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DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The chemical reactivity of ethylene (EO), propylene (PO) and butylene (BO) oxides was determined by reacting with nicotinamide in vitro and comparing their apparent zero order rate constants. The order of reactivity was EO>PO>BO. The ratios of reactivity of EO/PO, EO/BO and PO/BO were 2.00, 2.49 and 1.25 respectively. Comparison of this sequence of chemical reactivity to the overall general toxicity reported in the literature leads one to the conclusion that toxicity is in a similar order (EO>PO>BO). However, when comparing specific toxicologic effects in complex multicellular systems, differences emerge in these qualitative biological effects. It is likely that these qualitative differences are a manifestation of differing capacities for detoxification and repair (both cellular and DNA) operating in complex biological systems. Therefore caution must be exercised in the simple extrapolation of biological effects of chemicals from parameters such as chemical reactivity.

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COMPARISON OF CHEMICAL REACTIVITY TO BIOLOGIC ACTIVITY OF THE ALKYL EPOXIDES

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ABSTRACT

Comparison of Chemical Reactivity to Biologic Activity of the Alkyl Epoxides. Fox, T. R., Reitz, R. H., and Watanabe, P. G.

The chemical reactivity of ethylene (EO), propylene (PO) and butylene (BO) oxides were determined by reaction with nicotinamide in vitro and comparing their apparent zero order rate constants. The order of reactivity was EO>PO>BO. The ratios of reactivity of EO/PO, EO/BO and PO/BO were 2.00, 2.49 and 1.25 respectively. Comparison of this sequence of chemical reactivity to the overall general toxicity reported in the literature leads one to a similar conclusion that toxicity is in a similar order EO>PO>BO. However, of toxicologic significance when comparing specific toxicologic effects such as mammalian mutagenicity, teratogenicity and carcinogenicity marked differences emerge in these qualitative biological effects. It is likely that these qualitative differences are a manifestation of differing capacities for detoxification and repair (both cellular and DNA) operating in complex biological systems. Therefore caution must be exercised in the simple naive extrapolation of similar biological effects to chemicals of closely related structure and chemical reactivity.

DOW CHEMICAL U.S.A.

BIOCHEMICAL RESEARCH LAB. MIDLAND, MICHIGAN 48640

TOX. NO. T2.2-193-(1

FILE NO. HET T2.02-193-000-001

REQUEST FOR TOXICOLOGICAL STUDIES

1803 BLDG.

NAME OF MATERIAL TO BE TESTED: ETHYLENE OXIDE (PROPYLENE OXIDE, 1,2-BUTYLENE OXIDE)

SUBMITTED BY (Signature): Richard J. Reitz | (Date): Nov. 30, 1982 | (Building): 1803 | (Phone): 6-5995

SUPERVISOR (Signature) | (Date and Building) | PROJECT SAFETY COORDINATOR | (Date)

ESTIMATED COST	ACCOUNT NUMBER	LEDGER / LOCATION GROUP / FUNCTION / SECTION / SUB. FUNC / SUFFIX	PROBLEM NO. TO BE USED BY TOXICOLOGY	PROBLEM INDEX NO. LAB. / PROBLEM
$12,000				

DO NOT WRITE BELOW THIS LINE — COMPLETE ALL INFORMATION ON REVERSE.

ROUTE OF ADMINISTRATION: IN VITRO

LENGTH OF TREATMENT: —— N.A.

SPECIES:

RAT ☐M ☐F | RABBIT ☐M ☐F | GUINEA PIG ☐M ☐F

DOG ☐M ☐F | MONKEY ☐M ☐F | HAMSTER ☐M ☐F

OTHER: IN VITRO

DOSES:

CHECK POINT	DATE
SAMPLE RECEIVED TOXICOLOGY	10/10/82
ESTIMATED STARTING TIME	11/30
ESTIMATED REPORTED DATE	1/30
REVISED ESTIMATE REPORTED	
REPORT DRAFTED	
REPORT ISSUED	

FATE OF SAMPLE	DATE
☐ RETURNED	
☒ DESTROYED	
☐ REFERENCE SAMPLE STORED	
☐ REFERENCE SAMPLE TO K STORAGE	

NOTES

DATE PROJECT COMPLETED

NOTES, CHANGES, ADVERSE EFFECTS NOTED

Ethylene Oxide : K-1708-(10)
Propylene Oxide: K-1709-(9)
1,2-Butylene Oxide: K-64969-(9)

