THE FATE OF 2, 4, 5-TRICHLOROPHENOXYACETIC ACID IN MAN

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2, 4, 5-トリクロロフェノキシ酢酸の人体内における運命

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The effectiveness of chlorinated phenoxyacetic acids as plant growth regulators has been recognized for a number of years. In earlier reports^{1~2)}, 2, 4-dichlorophenoxyacetic acid (2, 4-D) was noticed to be non-toxic to animals and man. But later, the toxicity of 2, 4-D and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) for experimental animals has been revealed by various papers^{3~6)}.

Although tremendous amounts of 2, 4-D, 2, 4, 5-T and the related compounds have been manufactured and used, clinical reports of poisoning are rare. Nielsen⁷⁾ reported a case of suicide with ingestion of diethylamine salt of 2, 4-D. He observed 6.0 g of 2, 4-D in the corpse of the victim, corresponding to 80 mg/kg. Seabury⁸⁾ reported another case. He administered to a patient, suffering from disseminated coccidioid mycosis, 3.6 g of sodium salt of 2, 4-D through intravenous infusion. The patient was troubled with twichings of the muscles and fell into stupor, but recovered.

A couple of years ago, the author found such symptoms and signs as nausea, vomiting, weight loss, headache, fatigue and skin rash among the workers of the test operation mixing an ammonium-sulfamate derivative, 2, 4, 5-T and two other kinds of chemicals. Though the epidemiological study revealed 2, 4, 5-T to be noxious, the author failed to support the finding because the fate of 2, 4, 5-T in the human body was obscure in those days.

Unsubstituted or p-chlorophenoxyacetic acids are excreted in urine by man, dog and rabbit in an intact form because of the ether bondage^{9~10)}. Clark¹¹⁾, using C¹⁴-labelled 2, 4-D, demonstrated that 96% of orally administered 2, 4-D was excreted unchanged by sheep within 72 hours. Leigh¹²⁾ et al. reported the fate of 2, 4, 5-T in dairy cows. Out of 454 mg fed, 430.7 mg of the 2, 4, 5-T was

recovered from the urine. These facts suggest that analogically in the case of man, orally administered 2, 4, 5-T will be excreted in the urine. The author examined whether or not ingested 2, 4, 5-T was excreted in the urine unchanged and investigated the fate of it from the pharmacodynamic point of view.

Subjects

A volunteer (28-year-old male) was ingested with 150 mg (2.2 mg/kg) of 2, 4, 5-T after breakfast. Blood samples were collected at 1-to 2-hour intervals during the first 6 hours and less frequently thereafter.

Two volunteers (27- and 28-year-old males) were given 100 mg of 2, 4, 5-T orally. Urine samples were collected at 3- to 6-hour intervals for 72 hours.

Collections of 24-hour urine were obtained from 21 workers of a chemical manure factory.

Methods

1) Determination of 2, 4, 5-T

Determination of 2, 4, 5-T was carried out by Nielsen's method with modifications as follows.

Twenty milliliters of diluted urine (two- or threefold) or diluted blood plasma (three- or fourfold) was taken into a 50 ml-centrifuging tube with a glass stopper. After adding 1 g of crystalline tartaric acid, 4 g of anhydrous Na₂SO₄, 8 ml of chloroform and 10 ml of ethyl ether, the tube was vigorously shaken for 5 minutes and centrifuged for 5 minutes at 1,200 G. The organic phase was decanted and extraction of the residue was performed twice more with 8 ml of ether. The combined organic phase was extracted twice with 16 ml and 8 ml of 1/30 M phosphate buffer (pH 7.4). After being washed with half the volume of chloroform, the combined phosphate buffer extract was rendered strongly acid with 1.5 ml of 8N-H₂SO₄ to be extracted twice with chloroform. The chloroform solution was extracted with 20 ml of borate buffer (pH 10.6). The aqueous solution was extracted

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with 20.0 ml of chloroform after it had been made strongly acid with 1.5 ml of 8N-H₂SO₄. To remove the turbidity, the organic extract was filtrated through anhydrous Na₂SO₄.

One milliliter or two (or more, in the case of low concentrations) of the chloroform solution was poured into a small flask to make it evaporate cautiously just to dryness. Two milliliters of chromotropic acid in conc. H₂SO₄ solution was poured into the flask, which was prepared by dissolving 0.10 g of the sodium salt of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in 100 ml of concentrated sulfuric acid. Ten milliliters (or more according to the concentrations) of SnCl₂ solution prepared by dissolving 25 g of stannous chloride, SnCl₂·2H₂O, in 500 ml of 8N-H₂SO₄ was added to the flask after it had been heated at 143±2°C for ten minutes. Concentration was estimated at 575 mμ.

