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# Metabolism and alkylating activity of thio-TEPA in rat liver slice incubation*

**Bjørn Hagen[1], Ola Dale[1], Gunhild Neverdal[1], Shana Azri[2], and Odd G. Nilsen[1]**

[1] Department of Pharmacology and Toxicology, University of Trondheim, Trondheim, Norway
[2] Department of Anesthesiology, University of Arizona, Tucson, AZ, USA

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**Summary.** Precision-cut rat-liver slices were used to study the metabolism of the alkylating agent *N,N′,N″*-triethylenethiophosphoramide (thio-TEPA). Exposure to high concentrations (1–10 mM) of thio-TEPA for 6 h did not prove to be toxic to the liver slices as indicated by insignificant leakage of potassium from the cells. The time course of the disappearance of thio-TEPA (initial concentration, 5.2 μM) from the buffer during incubation followed first-order kinetics. Formation of *N,N′,N″*-triethylenephosphoramide (TEPA) apparently accounted for the elimination of thio-TEPA. Pretreatment of the rats with phenobarbital significantly increased the reaction rate. Conversely, pretreatment with the cytochrome P-450 inhibitor allylisopropylacetamide significantly reduced the metabolic rate. The elimination of thio-TEPA and formation of TEPA occurred independently of thio-TEPA concentration, which ranged from 5.2 to 104 μM. Thio-TEPA's oxo-analogue TEPA, which was not further metabolized, was the only metabolite identified. However, a significantly time-related increase in 4-(nitrobenzyl)-pyridine (NBP) alkylating activity was observed following incubation of liver slices with thio-TEPA but not after their incubation with TEPA. This may possibly indicate the formation of unknown active metabolites.

## Introduction

It has recently been shown that the alkylating agent *N,N′,N″*-triethylenethiophosphoramide (thio-TEPA) is metabolized to its oxo-analogue *N,N′,N″*-triethylenephosphoramide (TEPA) in man [12, 19], as had previously been demonstrated in various animal species [7]. Thus far, no other metabolites have been found. However, in urine from human patients who have been treated with thio-TEPA, <2% of the dose is recovered as thio-TEPA and TEPA, accounting for only a small part of the total 4-(nitrobenzyl)-pyridine (NBP)-alkylating activity found in their urine [6, 17]. This gap has been suggested to be due to active metabolites other than TEPA. Further evaluation of this gap by measurement of total NBP-alkylating activity in serum from thio-TEPA treated patients has not been possible due to methodological difficulties [1, 17].

Recent experimental studies have shown that the conversion of thio-TEPA to TEPA is catalyzed by hepatic cytochrome P-450 enzymes [25]. Studies using isolated rat-liver microsomes and reconstituted cytochrome P-450 enzyme systems have demonstrated that the phenobarbital-inducible P-450 subfamily IIB dominates the biotransformation of thio-TEPA. These enzymes also seem to be subject to inactivation as a result of the thio-TEPA metabolism [20]. Metabolic activation of thio-TEPA in vivo as shown by its increased cytotoxicity has been observed [13, 24] and subsequently demonstrated to be mediated by liver enzymes. This activation, however, could not be accounted for by the formation of TEPA [25]. This supports the hypothesis that active metabolites of thio-TEPA other than TEPA may be formed.

New techniques for the preparation and incubation of liver slices have been developed and applied in biotransformation studies [21]. In contrast to isolated hepatocytes or subcellular fractions, slices partly maintain tissue integrity. Slices have been found to remain viable for at least 4 h in a nutrient-free medium [9] and for at least 24 h in a supplemented incubation medium [22]. Tissue from various species, including humans, have been used in liver-slice incubation studies [5].

The aim of the present study was to establish the viability of precision-cut rat-liver slices in the presence of thio-TEPA and to use this model for further characterization of the in vitro metabolism of thio-TEPA and TEPA. Concentrations of thio-TEPA and TEPA and the total alkylating

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*Offprint requests to:* Bjørn Hagen, Dept. of Gynecology & Obstetrics, Trondheim Regional Hospital, N-7006 Trondheim, Norway

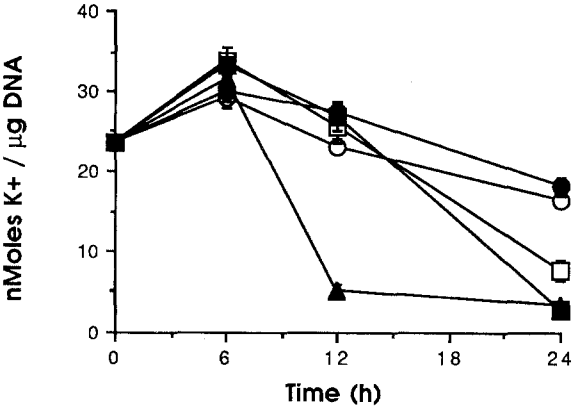


**Fig. 1.** The time course of the effect of thio-TEPA [○, control; ●, 1 mM; □, 2 mM; ■, 5 mM; ▲, 10 mM] on the intracellular content of $K^+$ in rat-liver slices during incubation. Each point represents the mean ± SD of duplicate experiments using slices from 4 individual rats

activity were determined in the incubation medium so as to explore their interrelationships.