DO 142642
CONFIDENTIAL

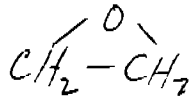
SAMPLE INFORMATION

NAME: ETHYLENE OXIDE, (PROPYLENE OXIDE, 1,2-BUTYLENE OXIDE)

MOLECULAR FORMULA	MOLECULAR WEIGHT	SAMPLE REFERENCE, SOURCE AND OR IDENTIFICATION
C_2H_4O	44.053	LOT # J11-0517

SYNONYMS: OXIRANE

STRUCTURAL FORMULA OR COMPOSITION



PHYSICAL AND CHEMICAL PROPERTIES

ESTIMATED PURITY OF SAMPLE	SAMPLE ANALYSIS	RESULTS
>95%	[X] YES [ ] NO	

BOILING POINT	EXPLOSIVE LIMITS (% BY VOLUME IN AIR)	FLASH POINT	IGNITION TEMP.	MELTING POINT	VAPOR PRESSURE
10.5 °C 760 mmHG	3%	25 °F	571 °C	NA °C	1.71 ATM mmHG 25°C

CORROSIVENESS (to common metals)	PHYSICAL STATE	COLOR
LOW	GAS	CLEAR

CHEMICAL REACTIVITY	ODOR (include concentration in air)
HIGH	NONE

STABILITY (to pH change, heat, light): GOOD

Why are toxicological studies needed? Include stage of development, proposed use, method of handling, and amount handled. Do you know of any adverse effects from human exposure?

In vitro studies will be carried out to objectively assess the reactivity of Ethylene oxide, Propylene Oxide and 1,2-Butylene oxide under identical reaction conditions.

	DATE		DATE		DATE
[ ] PROTOCOL COMPLETED		[ ] STATISTICS DONE		[ ] SLIDES TO BE READY	
[ ] ANIMALS ORDERED		[ ] CLINICAL TESTS REPORTED		[ ] SLIDES READ	DO 142643 CONFIDENTIAL
[ ] ANIMALS STARTED ON TEST		[ ] HEMATOLOGY TESTS REPORTED		[ ] HISTOPATH REPORTED	
[ ] NECROPSY DONE		[ ] SLIDES STARTED		[ ] WORK ALL DONE, READY TO WRITE	

INTRODUCTION

Ethylene oxide (EO) has been reported to increase the frequency of spontaneously occurring tumors in animals (Snellings et al., 1981) and preliminary information from Europe (Reuzel et al., publication in progress) indicates that propylene oxide (PO) may have a similar effect although PO is less potent than EO in this respect.

Butylene oxide (BO) is a structural analogue of EO and PO, but appears to be less toxic (acutely and subchronically) than either EO or PO (Hine et al, 1981; Miller et al., 1981). An inhalation bioassay of BO for carcinogenicity is presently being conducted by the National Toxicology Program (Contract #600253, agent C5527), and the results will probably be reported sometime in 1983-4 (personal communication, Dr. Thomas Cameron, NCI).

It appears that the toxicity of the alkyl epoxides may be related to the capacity of these materials to covalently react with nucleophilic sites within biological macromolecules. In order to objectively assess the relative potency of these three epoxides, the relative reactivity of these materials were determined under identical conditions.

Nicotinamide is readily alkylated by the alkyl epoxides in an aqueous solution at physiological pH (Ellis et al., 1982). The N-alkylnicotinamides formed by such a reaction can be converted to cyclic products which display absorbence maxima around 360 nM. Such a reaction is ideally suited to compare the *in vitro* reactivity of EO, PO and BO.

There are limitations in using *in vitro* measurements to try to estimate *in vivo* effects. However, determining the reactivity of EO, PO and BO with nicotinamide should provide an objective quantitative measure of the chemical's potential to react with biological macromolecules.

MATERIALS AND METHODS

Samples of the alkyl epoxides PO and BO (production sample, 1,2-butylene oxide, Lot #167) were obtained from the production facilities of the Dow Chemical Company. A cylinder of EO (Lot #J11-0517) was purchased from Valley Oxygen (Bay City, MI). Nicotinamide was obtained from the Sigma Chemical Company (St. Louis, MO). All other chemicals

used were of standard reagent grade and obtained from commercial suppliers.

The in vitro measurements of the alkylation of nicotinamide by EO, PO and BO were conducted essentially as stated by Ellis et al. (1982). The reactions were carried out in 1.8 ml Teflon* lined screw capped vials in a total reaction volume of 1.0 ml. The composition of the reaction mixture is described below:

700 µl 0.1 M Phosphate buffer (pH 7.4) containing 7.1% ETOH

200 µl Nicotinamide (250 µM/ml) in above buffer

100 µl Ethanolic epoxide solution

The ethanolic BO and PO solutions were prepared directly by addition of epoxide to dry ice-chilled ethanol at a concentration of 100 umoles/ml.

