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A threshold level for theophylline in urine : preliminary results

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Abstract

A HPLC method is used to quantitate methylxanthines including theobromine, paraxanthine, theophylline and caffeine in urine. After the oral administration of two sustained release preparations containing 150 and 250 mg theophylline respectively, between 10 and 20 % of the drug is recovered unchanged in the urine. Interindividual variation is noted, resulting in a wide range of peak concentration values from 4.9 till 33.3 μg/ml.
The results of the determination of methylxanthines in a sedentary population (N=200) and in samples collected for doping analysis (N=545) indicate that less caffeine containing beverages are consumed by athletes compared to non-athletes. With a detection limit of quantitation of 0.25 μg/ml theophylline is found in 71 and 44 % of the urine samples from the normal population and from athletes, respectively. The mean theophylline concentration is 0.68 μg/ml and 0.48 μg/ml, respectively. Based on these results the calculated threshold value ($p<0.0013$) for theophylline is 5 μg/ml. However the ratio theophylline-paraxanthine as an indicator for the intake of theophylline appears to be more reliable. A threshold level for this ratio could be 0.3.

Introduction

Caffeine, theophylline and theobromine share in common several pharmacological actions of therapeutic interest. They relax smooth bronchial muscle, stimulate the central nervous system, stimulate cardiac muscle and produce diuresis. Traditionally caffeine has been considered the most potent of the methylxantines. However theophylline produces more profound and potentially more dangerous CNS stimulation than does caffeine. Theophylline is on the list of forbidden doping substances as issued by the Flemish Ministry of Health (1).
As theophylline is also a metabolite of the social drug caffeine (2-4) a threshold value for theophylline is needed for doping analysis purposes. Therefore an excretion study after therapeutic administration of theophylline was started and normal levels of the methylxanthines were measured in a normal population and in samples collected for doping control.

Materials and methods

Experimental design and subjects
The study was performed on three healthy volunteers. The nature and the purpose of the study was explained to each volunteer before asking their concert to participate.
One tablet of the sustained release preparation THEO-2® (Galephar, Brussel, Belgium) containing 150 mg theophylline was administered orally after a light breakfast (3 subjects). Two weeks later one tablet of the sustained release preparation THEOLAIR® L.A. 250 (Riker Benelux, Diegem, Belgium) was taken orally by two subjects.
Total urine was collected in capped bottles before (0 h) and 2, 4, 6, 9 and 12h after administration of theophylline. After 24, 30, 36, 48, 60 and 72 h an aliquot of urine was also taken.
In each experiment, pre-administration urine (8 aliquots during the day preceding the experiment) was taken in one subject.
Urine samples from a sedentary population, consisting of members of the University were analysed for methylxanthines (N = 200). From October 93 till March 94 all urine samples collected for

doping analysis (N = 545) were controlled for theobromine, theophylline, paraxanthine and caffeine.
All samples were analysed in duplicate. Each batch of samples was preceded by the analysis of a quality control sample.

Reagent and apparatus
Theophylline, theobromine and caffeine were gifts from MERCK (Darmstadt, Germany).
Paraxanthine and β-OH ethyltheophylline were obtained from SIGMA (St Louis, MO, USA).
HPLC grade tetrahydrofurane was from MERCK (Overijse, Belgium). Aqueous HPLC solvent was prepared using water obtained from a MILLI-Q water purification system from Millipore (Brussel, Belgium). Ammonia buffer (pH 9.5) was prepared by the addition of ammonia to a saturated ammonium chloride solution.
The chromatographic system consisted of a model SP 8800 solvent delivery system, a model SP 8880 autosampler and a FOCUS forward optical scanning UV detector set at 275 nm (TSP, Fremont, CA, USA). Chromatographic data (peak heights) were generated through the LABNET (TSP) communication system with a PS2/386 computer (IBM). The column was a Hypersil-C18, 100 x 3 mm i.d., 5 μm (Chrompack, Antwerpen, Belgium) with an appropriate pre-column. The column was maintained at ambient temperature. The loop volume was 20 μl. The mobile phase was THF/H_2O (1:100 v/v) at a flow rate of 1 ml/min .

Determination method
A small amount of sodium chloride was added to 1 ml urine followed by 0.1 ml ammonia buffer and 50 μl internal standard (β-OH-ethyltheophylline 0.1 mg/ml). Extraction was performed by rolling with 5 ml CH_2Cl_2:MeOH (9 : 1, v/v) for 10 min. After centrifugation the organic phase was evaporated under nitrogen at 40 °C. The residue was redissolved in 200 μl mobile phase and transferred to an autosampler microvial.
A standard curve was constructed by analysing aqueous solutions (range 0-10 μg/ml) in quadruplicate for each concentration.