## Materials and methods

*Drugs and chemicals.* Thio-TEPA was a gift from Lederle Laboratories (American Cyanamid, Pearl River, N.Y. USA). TEPA was purchased from Polysciences Limited (Northampton, UK). The purity of thio-TEPA and TEPA was assayed by thin-layer chromatography (TLC). The solvent systems used were 8 : 2 (v/v) chloroform: acetone for thio-TEPA and 1 : 9 (v/v) methanol: dichloromethane for TEPA. The chromatography was run for 1 h, the TLC plates were dried, and silica was scraped off in segments above the application points and then dissolved in ethanol. The amounts of thio-TEPA and TEPA in each segment were determined by gas chromatography and compared with equal amounts of thio-TEPA or TEPA that had been applied on TLC plates and run through the same procedure of drying and subsequent dissolution in ethanol. Uniform migration and complete recovery was demonstrated, with retardation factor ($R_f$) values of 0.81 and 0.64 for thio-TEPA and TEPA, respectively, being found. Sodium phenobarbital was purchased from Hydro Pharma (Oslo, Norway), and allylisopropylacetamide (AIA) was a gift from La Roche (Nutley N.J. USA). Waymouths, MB 752/1 powdered medium was purchased from Sigma Chemicals Co. (St. Louis, Mo., USA), and gentamycin was purchased from Gibco Laboratories (Boston, Mass., USA). 4-(Nitrobenzyl)-pyridine (NBP) was purchased from Aldrich-Chemie (Steinheim, FRG).

*Animals and animal treatment.* Male Sprague-Dawley rats (Møllegaard, Denmark) weighing 250–350 g were used. They were fed a standard pellet/water diet ad libitum and maintained on a 12-h light/dark cycle. The rats were killed by decapitation. To induce the cytochrome P-450 activity, 80 mg/kg phenobarbital in NaCl i.p. to 3 days was given [26]. To inhibit cytochrome P-450 activity, 100 mg/kg AIA in corn oil i.p. was given at 1 h prior to animal decapitation [3].

*Liver-slice preparation and incubation.* Excised livers were immediately placed in cold buffer (pH 7.4, 4° C) under constant aeration (95% $O_2$, 5% $CO_2$). Liver slices exhibiting a dry weight of 7.5–10 mg, corresponding to 25–30 mg wet weight, were produced in a standardized manner according to the method of Krumdieck et al. [18] using a modified device [9, 21]. Two slices were placed on the inside wall of cylindrical stainless-steel roller screens. These were placed inside 20-ml glass scintillation vials containing 1.7 ml incubation medium supplemented with drug. After undergoing gassing for 20 s (95% $O_2$, 5% $CO_2$), vials were screw-capped and rotated horizontally on a vial rotator at 4 rpm at 37° C. For

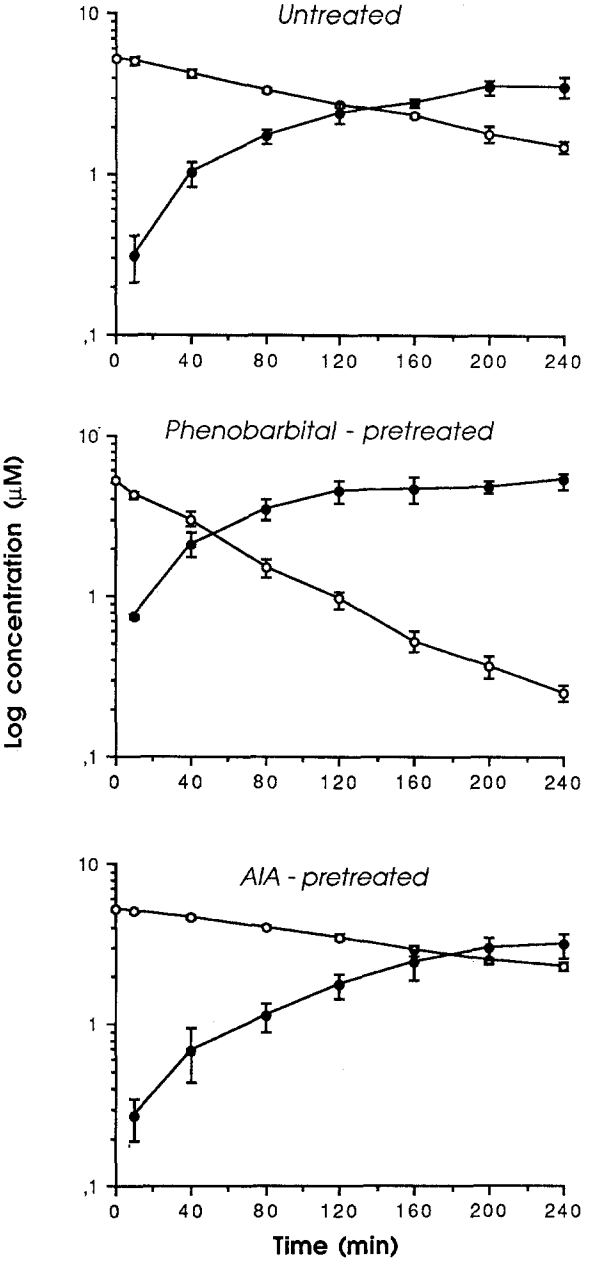


**Fig. 2.** The time course of thio-TEPA disappearance (○) and TEPA formation (●) during incubation of 5.2 µM thio-TEPA with liver slices from untreated, phenobarbital-pretreated, and allylisopropylacetamide-pretreated rats. Each point represents the mean ± SD of duplicate experiments using slices from 4 individual rats

