SYNTHESIS OF (^{3}H—METHYL) TRINITROTOLUENE AND ITS APPLICATION TO TOXICOLOGICAL STUDY

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ABSTRACT

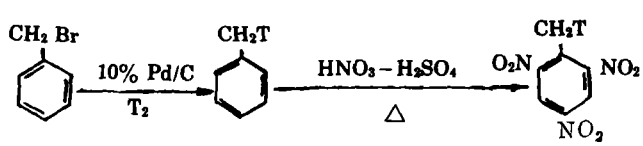
In this paper, (^{3}H- methyl) toluene was prepared by catalysed halogen- tritium substitution method from benzyl bromide, then it was nitrated to produce (^{3}H- methyl) trinitrotoluene. The tritiated product was purified by thin- layer chromatography. At last, the pure ^{3}H- TNT was obtained with specific radioactivity of 3.77 GBq/mmol. Radiochemical purity was over 98% and the ultraviolet absorption spectrum of tritiated TNT was conformed with that of standard sample. Using ^{3}H- TNT as a tracer, its toxicokinetics was studied in rats. The results showed that the toxicokinetics characteristics of TNT were quickly absorbed into the blood, $V_d > 2$L/kg.h, long $T_{1/2}\beta$ and fixed accumulation with four routes of administration, TNT and its metabolites were mainly excreted by the urine. The half- life of TNT in the urine were 1,1- 24h. A trace of radioactivity of ^{3}H- TNT and its metabolites could be detected in the urine on 7th day after administration (9.25×10^6Bq/kg).

Keywords: (^{3}H- methyl) toluene (^{3}H- methyl) trinitrotoluene Catalysed halogen- tritium substitution method Toxicology Toxicokinetics

I. INTRODUCTION

Trinitrotoluene (TNT), as a standard explosive, has been universally used in munitions, mining, chemical industries and so on, for about one hundred years. There were many cases of death by toxic hepatitis and aplastic anemia in TNT workers in large TNT workshops before 1950's[1-2]. In our country, middle and small explosive factories are widely distributed in 27 provinces and cities.

Although in recent years the concentration of TNT in working place has been markedly lowered and severe poisoning of TNT has seldom happened, the dermal contamination of TNT exposed workers were significant. The chronic poisoning and TNT cataract have not been controlled effectively. In this paper, ^{3}H- TNT is prepared by chemical synthesis. The chemical synthesis scheme is shown as follows:



Using ^{3}H- TNT as a tracer, the toxicokinetics of TNT is studied in rats in order to explicate the quantitative change rule of absorption, distribution and excretion of TNT in organism.

Ⅱ. EXPERIMENTAL

1. The synthesis of (^{3}H—methyl) trinitrotoluene

1) The preparation of (^{3}H- methyl) toluene The labelling of (^{3}H- methyl) toluene was carried out in an all glass vacuum apparatus. A small reaction flask containing 25 μ l (0.21mmol.) benzyl bromide, 1 ml (9 mmol.) toluene, 0.2ml 2.5 mol/l NaOH solution, 30mg 10% Pd/C catalyst and a magnetic stirring bar was connected to tritiated system. The contents of the reaction bottle were mixed fully and cooled with liquid nitrogen. The system was cautiously evacuated to 0.133332 Pa, T_2 was introduced into the system. The catalysed halogen- tritium replacement reaction was then carried out at 25°C under ordinary pressure for 45 min with good agitation. At the end of tritiating reaction, 5.29 ml tritium gas was consumed. After reaction stopped, unused tritium was recovered by active uranium. The catalyst was completely removed by centrifugalizing, the supernatant liquid is collected and the catalyst was washed with 3 ml toluene. Combining the washed liquid and the supernatant liquid, (^{3}H- methyl) toluene was obtained.