Results and discussion

Under the chromatographic conditions described theobromine, paraxanthine, theophylline, β-OH-theophylline (IS) and caffeine gave peaks with retention times of 2.12, 3.56, 4.05, 5.16 and 7.45 min, respectively. The calibration graphs showed good linearity between peak-height ratios and concentrations of 0-10 μg/ml. The lower limit for accurate quantitative determination (signal-to-noise ratio = 3) of theophylline was 0.25 μg/ml.

The concentrations of theophylline after the intake of THEO-2 by three subjects are shown in fig 1 and 2. From fig 1 where pre-administration values are also illustrated it is obvious that theophylline concentration starts to increase 2h after the intake. Maximal values are obtained after 9-24 h and range from 5 to 8 μg/ml. Great individual differences in concentration are noticed after the ingestion of 250 mg tablets (fig 3). Peak concentrations are obtained somewhat earlier than after THEO 2. Increased theophylline values are found during at least 24 h.

The distribution of theophylline in a sedentary population (N=200) is shown in fig 4. In 55 out of 200 subjects no theophylline could be detected. The mean value for theophylline in this population is 0.68 μg/ml (range 0 - 2.78 μg/ml). In 239 of the 545 samples collected for doping analysis (fig 5) the range varies from 0.16 to 7.85 μg/ml, and the mean value (0.48 μg/ml) is lower than in the normal population.

Based on the mean theophylline values found in the sedentary population and in athletes respectively , the following threshold levels (mean + 3 S.D.) for theophylline could be applied :

i) normal population : 4.7 μg/ml

ii) athletes (doping control) : 2.7 μg/ml

However taking into account the caffeine metabolism (2-4) , another parameter that is calculated is the ratio theophylline-paraxanthine (TP/PX). Before the intake of a theophylline

sustained release preparation by a coffee drinker (Subject 1) the ratio TP/PX varies from 0.047 to 0.073 (fig 6). Two hours after the ingestion of 150 mg theophylline (THEO-2), this ratio increases and remains high during at least 24 h. A similar pattern is also noticed in TP/PX for the other subjects. Similarly, after THEOLAIR (250 mg theophylline), TP/PX increases with peak levels of 10-11 after 3-6 h (fig 7).
The distribution of TP/PX in a normal population and in athletes is represented in fig 8, the mean value is 0.077 and 0.072, respectively. Based on these values following TP/PX threshold levels (mean + 3 S.D.) could be calculated :
i) normal population : 0.25
ii) athletes controlled for doping :0.23

In conclusion based on these results the following preliminary levels for the theophylline can be proposed : either TP/PX > 0.3 or when no paraxanthine is found a concentration threshold for theophylline of 5 μg/ml could be applied.
The results of the application of these levels after the intake of theophylline containing preparation in the different subjects is given in Table 1. As the ratio TP/PX is not dependent on the specific gravity of the urine and results in much longer detections times, this ratio should be prefered as a threshold value for the intake of theophylline.

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Tabel 1 : Detection times of theophylline based on theophylline concentration (TP) and theophylline - paraxanthine ratio (TP/PX) after the oral administration of sustained release preparations.

	150 mg (THEO 2®)			250 mg (THEOLAIR®)	
	Subj 1	Subj 2	Subj 3	Subj 1	Subj 2
TP	4, 6, 9 h	6, 9, 12, 24 h	negative	from 3 → 9 h	from 2 → 30 h
TP/PX	from 1 → 24 h	2 → 48 h	2 → 48 h	from 1 → 24 h	from 1 → 48 h

Fig 1 : EXCRETION OF THEOPHYLLINE AFTER THEO 2 (150 mg)

