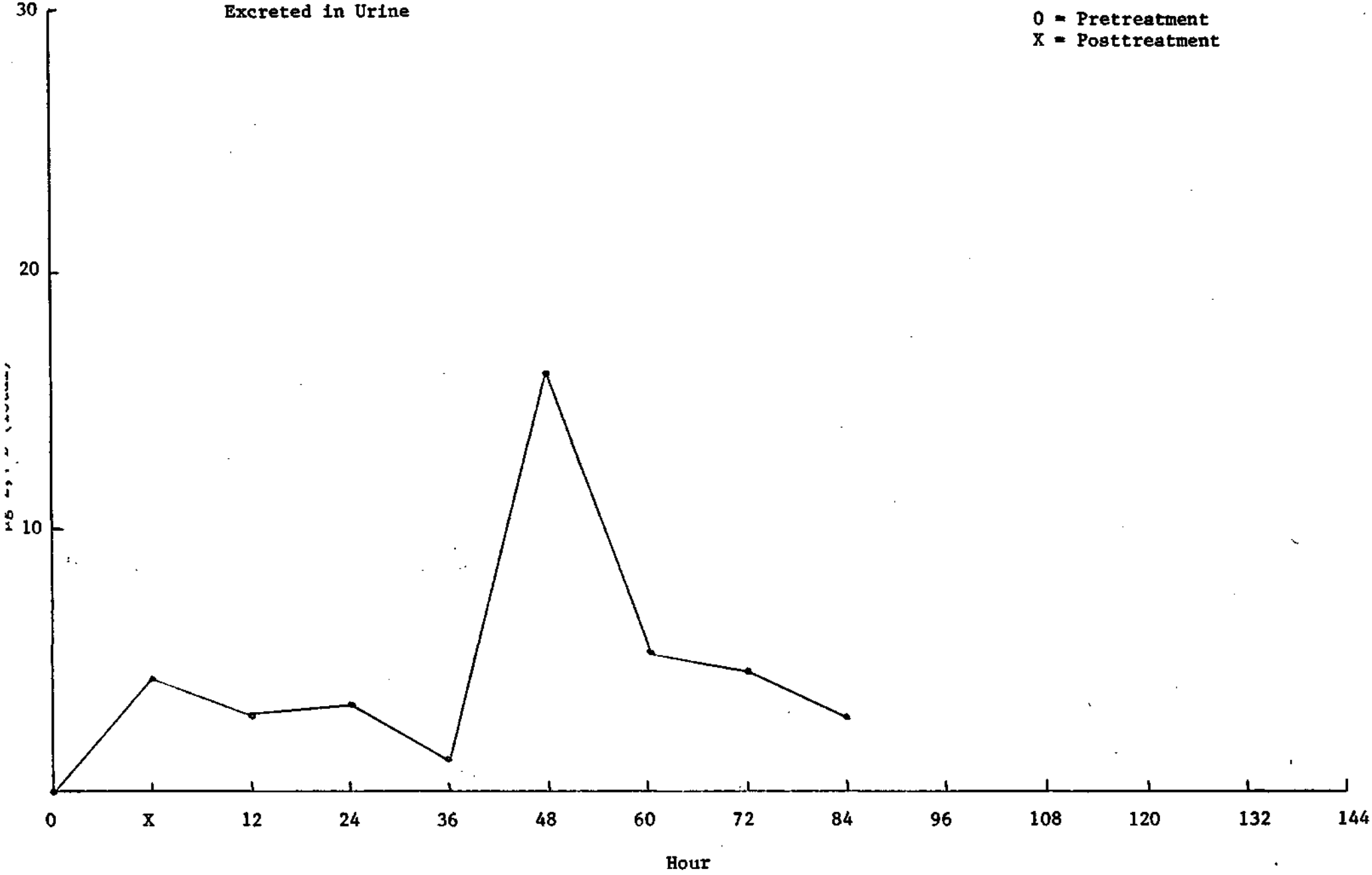


Figure 22. Amount of Total 2,4-D and Endogenous Phenolic Compounds Excreted in Urine