2) Identification of extracted 2, 4, 5-T

2, 4, 5-T in the urine was identified by the color reaction with chromotropic acid and by the spectrophotometric measurement of chloroform extracts. Gas-chromatographic identification was accomplished after methyl ester of 2, 4, 5-T with boron trifluoride being made¹⁰. Experimental conditions of gas-chromatographic analysis were shown in the foot note of Fig. 2.

3) Estimations of 2, 4, 5-T in the surroundings of a chemical manure factory

2, 4, 5-T in the surroundings was sampled with a high volume air sampler (Gelman, Type A). 2, 4, 5-T at the breathing point of the workers was sampled with a personal air sampler with a glass fiber filter. After sampling, filter papers were removed into a continuous extractor, containing approximately 100 ml of chloroform. 2, 4, 5-T, extracted in chloroform was estimated with the method mentioned above.

Results

1) Determination of 2, 4, 5-T in the urine

For a preliminary experiment, 500 and 100 μg of 2, 4, 5-T had been added to 100 ml of the urine of an adult and the urine was analyzed to obtain the recovery rates as high as 95.6, 92.1% respectively.

2) Identification of extracted 2, 4, 5-T

A peculiar wine-purple color developed when the extract was heated with chromotropic acid in the presence of concentrated sulfuric acid. The absorption spectrum of the color is presented in Fig. 1, showing the maximum absorption rate at 575 mμ. Spectrophotometric measurement of chloroform solution, one step before the last of the procedure,

Fig. 1. Absorption spectrum of 2, 4, 5-T extracted from the urine when reacted with chromotropic acid at 143 to 145°C for 10 minutes.

Fig. 2. Gas-chromatogram of 2, 4, 5-T obtained from the urine.

yielded the characteristic absorption curve of 2, 4, 5-T. The result of gas-chromatographic analysis is represented in Fig. 2, on which the sample coincided with pure chemicals in retention time of about 8 minutes. It is clear from these observations that 2, 4, 5-T was excreted in the urine in an intact form.

3) Distribution and elimination of 2, 4, 5-T

As will be seen in Fig. 3, the plasma concentra-

Fig. 3. Concentrations of 2, 4, 5-T in the blood plasma.

tion of 2, 4, 5-T rose rapidly during the first 3 hours and the peak plasma level ($21.1 \mu\text{g/ml}$) was attained at 4th hour after administration. The semi-logarithmic concentration-time curve was linear after 4 hours' post treatment, indicating a first order elimination rate with a gradient of -0.065 obtained with the method of least squares. Zero-time plasma level ($28.0 \mu\text{g/ml}$) was obtained by extrapolating the linear part of the plot.

The differences between the observed concentrations (C) and the respective ones obtained from extrapolation ([C]) were able to be plotted on a

Fig. 4. Excretion of 2, 4, 5-T in the urine.

line with a gradient of -0.605 , which indicate that the absorption is also of the first order.

The excretion of 2, 4, 5-T in the urine after a single oral administration was represented in Fig. 4 and Fig. 5. A fairly large amount of 2, 4, 5-T was recovered from the initial sample but only about 45% of original dose was found in the urine collected during the first 24 hours after administration. Thirty-six hours after treatment, 60% had been

Fig. 5. Excretion of 2, 4, 5-T in the urine.

recovered and at the end of 72 hours more than 80% was recovered.

4) Estimations of 2, 4, 5-T in the urine as a practical trial

Table 1 demonstrates that a relatively small amount of 2, 4, 5-T was found in the urine of workers. In some of them, it was impossible to detect it with this method. The concentrations of 2, 4, 5-T in the working area were listed in Table 2 and Table 3. None but the value obtained in the weighing box of 2, 4, 5-T exceeded the recommended value by A.C.G.I.H., which is 10 mg/m^3 .

Discussion

Two major bands of absorption by the phenoxy-acetic acids are found in ultraviolet region¹³⁾. The one band occurring in the region of $220\text{--}230 \text{ m}\mu$ is probably due to the carbonyl group of the side chain, while the other occurring around $280 \text{ m}\mu$ is due to the benzen ring. Nielsen⁷⁾ analyzed the concentration of 2, 4-D within the range of $200\text{--}350 \text{ m}\mu$. In the method, which relies upon the absorp-

Table 1. 2,4,5-T in the urine of workers of a chemical manure factory.