2) The preparation of (^{3}H—methyl) trinitrotoluene 4 ml (^{3}H- methyl) toluene, 10 ml concentrated sulfuric acid (sp.gr. 1.84) was added into a flat bottom flask respectively with stirring. The mixture was heated to 60- 70°C, and 10 ml fuming nitric acid (sp.gr. 1.50) was added into the flask with vigorous agitation. The reaction temperature was then warmed to 110-120 °C and maintained for 60min, the flask was cooled to 40- 50°C and 12g 25% SO_3 fuming sulfuric acid and 4 ml fuming nitric acid was added at 50°C. The reaction mixture was then heated to 120°C and maintained for 60min. When reaction mixture was cooled to room temperature, water was added to the bottle and the crude product was precipitated, the precipitate was then dissolved in 50- 100 ml 5% boiling sodium sulfite solution and cooled to room temperature, the slightly yellow solid- ^{3}H- TNT was precipitated, filtered, washed with hot water (60- 70°C) and recrystallized from absolute alcohol. The product was purified by TLC (solvent system: ethylacetate: petroleum ether = 30:90 *V*/*V*), the purified product (77.8mg) was obtained with specific radioactivity 3.77GBq/mmol, radiochemical purity over 98%

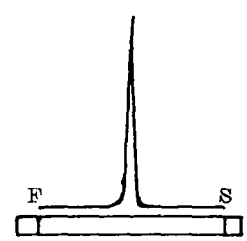


Fig.1 Radioscanning of ^{3}H—TNT on TLC plate

(Fig.1). The UV absorption spectrum of tritiated TNT was in conformed with that of standard sample.

2. the toxicokinetics of ^3H—TNT in rats

^3H- TNT alcohol normal saline (2:1) solution (1.85×10^7Bq/ml, containing ^3H- TNT 1.11mg) was used as intravenous injection and dermal application. The solutions diluted 1 and 10 times with saline were used for intratracheal injection and gavage.

16 male wistar rats (180- 230g/one rat) were divided randomly into four groups (4 rats/group), which were administrated by intravenous injection, intratracheal injection, gavage or dermal application respectively with 9.25×10^6Bq/kg of ^3H- TNT solution. Blood, urine and feces samples of various period were collected during seven days after administration.

Taking 0.1ml blood and 0.1ml urine and 50mg feces of rat were used as analytical samples. The radioactivity of samples was measured with a liquid scintillation counter. Sealed ^3H quenching standard was used for dpm. Standardization. TNT and its main metabolites in urine were isolated by thin- layer chromatography, and their radioactivity were determined.

After intravenous injection, intratracheal, gavage or dermal application the ^3H- TNT concentration in blood vs time curves obtained were fitted with two- compartment open model, the kinetics parameters were calculated in Apple- II computer by Gauss- Newton or Marquardt algorithm for nonlinear least squres approximation programs[3].

III. RESULTS AND DISCUSSION

1. Time curves and kinetic parameters of ^3H—TNT concentration in blood.

According to two- compartment model fitting, the real measured data in Fig.2 are close to the theoretically predicted data and the degrees of fittness are very well ($\bar{R}$=0.99, 0.97, 0.93 and 0.90 respectively).

Table 1

Kinetic parameters of ^3H—TNT concentration in blood

	$T_{1/2}\alpha$ (h)	$T_{1/2}\beta$ (h)	Ke (h^{-1})	K_{12} (h^{-1})	K_{21} (h^{-1})	Cl (L/kg · h)	V_d (L/kg · h)	AUC (mg/L · h)
i.v.	0.14	9.30	0.29	4.26	1.59	0.24	2.95	4.05
intratracheal injection	0.19	10.26	0.47	2.90	0.87	0.25	3.31	3.57
gavage	0.39	38.63	0.22	1.46	0.16	0.31	17.11	2.89
dermal application	2.53	45.28	0.05	1.75	0.75	0.28	17.92	3.06