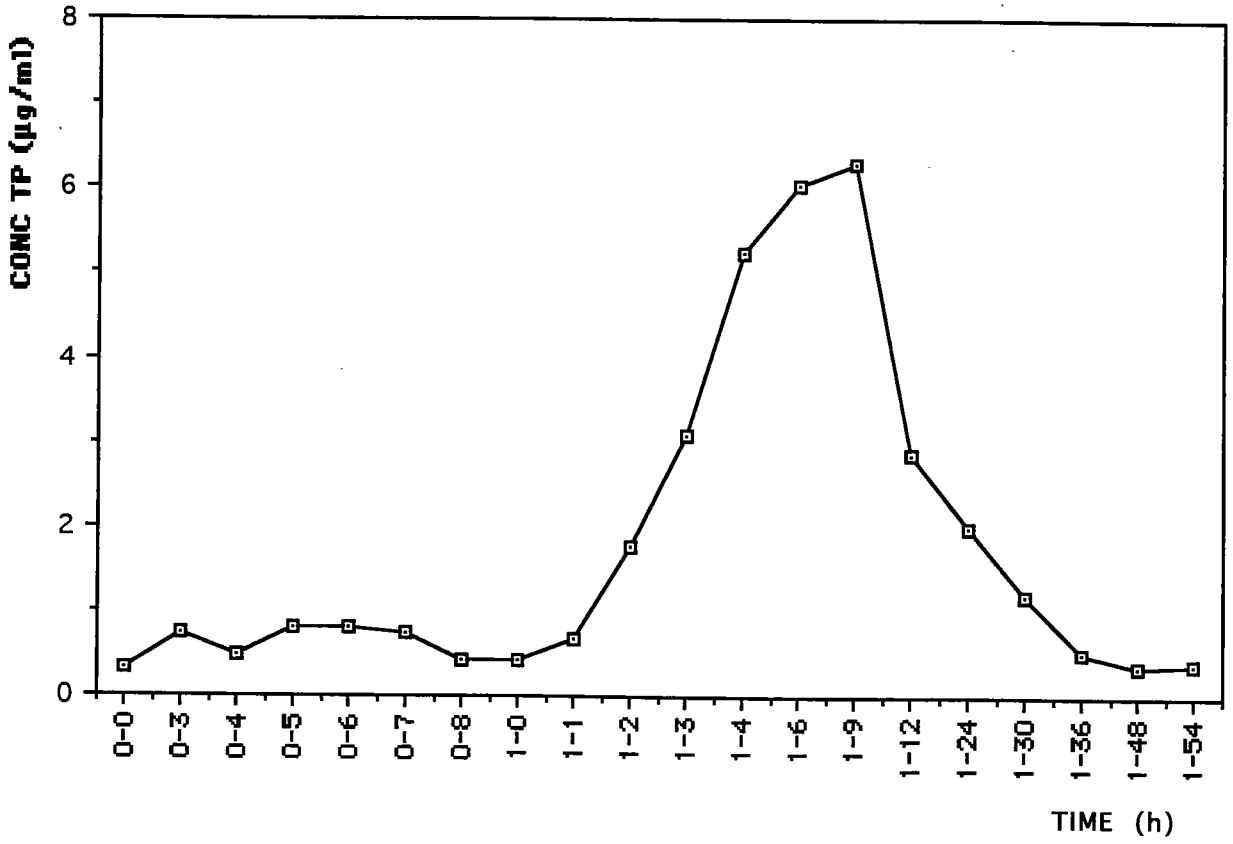
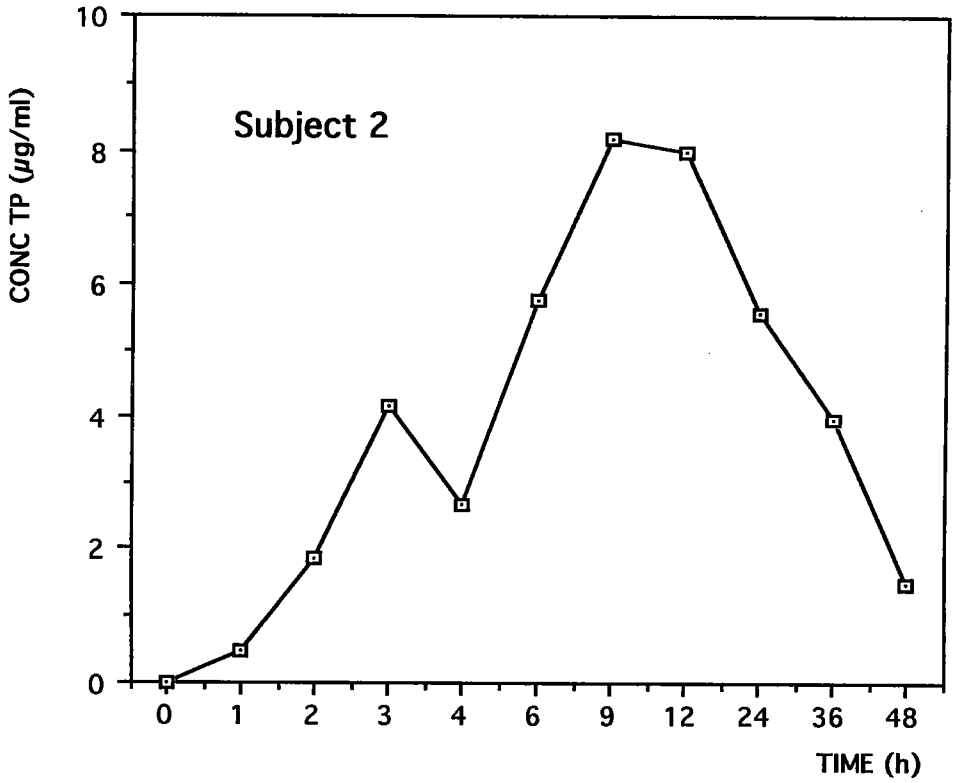