saturation of the tissue uptake of drug in slices, all experiments were preceded by a preincubation period of 30 min; thereafter, the roller screens were transferred into new scintillation vials containing fresh buffer supplemented with drug [8]. The preincubation also enables the removal of debris from the cutting process and allows the slices to recover biochemically [9].

*Slice viability study.* The effect of thio-TEPA on slice viability was studied by the determination of intracellular potassium ($K^+$) leakage [10, 23]. Intracellular $K^+$ was measured by flame photometry and was expressed in relation to the DNA content as measured using fluorometry [2]. Thio-TEPA was added at concentrations of 1, 2, 5, and 10 mM and

**Table 1.** The concentration in the medium and the total content of thio-TEPA in rat-liver slices during their incubation in Waymouth's MB 752/1 medium

| Thio-TEPA added (mM) | Incubation time | | | | | |
|---|---|---|---|---|---|---|
| | 3 h | | 6 h | | 9 h | |
| | Medium[a] (mM) | Slice[b] (nmol) | Medium[a] (mM) | Slice[b] (nmol) | Medium[a] (mM) | Slice[b] (nmol) |
| 2 | 1.68 ±0.14 | 2.67 ±0.2 | 1.92 ±0.43 | 2.58 ±0.1 | 2.1 ±0.4 | 2.77 ±0.13 |
| 5 | 4.28 ±0.43 | 4.72 ±0.15 | 4.57 ±0.22 | 5.13 ±0.21 | 3.65 ±0.78 | 4.39 ±0.32 |
| 10 | 6.48 ±0.2 | 8.64 ±0.55 | 9 ±1.1 | 9.98 ±0.67 | 9.5 ±0.12 | 12.39 ±0.32 |

Values represent the mean (±SD) of duplicate experiments using slices of liver from 4 individual rats in each group
[a] Concentration of thio-TEPA in medium
[b] Total content of thio-TEPA in whole slices

**Table 2.** The amount of thio-TEPA and TEPA present in the incubation medium at various time points during incubation of 5.2 μ*M* thio-TEPA with liver slices from untreated, phenobarbital-pretreated, and allylisopropylacetamide-pretreated rats

| | Incubation time | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 min | | | 80 min | | | 160 min | | | 240 min | | |
| | | U | PB | AIA | U | PB | AIA | U | PB | AIA | U | PB | AIA |
| Thio-TEPA remaining (nmol) | 8.84 | 8.59 ±0.12 | 7.19 ±0.32 | 8.53 ±0.12 | 5.68 ±0.15 | 2.58 ±0.34 | 6.89 ±0.14 | 4 ±0.15 | 0.9 ±0.14 | 4.88 ±0.32 | 2.6 ±0.24 | 0.43 ±0.05 | 3.93 ±0.24 |
| TEPA formed (nmol) | – | 0.53 ±0.17 | 1.28 ±0.03 | 0.46 ±0.14 | 2.94 ±0.31 | 5.93 ±0.85 | 1.89 ±0.37 | 4.74 ±0.29 | 7.92 ±1.56 | 4.06 ±0.88 | 6.09 ±0.94 | 9.03 ±1.04 | 5.3 ±0.87 |
| Recovery (%) | | 103 | 96 | 102 | 98 | 96 | 99 | 99 | 100 | 101 | 98 | 107 | 104 |

Values represent the mean (±SD) of duplicate experiments using slices of liver from 4 individual rats in each group. Recovery at each time point is calculated as the sum of thio-TEPA and TEPA, expressed as a percentage of the amount of thio-TEPA added. U, Untreated rats; PB, phenobarbital-pretreated rats; AIA, allylisopropylacetamide-pretreated rats

slices were incubated for 6, 12, and 24 h. The results were compared with the values found for drug-free controls. Thio-TEPA in medium and slices was measured following 3, 6, and 9 h incubation at the three highest concentrations. Krebs-HEPES buffer was used as the slicing buffer and Waymouth's MB 752/1 medium supplemented with 10 μg gentamycin was used as the incubation medium.

The quantity of thio-TEPA used in the slice viability studies was measured by gas chromatography using the method of Hagen et al. [15], with modifications. A 1-ml aliquot of medium or slice homogenate was added to 1 ml ethyl acetate. Samples were vigorously agitated and then centrifuged at 600 *g* for 5 min. The organic layer was removed and a 1-μl volume was injected onto a 5-ft. ×2-mm column containing 3% OV-17 on a 100- to 120-mesh Gas Chrom Q (Supelco) in a Varian gas chromatograph (model 3700; Palo Alto, Calif. USA) equipped with a flame ionization detector (FID). A standard curve for thio-TEPA was used to calculate the concentrations in slices and medium. Although a nitrogen-phosphorus detector is the most sensitive device for the detection of this compound [15], the higher concentration of thio-TEPA used in the slice viability studies was readily detected by the FID.