Because EO is a gas, it was necessary to prepare the stock solution by bubbling 10 ml of gas into 4 ml of dry-ice chilled ethanol to obtain a concentration similar to the BO and PO solutions.

The samples were incubated at 50°C. The color was then developed by adding 0.5 ml acetone and 0.2 ml 6 M KOH, waiting 5 minutes, and adding 2.0 ml distilled water. After 11 minutes the optical density was read at 358 nM in a Beckman Acta CIII spectrophotometer.

The concentration of the epoxide in the liquid phase of the reaction vials was determined analytically by flame ionization gas chromatography using a Varian 3700 gas chromatograph. Separation was achieved on a 3.5 m x 2 mm, 20% Carbowax 20 M on Chromosorb WAW-DMCS (80-100 ml) column. The column temperature was varied between 80-100°C isothermal depending on the epoxide analyzed. Nitrogen carrier flow was at 15 ml/min. Injection port and detector temperatures were 120°C and 250°C respectively.

The extinction coefficients of the N-alkyl-nicotinamides were determined by incubating the nicotinamide (6.22×10^{-4}M) with a 20 fold excess of epoxide under the conditions described for the rate determinations. The optical density was then measured when the reaction had reached completion. The molar extinction coefficients were calculated directly using Beer's law.

*Trademark of the DuPont Chemical Company

RESULTS

The three epoxides used in this study have fairly high vapor pressures at the 50°C incubation temperature and in an aqueous environment might undergo hydrolysis. Therefore it was important to analytically determine the epoxide concentration prior to and at the termination of the incubation period. The concentration of the three epoxides as measured by GC analysis is listed in Table 1. At the beginning of the incubation the concentrations were EO (5.1 mM), PO (8.7 mM), BO (9.2 mM). In a set of replicate vials incubated without nicotinamide it was observed that the EO concentration had decreased by 8 percent and the PO and BO concentration by 3 percent at the end of the 80 minute incubation. These decreases are not considered significant and did not impact on the experimental results.

The reaction rates of EO, PO and BO with nicotinamide were determined by measuring the initial rate of change in optical density resulting from the production of the nicotinamide adduct. To make this direct comparison of reactivity based on a comparison of the slopes of the molar absorbence versus time graph, the molar extinction coefficients must be the same. These coefficients were determined by reacting a 20 fold excess of epoxide with nicotinamide and measuring the optical density when the reaction had reached completion. Using Beer's law, the molar extinction coefficients of the three N-alkylnicotinamide adducts were computed and are essentially identical (Table 2).

The relative reactivity of EO, PO and BO can be determined by comparing the apparent zero order rate constants. Figure 1 represents a plot of the molar absorbence (MA) versus time of incubation for the reaction of the three epoxides with nicotinamide. Because the concentration of the epoxides varied between EO, PO and BO, the optical densities were normalized on a per mole per liter basis. As can be seen in Figure 1, a linear relationship exists between the molar absorbency and time for all three epoxides thereby satisfying the zero order conditions (only 2-5 % of either reactant was consumed). Linear regression data for the three plots is tabulated in Table 2.

The relative reactivity of the three epoxides is determined by computing the ratio of the rate constants derived from the slopes of the linear plots in Figure 1. These ratios, found in Table 3, indicate that

EO is about 2.5 times more reactive than BO and 2 times more reactive than PO. A comparison of the rate constants between PO and BO showed PO to be slightly more reactive (1.25 times) than BO.

DISCUSSION

It has been suggested that the toxic, mutagenic and carcinogenic potential of a chemical can be related to chemical reactivity or alkylation capacity. In this study the in vitro reactivity of EO, PO and BO with nicotinamide was determined. The results indicated that EO was about 2.5 and 2.0 times more reactive than BO and PO respectively. A comparison between the reactivity of PO and BO resulted in PO being 1.25 times more reactive than BO.

Recently the assessment in the potency of chemical toxicity has been made using a wide spectrum of biological endpoints from simplistic in vitro bacterial systems to more complex animal bioassays. In order to put the reactivity data in perspective with the existing toxicity data it is useful to examine several of these systems in which the epoxides have been tested.