Fig 2 : EXCRETION OF THEOPHYLLINE AFTER THEO 2 (150 mg)



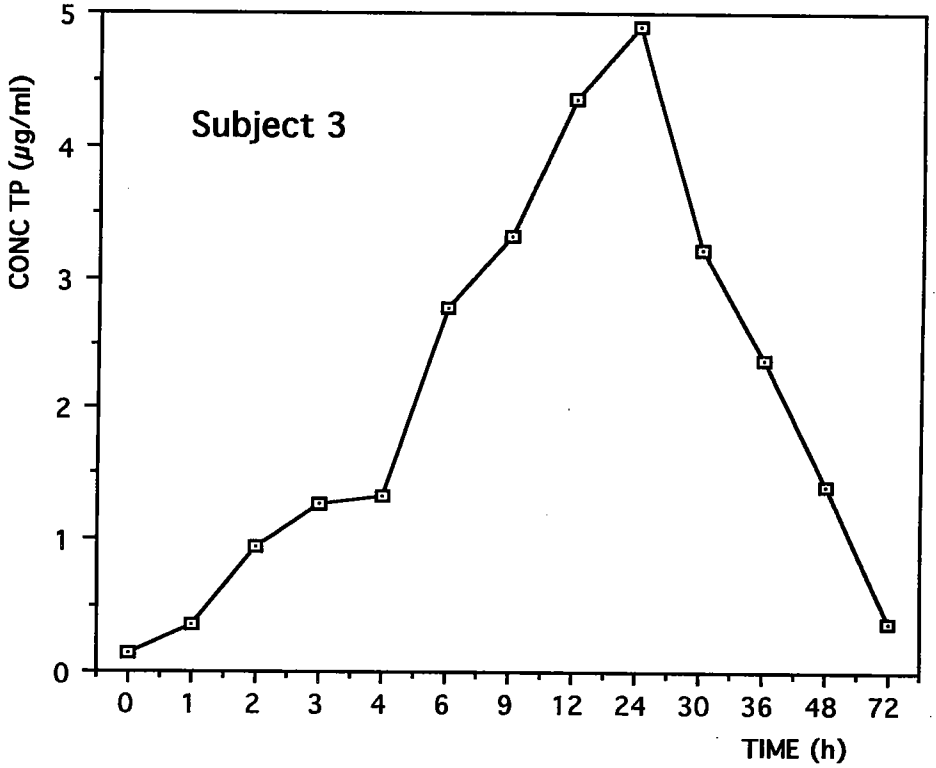
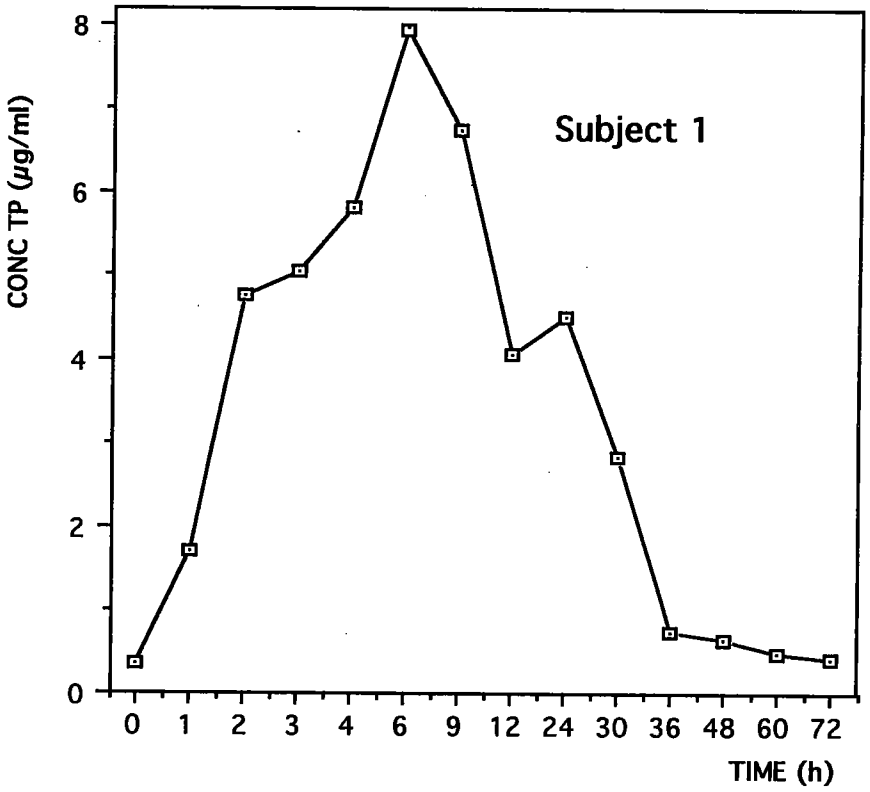