*Drug metabolism studies.* Incubations were carried out for up to 4 h in a Krebs-Henseleit buffer. Experiments were performed using liver slices from untreated animals and rats that had been pretreated with phenobarbital or AIA. Duplicate incubations using slices from 4 individual animals were carried out for each set of experiments. Control incubations were performed in duplicate in the absence of liver slices; these were always run parallel to the liver-slice incubations.

Time-course experiments were carried out at a thio-TEPA concentration of 5.2 μM (1 μg/ml). Buffer concentrations of thio-TEPA and TEPA were measured at 10, 40, 80, 120, 160, 200, and 240 min after the start of incubation. The half-life of thio-TEPA disappearance from the incubation buffer was derived from the equation $t_{1/2} = 1n2/K_e$, where $K_e$ represents the slope of the linear regression line in the thio-TEPA concentration-time plot. Identical experiments were performed using TEPA.

Dose-related experiments involved incubations of thio-TEPA at concentrations of 5.2, 26, 52, and 104 μM (1–20 μg/ml) for 4 h, after which the buffer concentrations of thio-TEPA and TEPA were determined. The NBP-alkylating activity was determined for the three highest concentrations and was compared to control values obtained in the absence of liver slices. Identical experiments were performed using TEPA. The time course of NBP-alkylating activity was examined for thio-TEPA (26 μM) and TEPA (28.75 μM) using liver slices from untreated animals.

*Assays of thio-TEPA, TEPA, and alkylating activity.* All drug metabolism analyses were performed in the incubation buffer medium. Concentrations of thio-TEPA and TEPA were measured by gas chromatography as previously reported [15, 17]. Neither the incubation buffer medium nor the liver slices produced interfering peaks in the chromatograms. The detection limits were 26 and 115 nM for thio-TEPA and TEPA, respectively. NBP-alkylating activity was determined by colorimetry [14].

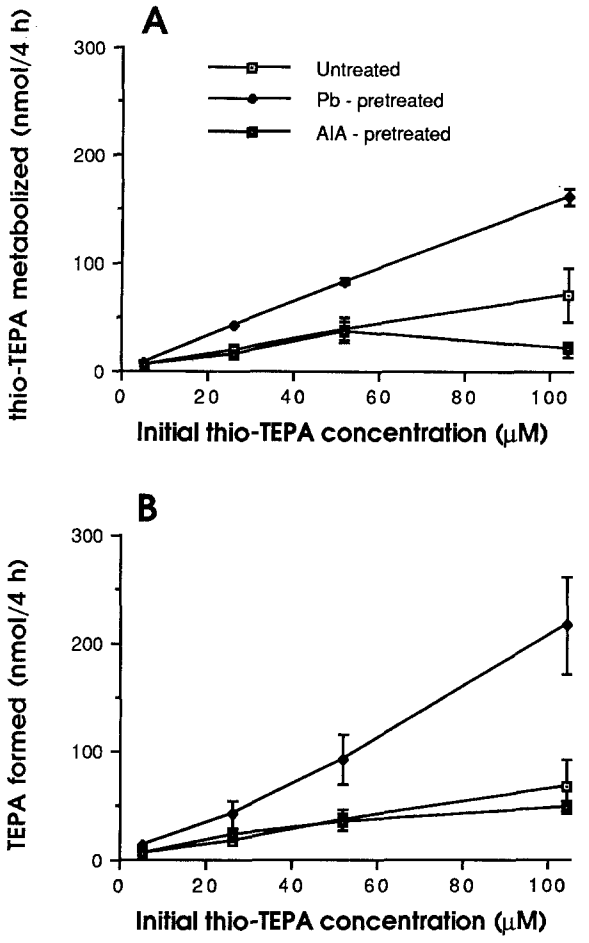


**Fig. 3 A, B.** The amounts of **A** thio-TEPA metabolized and **B** TEPA formed after 4-h incubations of thio-TEPA at concentrations ranging from 5.2 to 104 μM with liver slices from untreated, phenobarbital-pretreated (*Pb-pretreated*), and allylisopropylacetamide-pretreated (*AIA-pretreated*) rats. Each point represents the mean ± SD of duplicate experiments using slices from 4 individual rats