Applicator No. 11

0 = Pretreatment
X = Posttreatment



Appendix

Methodology for 2,4-D Analysis

Methods for Analysis of 2,4-D

In order to select the most recent analytic techniques for 2,4-D assay in urine, serum, gauze patches, vegetation and soil samples, a literature survey was done. The following methods were used in our investigation:

a. Urine: Out of the total volume of urine samples, 10 ml was taken and 2 ml of 1N HCl was added to it. The acidified urine was then mixed with 5 ml of diethyl ether and was shaken for 10 minutes on a Buchler-Vortex evaporator (Vortex setting was kept at 5). This extraction method was followed three times. The ether extracts were pooled together and evaporated to complete dryness at a temperature not higher than 45°C. The dry residue was dissolved in 1 ml of methanol first and then mixed thoroughly with 1 ml of 15% solution of BF_3 in methanol. The resultant mixture was heated in a water bath at 70°C for 10 minutes for complete methylation. The methylated solution was cooled and again extracted thrice with 1 ml of n-hexane. N-hexane extracts were either injected (1 µl) immediately in a G.C. or stored at -20°C.

b. Blood Serum: One ml of isolated serum was diluted with 4 ml of distilled water and then acidified with 1 ml of 1N HCl. The acidified serum was then treated (i.e. extracted and methylated) in exactly the same manner as was the urine. One µl of n-hexane extract was injected in a G.C.

c. Gauze Patches: Immediately after the day's work, the patches (from the front, back and head cover) were soaked separately in 100 ml of methanol and kept overnight at 4°C. After washing the patches thoroughly with methanol, 50 ml of the wash (from each group) was evaporated to dryness at less than 45°C temperature. The dry residue was dissolved in 1 ml of methanol and then methylated and finally extracted as above. The methylated compound, however, was extracted three times with 4 ml, 3 ml and finally 3 ml of n-hexane. The pooled extractant was diluted when needed before G.C. analysis or stored as before at -20°C.

d. Air Filter: Each air filter was washed with 50 ml of methanol immediately after the day's work and was kept at 5°C overnight. Clear methanol was removed and the filter was further rinsed twice with 10 ml of methanol. The pooled methanol was treated in the same way as the gauze patches.

e. Vegetation: Ten grams of freshly collected leaves were chopped and homogenized in 50 ml of 95% hot ethanol. The homogenate was centrifuged at 5,000 rpm in a fixed angle rotar for 30 minutes. Supernatant was carefully removed and saved. The pellet was thoroughly washed with 50 ml of 80% hot ethanol and recentrifuged three times as before. Each supernatant fraction was pooled together in a round bottom flask and was evaporated to bring the final volume to 20 ml. The concentrated supernatant was adjusted to pH 3 by adding exactly 10 drops of H_3PO_4. This acidified solution was extracted first with 50 ml of diethyl ether and then with 25 ml and finally with 25 ml of diethyl ether. The total extractant was evaporated to dryness under a current of nitrogen at 45°C. The dry residue was again extracted three times with 3ml, 1 ml and 1 ml of methanol. The combined methanol extract was treated with 1 ml of 15% BF_2 in methanol and kept under a nitrogen atmosphere in a water bath at 70°C until concentrated to 2 ml. This methylated solution was further extracted with n-hexane as described above. N-hexane was diluted when needed for G.C. analysis.

f. Soil: Fifty grams of soil sample was mixed with 40 ml of 1N H_2SO_4 to prepare a slurr. To the acidified soil, 45 ml of diethyl ether was added and was taken in a separating funnel for extraction. The separating funnel containing the soil sample was shaken for a total of fifteen minutes, but for not more than one minute at a time. The solvent layer was decanted and passed through glass wool and the aqueous layer containing soil particles was rejected. The clear solvent layer was then mixed with 25 ml of 1N NaOH and shaken vigorously in a separating funnel for 1 minute. The organic layer was rejected and the alkaline aqueous layer was cleaned by partition method with 25 ml chloroform. The clean aqueous layer was further acidified with 1 ml of conc. H_2SO_4 and extracted with 25 ml of ether. The ether extract was dried completely and redissolved in 5 ml of methanol. Methylation and final n-hexane extraction were made as in the case of the vegetation samples.

The following gas chromatographic conditions were used:

Detector, Electron capture Ni 63 temperature:	350°C
Gas: Methane: Argon	(5:95)
Flow rate through the column	25 ml/min
Sample volume injected	1 µl
Column material	3% OV 101 on 80/100 supelcoport
Length of the column	6 ft
I.D. of the column	2 mm
Column temperature	187°C
Injection port temperature	200°C