The results of Fig.2 and Table 1 indicated that the in- vivo process of ^3H- TNT through intravenous injection in rats is similar to that of intratracheal injection. The

concentration of ^{3}H- TNT in blood reached its peak immediately after administration. The in- vivo process of ^{3}H- TNT through digestive tract is similar to that of skin. In all four routes of administration, the absorption of TNT is fast, and the time of reaching the highest concentration is very short. ^{3}H- TNT in blood is transfered rapidly from central compartment to peripheral compartment, however, the rate which ^{3}H- TNT come back from peripheral compartment to central compartment is quite slow. All the ratio of K_{12}/K_{21} are more than 1. The apparent volumes of distribution (V_d) are more than 2L/kg.h. The clearances are small and the half- time of elimination phase are long. All these lead to that TNT has a certain affinity with some tissues in which it appeared to be accumulated. Based on the area under the TNT concentration in blood vs time curve through intravenous injection, the absorption fractions of TNT through respiratory tract, digestive tract and skin are 88.19%, 37.42% and 39.76% respectively. The results showed that TNT absorption is rather complete through respiratory tract. The absorption percentages through digestive tract and skin are also large. The respiratory tract and skin are main routes of entry of TNT in occupational exposing employees. Recently, the concentration of TNT in the workplace air have been controlled but the cutaneous protection of workers is often neglected and the dermal contamination of TNT in exposed workers is a serious problem. The prevalence rate of TNT cataract is constantly risen. Therefore, it must be emphasized the dermal protection for occupational exposing workers to provent chronic TNT poisoning.

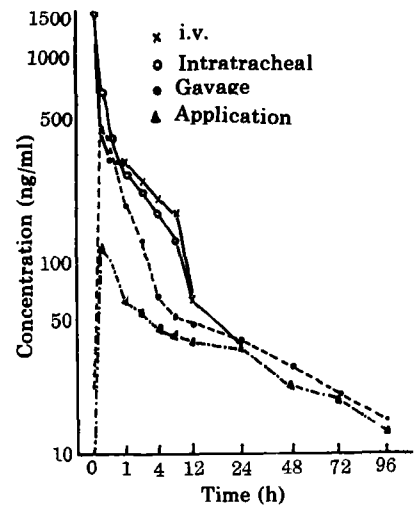


Fig.2 ^{3}H—TNT concentration in blood vs time curve

2. ^{3}H—TNT excreting kinetics in rats

Using urine- drug method[4], the kinetics parameters of urine and feces

Table 2

Excreting kinetic parameters of ^{3}H—TNT in urine and feces of rats

		$T_{1/2\beta}$ (h)	Ku(h^{-1})	Cl(L/kg · h)	T_{max}(h)
urine	i.v.	11.68	0.186	0.58	2.0
	gavage	24.11	0.056	0.25	2.0
	dermal application	20.09	0.013	0.33	11.40
feces	i.v.	10.65	0.009	0.12	11.29
	gavage	22.07	0.015	0.09	6.64
	dermal application	28.24	0.002	0.04	11.41

excretion of TNT are shown in Table 2.

In four routes of administration, the excretion of ^{3}H- TNT in rats urine is same as that in feces. The major parts of TNT and its metabolites are excreted through urine during the 0- 24 h after administration and reached its peak at 2- 11.4 h. The excretion ratio for urine:feces is 5:1 and the urine half- time of excreting is 11- 24 h. A trace of TNT and its metabolites can still be detected in the urine and feces at 7 days after administration.

The results in Table 3 showed that the major excretion composition was DNAT (2, 6- dinitro- 4- aminotoluene) in urine while TNT was relatively low. In three different adminstration, the results are very consistent.

Table 3

Excreting proportion of TNT and DNAT in urine

t (h)	i.v.	gavage	dermal application
24	1/75	1/21.2	1/3.4
48	1/5	1/1.9	1/7.9
72	1/3.4	1/1.2	1/9.3

3. The problem about preparing and purifying of ^{3}H—TNT

There are three nitro groups in 2,4,6 positions of trinitrotoluene molecule. Thus, the ^{3}H- TNT can not be obtained by tritium gas- liquid catalytic exchange method. At the same time, the presence of three nitro group in an aromatic nucleus markedly influences the H- T isotopic exchanging, so that, the tritiated water exchange method can not prepare ^{3}H- TNT in higher specific radioactivity[5]. In this method, if less amount of carrier toluene. The specific radioactivity of ^{3}H- TNT will be increased. To eliminate trinitrotoluene isomers and dinitrotoluene, the resulting product is purified with sodium sulfite solution and washed with not water (60- 70°C).

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