Date	Name	Age	Sex	Urine volume (ml/day)	2,4,5-T (mg/day)
Apr. 9	A. M.	28	Male	2,060	1.5
	S. S.	50	Male	1,400	—
	Y. I.	60	Male	1,570	—
	T. Ki.	40	Female	1,020	1.3
	S. H.	26	Female	1,020	1.2
	T. T.	20	Male	870	0.5
Apr. 15	A. M.	28	Male	2,000	3.6
	S. S.	50	Male	2,220	—
	Y. I.	60	Male	2,400	—
	S. H.	26	Female	1,260	—
	T. N.	38	Female	1,760	2.7
	S. K.	21	Male	1,400	3.6
Apr. 16	T. Ki.	40	Female	750	1.0*
	Y. I.	60	Male	1,670	—
	S. S.	50	Male	3,020	—
	S. N.	41	Male	1,470	2.2
Apr. 22	T. Ko.	58	Female	450	3.5*
	Y. O.	38	Female	250	—
	S. S.	50	Male	850	—
	T. Ki.	40	Female	180	1.9*
	Y. I.	60	Male	1,150	—

* milligrams per liter.

Table 2. Concentrations of 2, 4, 5-T in the surroundings of a chemical manure factory.

Sampling points	Concentrations*
Weighing box	15.4
Inlet of mixer	1.37
Outlet of mixer	0.82
Center of work-room	0.62

* milligrams per cubic meter.

Table 3. Concentrations of 2, 4, 5-T at the breathing location of workers.

Workmen	Concentrations*
A	0.21
B	0.31
C	0.67
D	0.38

* milligrams per cubic meter.

tion in the ultraviolet region, it is rather unsatisfactory to measure the concentrations lower than 1 mg/100 ml because of the low absorption rate. Using the color reaction with chromotropic acid, I can measure concentrations lower than that accurately.

On the other hand, industrially manufactured 2, 4, 5-T seems to be inevitably contaminated with 2, 4, 5-trichlorophenol, the precursor of 2, 4, 5-T, or other chlorophenol derivatives. As long as the measurement is carried out in the ultraviolet region, at 288 m μ , the contaminants possessing the benzen ring can not be excluded. Chromotropic acid enhances not only sensitivity but also specificity.

The development of the color depends upon a reaction between 2, 4, 5-T and chromotropic acid in the concentrated sulfuric acid. It is said that the heating of 2, 4, 5-T in the presence of concentrated sulfuric acid results in the formation of formaldehyde which then reacts with the chromotropic acid¹⁴. The reaction, highly sensitive to a small amount of phenoxyacetic acid, is relatively non-specific. A number of other compounds such as acetic acid, fats and waxes may react and give a similar color. But in this study, samples free from 2, 4, 5-T did not develop the characteristic purple color. Sometimes when the samples were heated above 150°C, they developed amber color, which was bleached by a stannous chloride solution. Since the wine-purple color of chromotropic acid had the maximum absorption at 525 m μ , the concentration was estimated in this region.

In preliminary experiments, the recovery of 2, 4, 5-T, added to the urine of an adult, was satisfactory and that of 2, 4, 5-T, added to the homogenate of the organs of rats, was shown elsewhere¹⁵.

The identity of 2, 4, 5-T, extracted from the urine, was judged by the following criteria: a characteristic color reaction with chromotropic acid representing the absorption maximum at 575 m μ , coincidence of the absorption maximum with pure 2, 4, 5-T in the ultraviolet range at 288 m μ , and coincidence of a retention time in gas-chromatographic analysis. The acetic acid of the side chain was demonstrated by the positive color reaction with chromotropic acid. The benzene ring was investigated spectrophotometrically in the ultraviolet region. Not only the intactness of ether bondage but also the intactness of the chlorine of the side chain were confirmed by gas-chromatograms. It can be concluded from these observations that ingested 2, 4, 5-T was excreted in the urine unchanged.

Orally administered 2, 4, 5-T was readily absorbed as indicated by the observation that the peak plasma

concentration was attained within a few hours after a single administration. Linearity of the semi-logarithmic concentration-time curve in its terminal course indicates that 2, 4, 5-T was eliminated from the blood plasma at the first order elimination rate. On the other hand, non-linear part of the plot represents the absorption of 2, 4, 5-T from the digestive tract. The absorption rate could be estimated indirectly by plotting the differences between the observed concentrations and respective ones obtained from extrapolation on semi-logarithmic scale against time, which stands for the absorption rate¹⁷⁾.

Distribution and elimination of 2, 4, 5-T have rarely been investigated. Only Kurt-Erne¹⁸⁾ reported that the plasma concentration time curves as well as the plasma half-life values of 2, 4, 5-T were similar to those obtained with 2, 4-D in experimental animals. Accordingly, it is of interest to compare the fate of 2, 4, 5-T in man with that of 2, 4-D in experimental animals. Distribution volume can be estimated from zero-time plasma level and the dosage given at 7.9% of the body weight, approximately as large as the total blood volume. It is said that 2, 4-D could regularly be detected in blood cells in concentrations ranging 10 to 20% of the corresponding plasmas. Based on these findings, two thirds of the orally administered 2, 4, 5-T would be distributed in the blood in earlier stages of elimination.