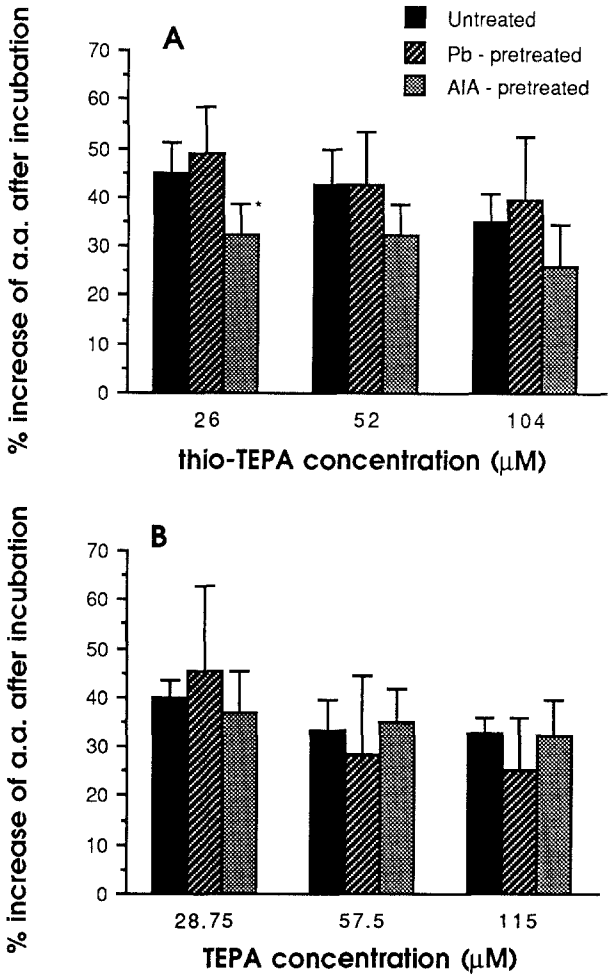


**Fig. 5 A, B.** The percentage of increase in NBP-alkylating activity after 4-h incubations of **A** thio-TEPA and **B** TEPA at 3 different concentrations with liver slices from untreated, phenobarbital-pretreated (*Pb-pretreated*) and allylisopropylacetamide-pretreated (*AIA-pretreated*) rats. Results are given as the mean ± SD of duplicate experiments using slices from 4 individual rats. * $P < 0.05$ vs the untreated group ($n = 4$)

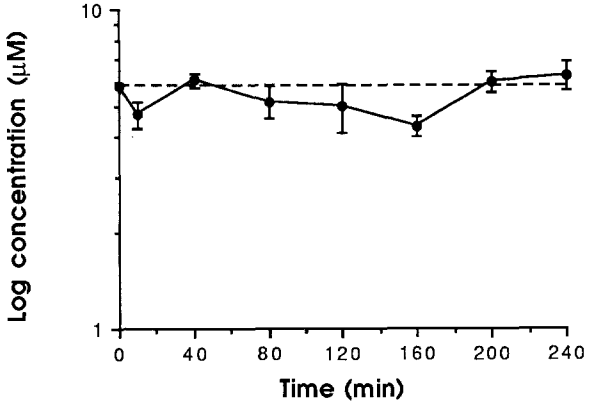


**Fig. 4.** The fate of TEPA during its incubation (5.75 μM) with liver slices from untreated rats. The *dashed line* represents the initial concentration of TEPA. Each point represents the mean ± SD of duplicate experiments using slices from 4 individual rats

Standard curves were constructed from known concentrations of thio-TEPA, and alkylating activity was expressed as thio-TEPA alkylating activity equivalents. The detection limit was 5.2 μM.

*Statistics.* Results were recorded as mean values (±SD, $n = 4$), and statistical analysis of differences between groups of observations was performed using Student's $t$-test. The correlation between the increase in alkylating activity and the duration of liver-slice incubation with thio-TEPA and TEPA was investigated by simple regression and was tested using analysis of variance. Values of $P < 0.05$ were considered to be significant.

## Results

The liver slices remained viable during 6 h incubation at all concentrations of thio-TEPA (Fig. 1). After 12 h, $K^+$ leakage was obvious at the highest thio-TEPA concentration (10 mM). Following 24 h incubation, a concentration-dependent degree of $K^+$ leakage from the liver cells was observed. However, at 1 mM, which was well above the drug concentrations used in subsequent biotransformation