Measurements of acute toxicity generally follow the same trend as observed for chemical reactivity. The LC_{50} in rats for EO has been determined to be 1460 ppm and the LC_{50} for PO is approximately 3600 ppm for a 4 hour exposure (Hine et al., 1981). BO is less toxic than PO and appreciably less toxic than EO. Rats can tolerate a single 7 hour exposure to 2600 ppm BO without any mortalities (unpublished data, Dow Chemical Company).

Bioassays for EO and PO have been completed (Snellings et al., 1980; Reuzel et al., 1982), and a bioassay of BO is underway under the auspices of the NTP (started in December, 1981). Administration of EO was associated with an increase in mononuclear cell leukemia in female F344 rats and peritoneal mesothelioma in male F344 rats, while administration of PO was associated with an increase in benign and malignant mammary tumors in female Wistar rats. These tumors occur at a fairly high spontaneous incidence in the corresponding strains in the absence of chemical treatment, although they appear to occur sooner and with higher multiplicity in the treated animals. In addition, both EO and PO produced a generalized increase in total tumors and total malignant

tumors at the top dose (100 ppm for EO; 300 ppm for PO). Thus EO and PO appear qualitatively similar in their ability to affect tumor incidences, although this comparison is complicated by the fact that the bioassays were conducted in different strains of rats.

The ability to cause mutation, a manifestation of electrophilic chemicals, has been linked to toxicity and the possible onset of carcinogenicity. The three epoxides used in this study have all been determined to be mutagenic in the Salmonella typhimurium bacterial mutagenicity assay (Embree et al., 1975; Bootman et al., 1979). No qualitative difference in response of the alkyl epoxides is evident in this simple in vitro system. However it is important to note that the bacteria used in this assay have deficient DNA repair mechanisms unlike those found in normal bacteria or other higher mammalian system.

However, if one examines several mammalian systems capable of detecting genetic damage, a greater disparity in effects caused by the three epoxides becomes evident. Exposure to EO caused an increase in the number of sister chromatid exchanges in rabbit lymphocytes (Yager et al., 1981), an increase in dominant lethality in rats and mice (Generoso et al., 1980; Embree et al., 1975), and an increased frequency of sex linked recessive lethal mutations in Drosophila (Glaser et al., 1979). While PO was also found to induce mutations in Drosophila the induction of dominant lethality in rats and mice was questionable. In addition, no evidence was found for mutagenic action of PO on mice sperm at 50 and 250 mg/kg/day (Bootman et al., 1979; NTP, 1982). On the other hand, BO failed to produce any of these effects in similar studies (eg. dominant lethality, mouse sperm head morphology, Drosophila sex-linked recessive lethal, unscheduled DNA synthesis, or rat bone marrow cytology (NTP, 1982).

Reproductive and teratogenic effects have also been reported for EO. Significant reduction in fetal body weight and an increase in malformed fetuses were observed at a 150 mg/kg/day dose (LaBorde, 1980). In contrast, BO did not produce any significant embryotoxicity or teratogenicity at either 250 or 1000 ppm exposure in rats or rabbits (Sikov et al., 1980).

From the data presented, the quantitative differences in chemical reactivity between the three epoxides are relatively small. Using this

data alone one might postulate that there should be very little difference in the qualitative biological effects of the three epoxides. However, in examination of several biological endpoints ranging from simple bacterial mutagenicity to more complex multicellular systems this simplistic hypothesis weakens as differences emerge in the observed qualitative biological effects. It is likely that this difference can be attributed to various biological barriers, the presence of enzymatic detoxification (GSH), and repair mechanisms (cellular and DNA) operating in these more complex systems. Therefore, caution must be exercised in simply extrapolating similar biological effects to molecules of similar structure and chemical reactivity.

Written by:

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Mar. 23, 1983

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23 Mar 1983

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TABLE 1

INITIAL AND FINAL CONCENTRATION OF ALKYL EPOXIDES

Compound	Conc. Before Incubation	Conc. After Incubation	% Change
EO	5.1 ± .48 mM*	4.7 ± .23 mM*	-8%
PO	8.7 ± .12 mM*	8.4 ± .12 mM*	-3%
BO	9.2 ± .26 mM*	8.9 ± .08 mM*	-3%

*n=3 for all determinations

TABLE 2

LINEAR REGRESSION ANALYSIS AND MOLAR EXTINCTION COEFFICIENT FOR REACTION OF ALKYL EPOXIDES WITH NICOTINAMIDE

Compound	Equation of Curve	Correlation Coefficient	Extinction Coefficient
EO	M.A.[a] = 3.74 (t)[b] -2.98	.9973	6,226 ± 115*
PO	M.A. = 1.87 (t) -0.7	.9992	6,293 ± 98*
BO	M.A. = 1.5 (t) +1.98	.9983	6,266 ± 72*