Clark¹¹⁾ described in his paper that the radioactivity of labelled 2, 4-D rose rapidly in the blood plasma of sheep during the first half-hour, reached the peak after 1 $\frac{1}{4}$ hours and diminished rapidly thereafter. About 15% of the original dose was excreted in the urine in the first 1 $\frac{3}{4}$ hours. Eight and a half hours after treatment, 50% had been recovered in the urine and by the end of 28 hours, over 90% of the dose had been excreted. In contrast to these observations of Clark, the peak plasma level of 2, 4, 5-T was attained 4 hours after administration and the plasma level declined gradually, indicating the plasma half-life as 11 hours. Within 24 hours, only 45% of the original dose was found in the urine of volunteers. Low elimination rate, therefore, would make 2, 4, 5-T more toxic in man than in experimental animals.

It is mighty favorable for prevention and treatment of 2, 4, 5-T intoxication that the agent is excreted in the urine unchanged. The intake of 2, 4, 5-T could be estimated by urinalysis. As a practical trial, the urinalysis of the workers was performed to find out that a comparatively small amount of 2, 4, 5-T was recovered. In some of

them, the agent could not be detected with the method mentioned above. It is to be accounted for by the low environmental 2, 4, 5-T concentrations, which were due to several kinds of improvement of operations. Furthermore, workers had been provided with such protectors as gloves, masks, hoods and protective suits and they took a bath at the end of duty. In the case of a well-controlled operation, the urinary excretions were occasionally lower than 1 mg/day. Thus, it would be inadequate to detect 2, 4, 5-T with the method mentioned, if the chronic toxicity of 2, 4, 5-T could not be ruled out in such levels.

Summary

To clarify the fate of 2, 4, 5-T in the human body, volunteers took 100 to 150 mg of 2, 4, 5-T orally. Blood and urine samples were collected and analyzed to find out that

- 1) orally administered 2, 4, 5-T was readily absorbed and eliminated gradually from the blood plasma, showing a first order elimination rate,
- 2) more than 80% of orally administered 2, 4, 5-T was excreted in the urine in an intact form within 72 hours, and
- 3) elimination from the blood plasma and excretion in the urine by man were slow, as compared with those in experimental animals.

According to the method elaborated, 2, 4, 5-T in the urine of workers of a chemical manure factory was analyzed.

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除草剤 2, 4, 5-トリクロロフェノキシ酢酸 (2, 4, 5-T) のヒトの体内における運命を知る目的で, これを経口的に投与し, 血中濃度の推移, 尿中排泄量を検討した。

生体試料中の 2, 4, 5-T の抽出定量には Nielsen らの方法を改良し, 濃硫酸下における 2, 4, 5-T とクロモトロープ酸との呈色反応を利用して定量した。

成人男子に 150 mg の 2, 4, 5-T を投与すると, すみやかに血漿中の濃度は高まり, 投与後 4 時間で最高値 21.1 $\mu\text{g/ml}$ に達し, 以後緩慢に低下した。血漿からの消失速度は一次であり, 生物学的半減期は 11 時間と計算された。

尿中より抽出した物質を次の三つの方法で検討した結果, 2, 4, 5-T はヒトの体内において分解および抱合等の質的变化をうけずに尿中に排泄されてくることがわかった。

1) 濃硫酸下においてクロモトロープ酸と反応し特有なブドー色に呈色した。

3) 抽出物のクロロホルム溶液が紫外部において最高の吸収を示す点は 288 $\text{m}\mu$ にあり標準品のそれと一致した。

3) 抽出物を三弗化硼素でメチルエステルとしてからガスクロマトグラフィーで検討した結果, 保持時間は約 8 分であり標準品のそれと一致した。

成人男子 2 名に 100 mg の 2, 4, 5-T を経口投与し, 72 時間にわたって採尿し尿中に排泄された 2, 4, 5-T を定量した。投与後 24 時間で投与量の約 45%, 48 時間で約 75%, 72 時間で 80% 以上が排泄された。これを実験動物における文献成績と比較すると, ヒトの場合には排泄により長時間を要するといえる。

これらの知見に基づいて産業現場で 2, 4, 5-T の体内侵入量を推定するための一段階として, ある化学肥料工場作業員の 24 時間尿について尿中 2, 4, 5-T を測定した。作業場環境中の 2, 4, 5-T の濃度は 0.62 mg/m^3 ~ 15.4 mg/m^3 であり, 測定した延 21 名中最高値は 3.6 mg/day であった。また約半数の尿中ではこの方法によって 2, 4, 5-T を検出することができなかった。