Fig 3 : EXCRETION OF THEOPHYLLINE AFTER THEOLAIR (250 mg)



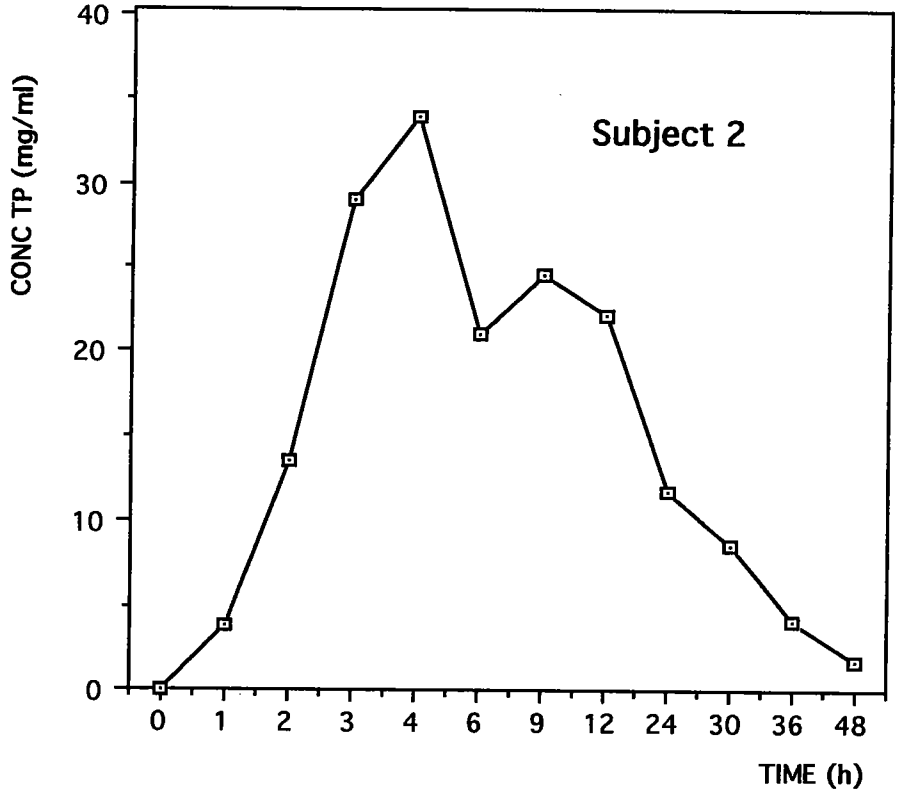


Fig 4 : LOG DISTRIBUTION OF THEOPHYLLINE