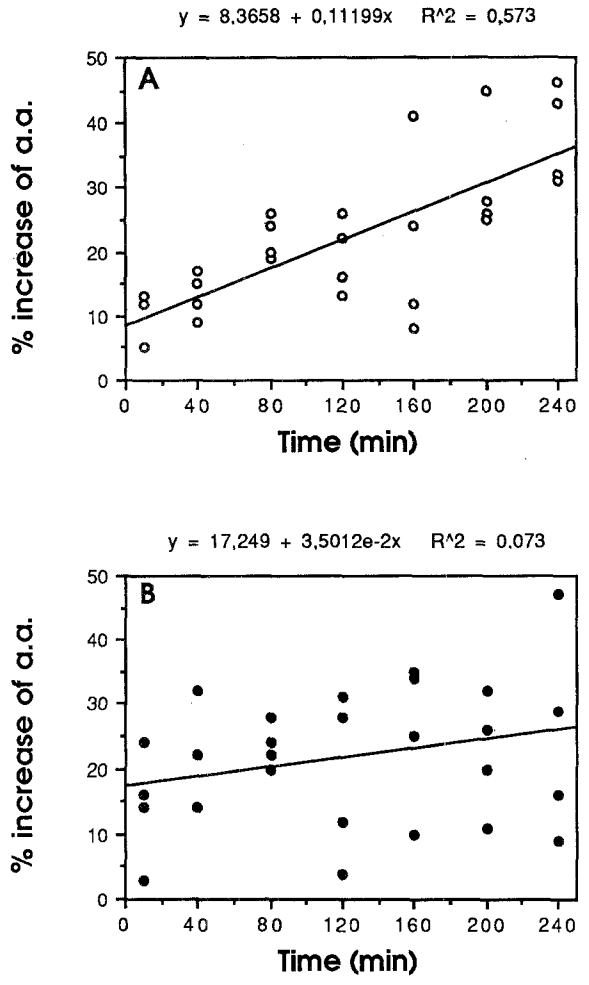


**Fig. 6 A, B.** The time-course of the percentage of increase in NBP-alkylating activity (*a. a.*) during incubation of rat-liver slices with **A** thio-TEPA (26 μM = 5 μg/ml) and **B** TEPA (28.75 μM = 5 μg/ml). Individual points representing 4 untreated rats in each experiment (duplicate incubations in each) are given, and the least-square regression lines are indicated

experiments, the $K^+$ leakage was not significantly different from that found in the control over the incubation period investigated. In the medium, more than two-thirds of the initial concentration of thio-TEPA remained even after 9 h, showing that the slices were in excess of thio-TEPA in the medium throughout the incubation. In the slices, the content of thio-TEPA was proportional to the concentration in the medium and remained stable throughout the study period, indicating that thio-TEPA was not accumulated in the slices (Table 1).

The time course of thio-TEPA disappearance and TEPA formation during incubation of liver slices with thio-TEPA is displayed in Fig. 2. The disappearance of thio-TEPA apparently followed first-order kinetics. Pretreatment of the rats with phenobarbital or AIA significantly increased or decreased, respectively, the rate of thio-TEPA conversion to TEPA. The half-life of thio-TEPA disappearance from the buffer incubation medium was $2.23 \pm 0.15$ h in slices from untreated animals as compared with $0.91 \pm 0.05$ h in slices from phenobarbital-pretreated animals ($P < 0.001$) and $3.23 \pm 0.27$ h in slices from AIA-pretreated animals ($P < 0.001$). The formation of TEPA was consistently accounted for by the removal of thio-TEPA throughout the incubation period (Table 2).

Figure 3 shows the relationships between the initial thio-TEPA concentrations and the disappearance of thio-TEPA and formation of TEPA, respectively, following 4 h liver-slice incubation. Linearity was found for slices from untreated and phenobarbital-pretreated animals, indicating that the removal of thio-TEPA followed first-order kinetics. Apparently, phenobarbital pretreatment increased the removal of thio-TEPA and the corresponding formation of TEPA, regardless of the initial thio-TEPA concentration. However, AIA pretreatment did not reduce the thio-TEPA disappearance/TEPA formation as compared with that observed in untreated controls, except at the highest initial thio-TEPA concentration.

The fate of TEPA during its incubation is shown in Fig. 4. No significant disappearance of TEPA from the buffer incubation medium was found. This also held true for liver slices from both phenobarbital- and AIA-pretreated animals (data not shown). The lack of TEPA metabolism was further demonstrated in 4-h incubations of TEPA at concentrations of 5.75, 28.75, 57.5 and 115 μM (1–20 μg/ml; data not shown). Control experiments carried out in the absence of liver slices revealed the stability of TEPA under the incubation conditions used.

Liver slice incubations at various concentrations of both thio-TEPA and TEPA produced an increase in NBP-alkylating activity in the buffer incubation medium that was on the order of 30%–50%, independent of drug concentration. Pretreatment of the animals with phenobarbital had no apparent effect on this phenomenon, whereas AIA pretreatment showed a tendency to reduce the increase in alkylating activity as compared with that observed in untreated controls. This effect of AIA was only seen following incubations with thio-TEPA (Fig. 5). The increased alkylating activity observed after liver-slice incubation was further investigated by determination of its time dependence. A significant correlation with time was found for thio-TEPA ($r^2 = 0.573$, $P = 0.0001$) but not for TEPA ($r^2 = 0.073$, $P = 0.165$, Fig. 6). Control experiments involving liver slice incubation in the absence of drug revealed that a background alkylating activity originating from the liver slices as such could be ruled out.

## Discussion

Incubation of precision-cut rat-liver slices seemed to be adequate for the study of thio-TEPA metabolism as judged from the lack of thio-TEPA toxicity in the system in terms of leakage of $K^+$ from the liver cells. $K^+$ leakage was observed only at drug concentrations and incubation times far beyond those used in the drug metabolism experiments. This indicates that liver cells remained intact throughout the incubation period.

This study supports the observation that the liver mediates the metabolism of thio-TEPA [4, 11]. Induction of an increase in the biotransformation of thio-TEPA to TEPA by phenobarbital pretreatment was evident from the results

of both the time-related and the dose-related experiments, supporting the findings of Ng and Waxman [20] that phenobarbital-inducible forms of cytochrome P-450 contribute significantly to the elimination of thio-TEPA and associated formation of TEPA.

