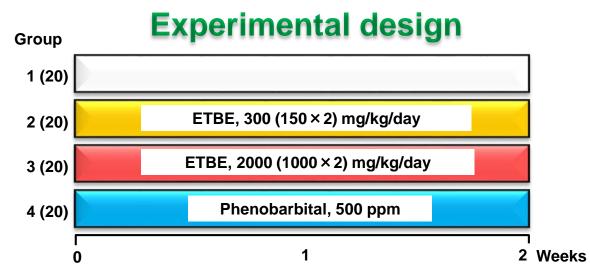
Test Material

Chemical name: Ethyl *tertiary*-butyl ether (ETBE), or 2-ethoxy-2-methylpropane

 $\begin{array}{c}
C H_3 \\
H_3C - C - C H_2 - C H_3 \\
C H_3
\end{array}$

Objectives of the study

- ➤ Elucidation of mode of action (MOA) of the increased incidence of rat liver tumors by ETBE administration:
- Formation of oxidative stress (OH*, P450, 8-OHdG)
- DNA repair
- Alteration to protein expression,
- Changes in cell ultrastructure,
- Alteration to apoptosis and cellular proliferation



Animals: 6-week-old F344 male rats;

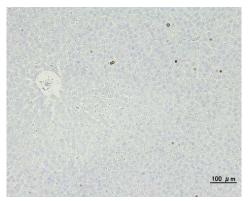
ETBE was administered at dose levels of 0 (control), 300 and 2000 mg/kg/day by gavage in olive oil for 1 and 2 weeks. Animals in group 4 were fed diet containing 500 ppm phenobarbital sodium (PB). Liver perfusion was performed in all rats.

Generation of hydroxyl radicals in the liver (ESR) Week 1 P<0.01 Week 2 0 PB, Control ETBE. ETBE. PB, Control ETBE, ETBE, **Proteome analysis** 300 500 2000 500 300 2000 mg/kg/day mg/kg/day ppm mg/kg/day mg/kg/day Week 2 ppm Induction of peroxisome proliferation Transcription PB, 500 ppm **ETBE, 2000** mg/kg/day TERT, RUNX1, Cytoskeleton in hepatocytes Week 2 ipid metabolisi **YWHAB** SPTBN2 PON1, APOE Transporters Xenobiotic metabolism APOE Xenobiotic metabolism CYP2E1, CYP2G1, CYP2B1, CYP2B2, CYP3A1, CYP3A2 FDX1,SOD2 CYP2C6, CYP2C55, CYP2A2, Cytoskeleton GSTA1, GSTA3, GSTA5, GSTM5, Xenobiotic ACTN1, PLEC, GPX1,UGT1A1, UGT2B1, UGT2B3, metabolism **MARCKS** UGDH, SOD1 Aminoacid and carbohydrate CYP2C11, CYP2C12, Aminoacid and GSTT2/GSTT2B, carbohydrate metabolism UGT1A6, ALDH1A1, ARG1, ASS1, metabolism Protein post-translational KHK, PDK2, **Aminoacid and** modification, folding, PGD, PNP, carbohydrate ubiquitination metabolism PDIA6, HSP90AA1, HSPA2 Lipid metabolism Signal transduction PGK1. SUCLG1 ACOX1, ECH1, ETBE, **Control** MARK3, M DLD SLC27A2, TOR, RAB3IP 2000 mg/kg/day SLC27A5 CYP2B1/2 and 8-OHdG coordinated elevation in hepatocytes Week 2 ETBE, ETBE, PB. Control 300 mg/kg/day 2000 mg/kg/day 500 ppm

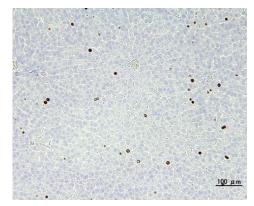
Immunohistochemical BrdU labeling indices in hepatocytes Day 3

Groups	Treatment duration (days)	No. of rats	BrdU labeling index in hepatocytes (%)
Control	3	3	16.94 ± 1.51
ETBE	3	3	$23.82 \pm 3.05 *$
Control ETBE	10 10	5 5	4.08±2.38 5.48±3.14
Control	17	5	2.12 ± 1.68
ETBE	17	5	3.03 ± 1.79
Control	28	5	0.89 ± 0.33
ETBE	28	5	$2.21 \pm 0.99 *$

Rats were administered ETBE by gavage, 1000 mg/kg b.w. twice a day with a 6-hour interval.



Control



ETBE, 2000 mg/kg/day

^{*}P<0.05 vs control group