IN A NORMAL POPULATION

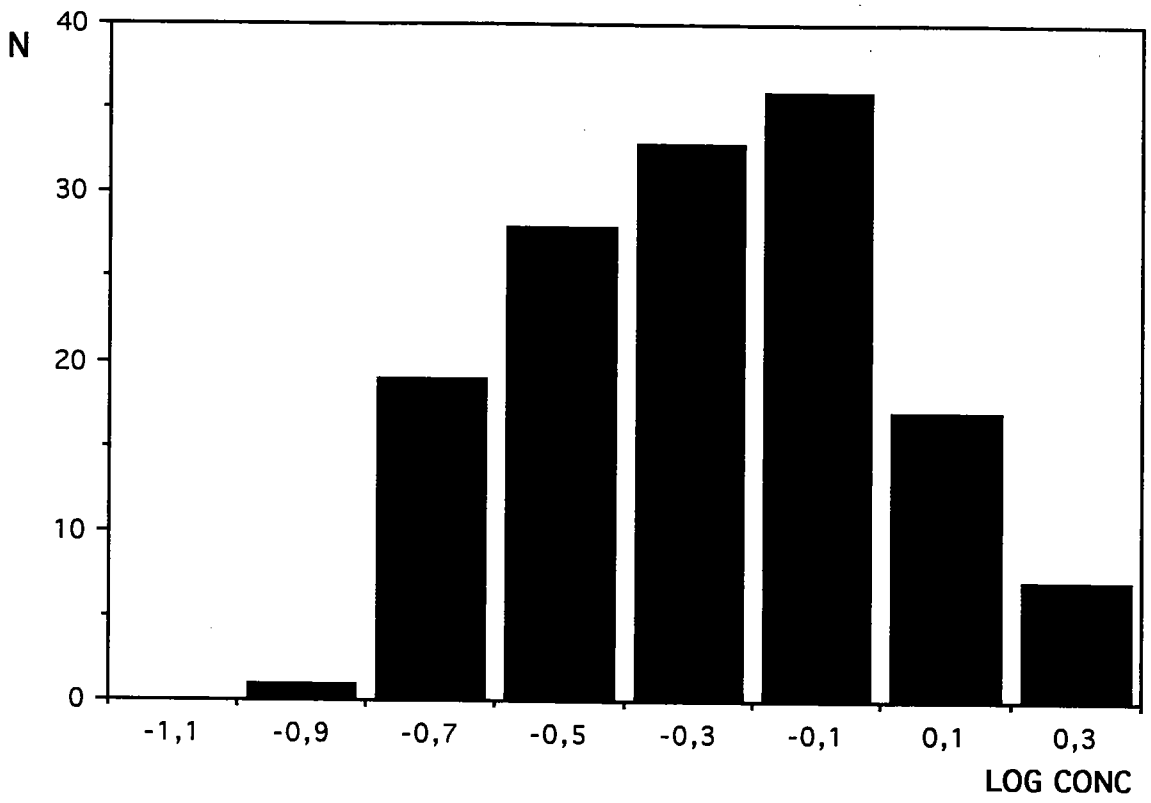


Fig 5 : LOG DISTRIBUTION OF THEOPHYLLINE IN ATHLETES