[a] M.A. = Molar Absorbence

[b] t = Time in minutes

*n=4 for all determinations

FIGURE 1

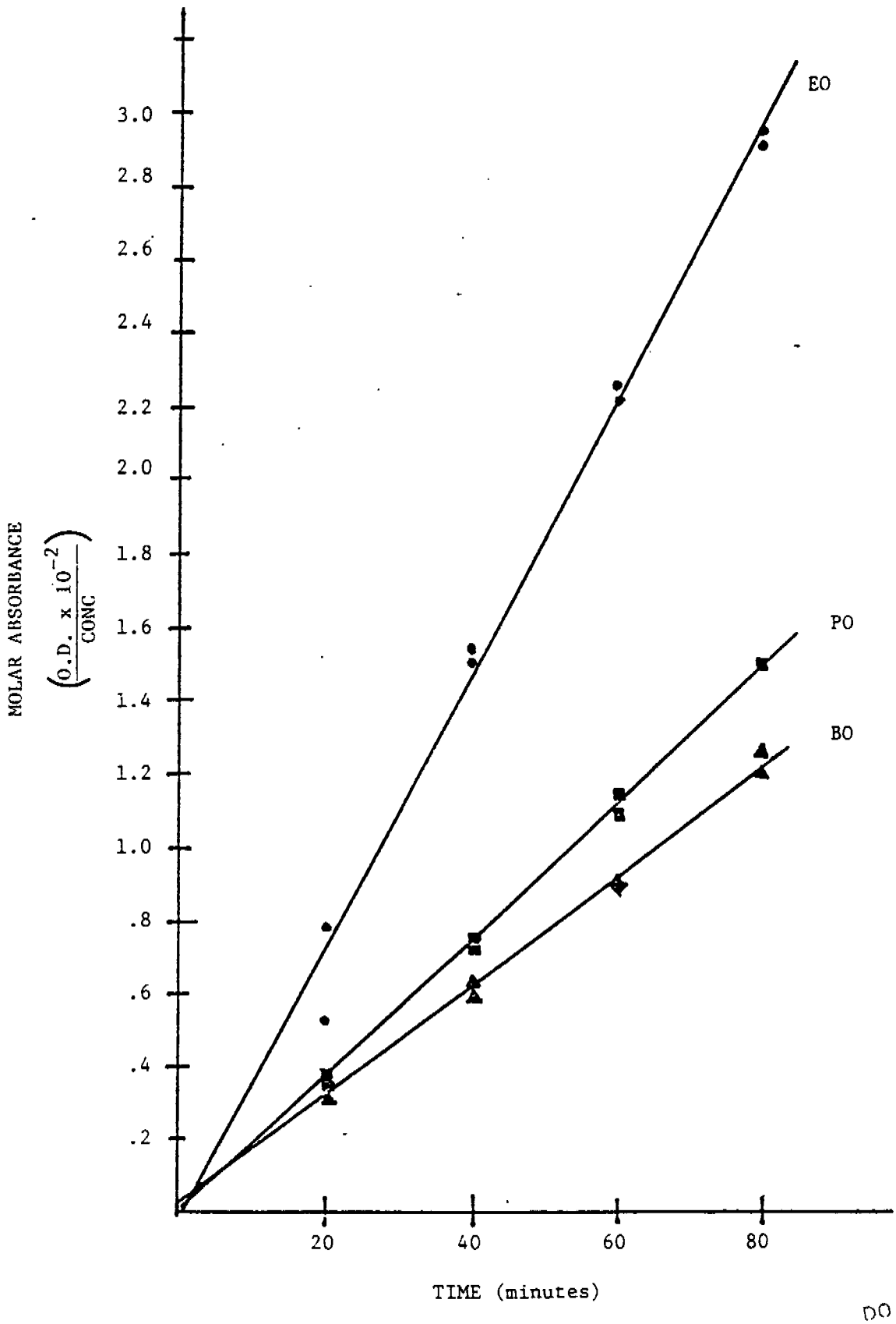

TABLE 3

RELATIVE REACTIVITIES OF n-ALKYL EPOXIDES WITH NICOTINAMIDE

Ratio of Rate Constants	Relative Reactivities
EO/BO = $\frac{3.74}{1.5}$	2.49
EO/PO = $\frac{3.74}{1.87}$	2.00
PO/BO = $\frac{1.87}{1.5}$	1.25