AIA, which acts by alkylating the prosthetic heme moiety of cytochrome P-450 [3], has previously been shown to reduce the toxicity of carbon tetrachloride toxicity to rat-liver slices by inhibition of its bioactivation [2]. In the present study, AIA pretreatment clearly reduced the rate of conversion of thio-TEPA to TEPA in the time-course experiments, whereas a reduction was found only at the highest dose in the dose-related experiments. The latter indicate AIA-induced saturation of the metabolic process, whereas the former demonstrate that the dose-related end-point experiments seemed to be less sensitive than the time-course experiments.

Ng and Waxman [20] reported suicidal inactivation of the major phenobarbital-inducible form of P-450 in their system of reconstituted cytochrome P-450. This was not confirmed in our study using intact liver slices, as thio-TEPA was metabolized according to first-order kinetics over a period of 4 h. A possible explanation for this difference may be that the rat-liver slices, with their partly preserved tissue integrity, could biotransform thio-TEPA and concomitantly detoxify possible harmful intermediates. Our results are compatible with human studies demonstrating unchanged thio-TEPA pharmacokinetics during repeated treatments over >20 weeks [16].

In the present study, no metabolites other than TEPA were identified by gas chromatographic analysis following thio-TEPA incubations. Our slice incubations with TEPA alone indicated that no further metabolism of TEPA occurred. This corresponds with the findings of Ng and Waxman [20], who could not identify metabolites of thio-TEPA other than TEPA using gas chromatography or thin-layer chromatography. However, Ng and Waxman [20] reported that only 50%–85% of the depleted thio-TEPA was recovered as TEPA in their purified and reconstituted cytochrome P-450 enzyme systems, whereas our data indicated 100% recovery as judged by the amounts of thio-TEPA and TEPA measured. This discrepancy may be explained by the different conditions used for the extraction of thio-TEPA and TEPA in the two studies prior to the gas chromatographic quantitation. In the former, extraction from lipid constituents in the reconstituted cytochrome P-450 reaction mixture may be incomplete, leading to an underestimation of recovery [20]. On the other hand, although the extraction from the aqueous incubation medium was complete in the present study, there may have been some contribution to the amount of TEPA from its formation in the slices during preincubation, leading to an overestimation of recovery.

Active metabolites of thio-TEPA other than TEPA have been postulated from human studies showing that urinary recovery of thio-TEPA and TEPA accounted for only a fraction of the total alkylating activity [6, 17]. This is supported by data demonstrating an increase in the cytotoxicity of thio-TEPA following incubation with rat-liver homogenate, despite the lower intrinsic cytotoxicity of the metabolite TEPA as compared with that of the parent drug [25]. Our finding of increased alkylating activity following liver-slice incubation, even when thio-TEPA and TEPA exhibited the same intrinsic alkylating activity, may indicate that additional active metabolites were formed. Although end-point studies demonstrated increased alkylating activity after incubation for both thio-TEPA and TEPA, a time-related correlation was seen only for thio-TEPA. This indicates that there is considerable nonspecific background activity in the system, which we have shown is not produced by the liver slices alone. Thus, the increased alkylating activity observed following liver-slice incubation with TEPA, although difficult to explain, was probably not caused by the metabolism of TEPA. In the case of thio-TEPA, both the observed time relation and the possible inhibitory effect of AIA suggest that the increased alkylating activity found following liver-slice incubation with thio-TEPA may reflect a metabolic process.

However, the question as to the identity of possible thio-TEPA metabolites other than TEPA remains unanswered. We are planning further studies to pursue our observations by introducing species other than the rat. In addition, the effect of enzyme-inducing and -inhibiting agents other than phenobarbital and AIA will be investigated.

In conclusion, the present in vitro study supports previous reports of liver-mediated metabolism of thio-TEPA. The biotransformation of thio-TEPA to TEPA follows first-order kinetics, and no further metabolism of TEPA occurs. Consistent with recent observations of liver-mediated increases in the cytotoxicity of thio-TEPA, incubation of liver slices with thio-TEPA produces an increase in NBP-alkylating activity that may reflect the formation of unknown active metabolites.

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## References

1. Ackland SP, Choi KE, Ratain MJ, Egorin MJ, Williams SF, Sinkule JA, Bitran JD (1988) Human plasma pharmacokinetics of thiotepa following administration of high-dose thiotepa and cyclophosphamide. J Clin Oncol 6: 1192
2. Azri S, Gandolfi AJ, Brendel K (1990) Carbon tetrachloride toxicity in precision-cut rat liver slices. In Vitro Toxicol 3: 127
3. Bornheim LM, Kotake AN, Correia MA (1985) Differential haemin-mediated restoration of cytochrome P-450 *N*-demethylases after inactivation by allylisopropylacetamide. Biochemistry 277: 277
4. Breau AP, Field L, Mitchell WM (1984) Thiono compounds: in vitro mutagenic and antineoplastic activity of TEPA and thio-TEPA. Cell Biol Toxicol 1: 21
5. Brendel K, Gandolfi AJ, Sipes IG, Krumdieck CL (1988) Precision-cut liver slices in organ culture: a new tool in human drug metabolism and hepatotoxicity studies. AAPS June
6. Cohen BE, Egorin MJ, Kohlhepp EA, Aisner J, Gutierrez PL (1986) Human plasma pharmacokinetics and urinary excretion of thiotepa and its metabolites. Cancer Treat Rep 70: 859
7. Craig AW, Fox BW, Jackson H (1959) Metabolic studies of $^{32}$P-labeled triethylenethiophosphoramide. Biochem Pharmacol 3: 42
8. Dale O, Gandolfi AJ, Brendel K, Schuman S (1988) Rat liver slices and diazepam metabolism: in vitro interactions with volatile anaesthetic drugs and albumin. Br J Anaesth 60: 692