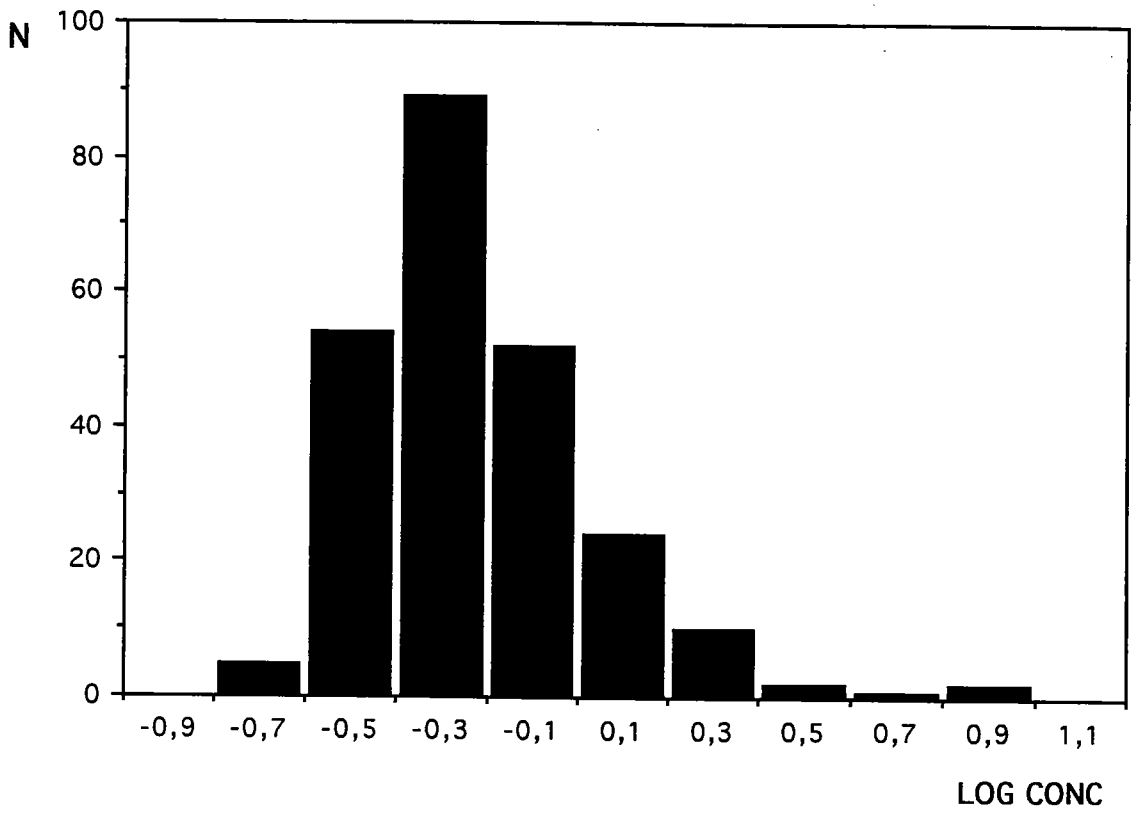


Fig 6 : TP/PX AFTER THEO 2 (150 mg)

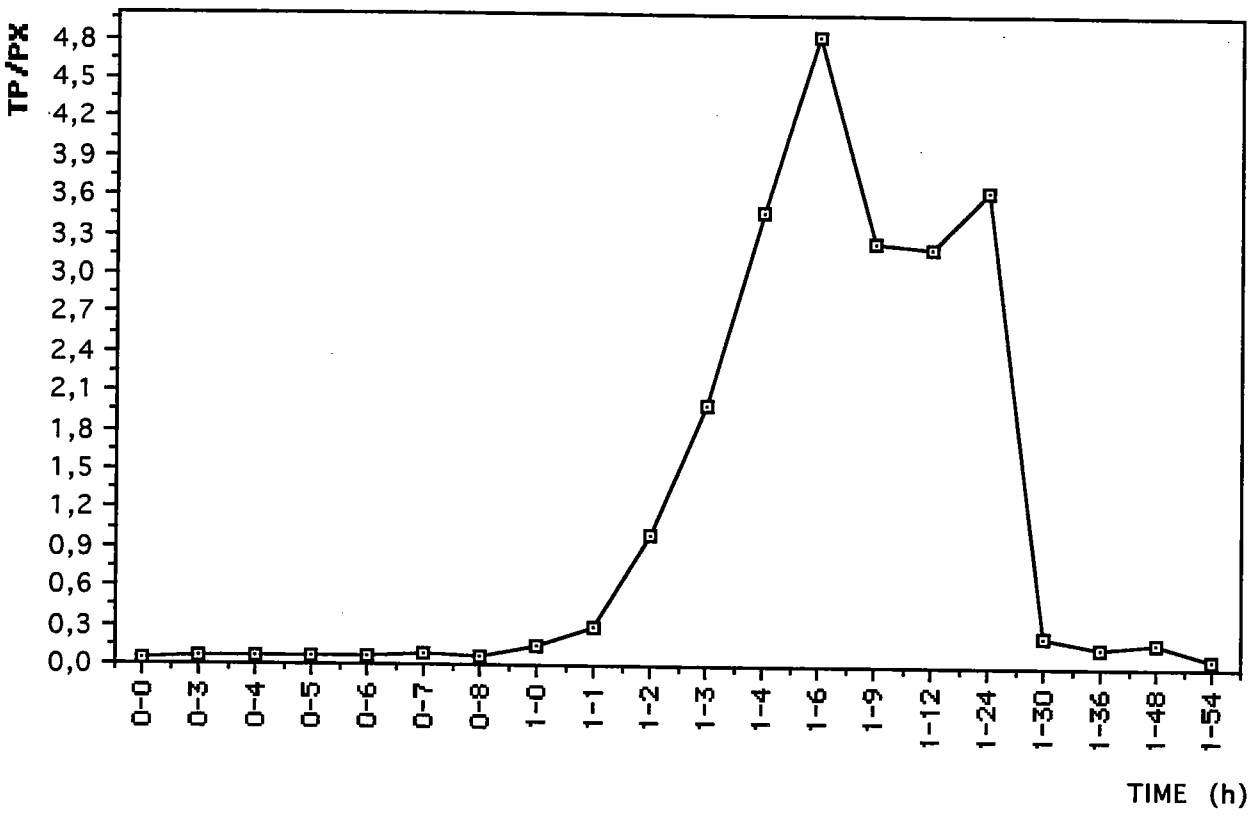
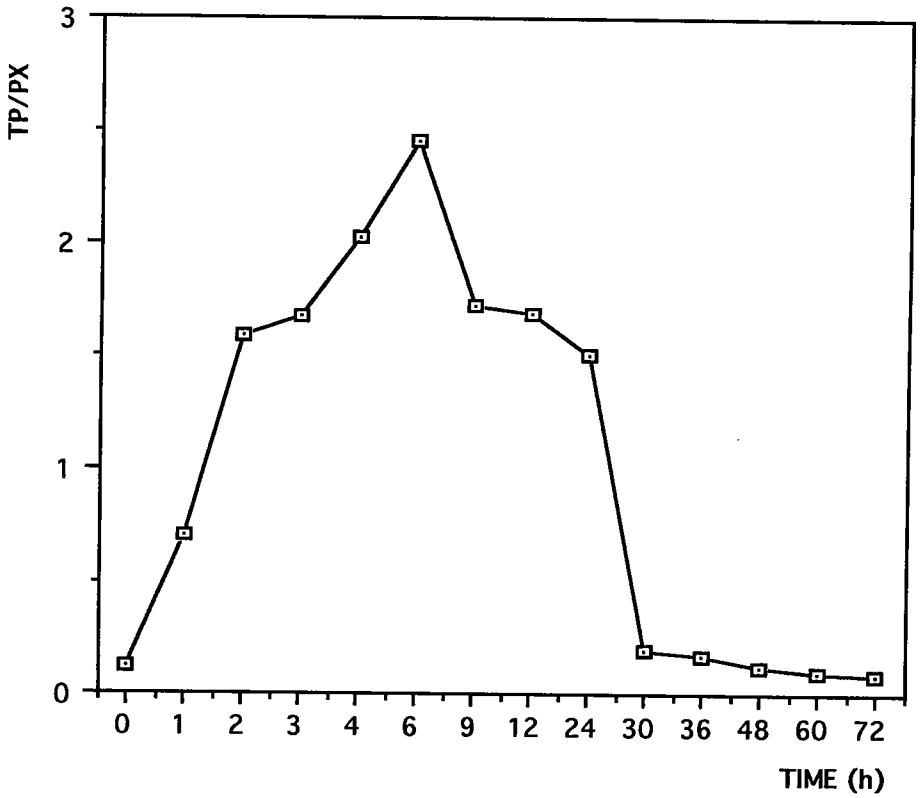


Fig 7 : TP/PX AFTER THEOLAIR (250 mg)



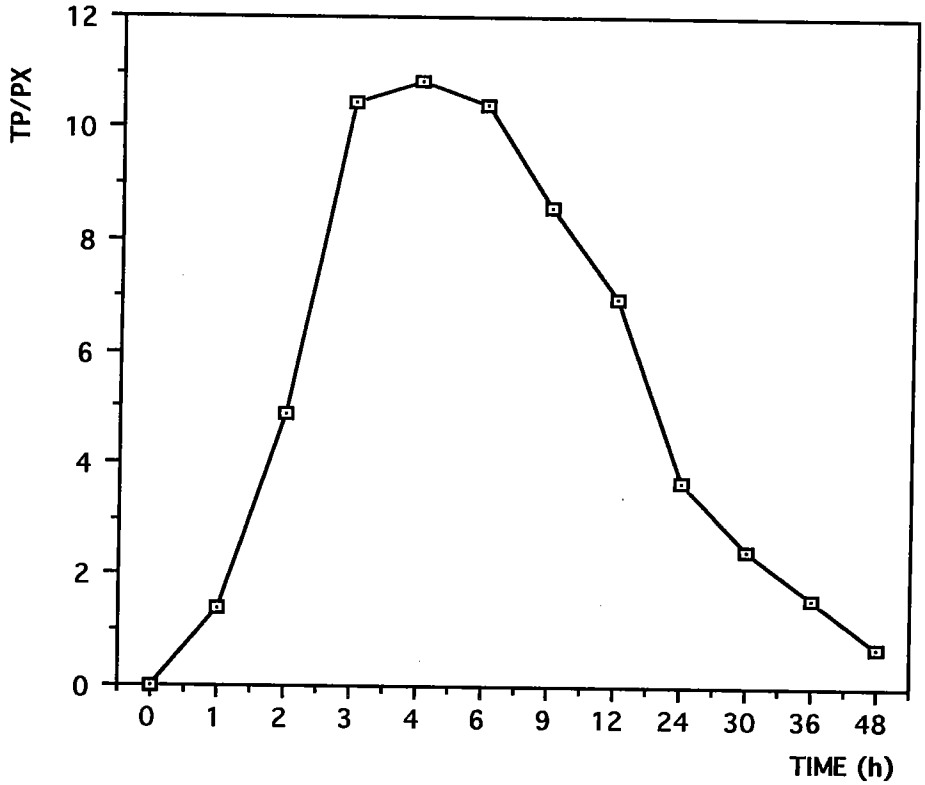


Fig 8 : LOG DISTRIBUTION TP/PX