9. Dale O, Holten T, Nørkov T (1990) Rat liver slices: metabolic changes during short time incubation in glucose-free buffer. Pharmacol Toxicol 67: 88
10. DiRenzo AB, Gandolfi AJ, Brooks SD, Brendel K (1985) Toxicity and biotransformation of volatile halogenated anesthetics in rat hepatocyte suspensions. Drug Chem Toxicol 8: 207
11. Egorin MJ, Akman SA, Gutierrez PL (1984) Plasma pharmacokinetics and tissue distribution of thio-TEPA in mice. Cancer Treat Rep 68: 1265
12. Egorin MJ, Cohen BE, Kolhepp EA, Gutierrez PL (1985) Gas-liquid chromatographic analysis of *N,N′,N″*-triethylenethiophosphoramide and *N,N′,N″*-triethylenephosphoramide in biological samples. J Chromatogr Biomed Appl 343: 196
13. Frei E III, Teicher BA, Holden SA, Cathcart KNS, Wang Y (1988) Preclinical studies and clinical correlation of the effect of alkylating dose. Cancer Res 48: 6417
14. Friedman OM, Boger E (1961) Colorimetric estimation of nitrogen mustards in aqueous media. Anal Chem 33: 906
15. Hagen B, Walseth F, Walstad RA, Iversen T (1985) Gas chromatographic assay of triethylenethiophosphoramide in serum and urine. J Chromatogr Biomed Appl 345: 173
16. Hagen B, Walseth F, Walstad RA, Iversen T, Nilsen OG (1987) Single and repeated dose pharmacokinetics of thio-TEPA in patients treated for ovarian carcinoma. Cancer Chemother Pharmacol 19: 143
17. Hagen B, Neverdal G, Walstad RA, Nilsen OG (1990) Long-term pharmacokinetics of thio-TEPA, TEPA and total alkylating activity following i. v. bolus administration of thio-TEPA in ovarian cancer patients. Cancer Chemother Pharmacol 25: 257
18. Krumdieck CL, Santos JE dos, Ho K-J (1980) A new instrument for the rapid preparation of tissue slices. Anal Biochem 104: 118
19. McDermott BJ, Double JA, Bibby MC, Wilman DEV, Loadman PM, Turner RL (1985) Gas chromatographic analysis of triethylenethiophosphoramide and triethylenephosphoramide in biological specimens. J Chromatogr Biomed Appl 338: 335
20. Ng S, Waxman DJ (1990) Biotransformation of *N,N′,N″*-triethylenethiophosphoramide: oxidative desulfuration to yield *N,N′,N″*-triethylenephosphoramide associated with suicide inactivation of a phenobarbital-inducible hepatic P-450 monooxygenase. Cancer Res 50: 464
21. Smith PF, Gandolfi AJ, Krumdieck CL, Putnam CW, Zukoski CF, Davis WM, Brendel K (1985) Dynamic organ culture of precision liver slices for in vitro toxicology. Life Sci 36: 1367
22. Smith PF, Fisher R, McKee R, Gandolfi AJ, Krumdieck CL, Brendel K (1989) Precision cut liver slices. A new tool in toxicology. In: McQueen CA (ed) In vitro models in toxicology. Telford Press, Caldwell, New Jersey, pp 93–130
23. Stacey NH, Priestly BG (1978) Dose-dependent toxicity of carbon tetrachloride in isolated rat hepatocytes and the effects of hepatoprotective treatments. Toxicol Appl Pharmacol 45: 29
24. Teicher BA, Holden SA, Cucchi CA, Cathcart NS, Korbut TT, Flatow JL, Frei E III (1988) Combination of *N,N′,N″*-triethylenethiophosphoramide and cyclophosphamide in vitro and in vivo. Cancer Res 48: 94
25. Teicher BA, Waxman DJ, Holden SA, Wang Y, Clarke L, Sotomayor EA, Jones SM, Frei E III (1989) Evidence for enzymatic activation and oxygen involvement in the cytotoxicity and antitumor activity of *N,N′,N″*-triethylenethiophosphoramide. Cancer Res 49: 4996
26. Toftegaard R, Nilsen OG (1982) Effect of xylene and xylene isomers on cytochrome P-450 and enzymatic activities in rat liver, kidney and lung. Toxicology 23: 197