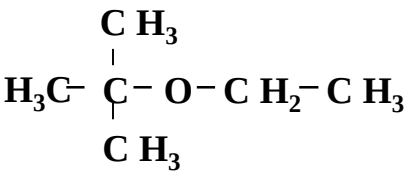
Test Material

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

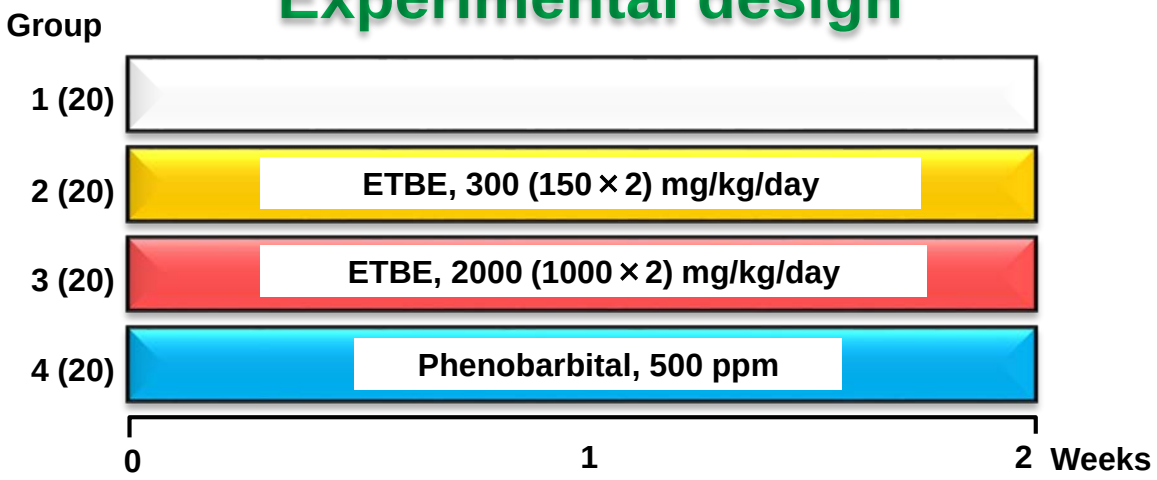


Objectives of the study

➢ **Elucidation of mode of action (MOA) of the increased incidence of rat liver tumors by ETBE administration:**

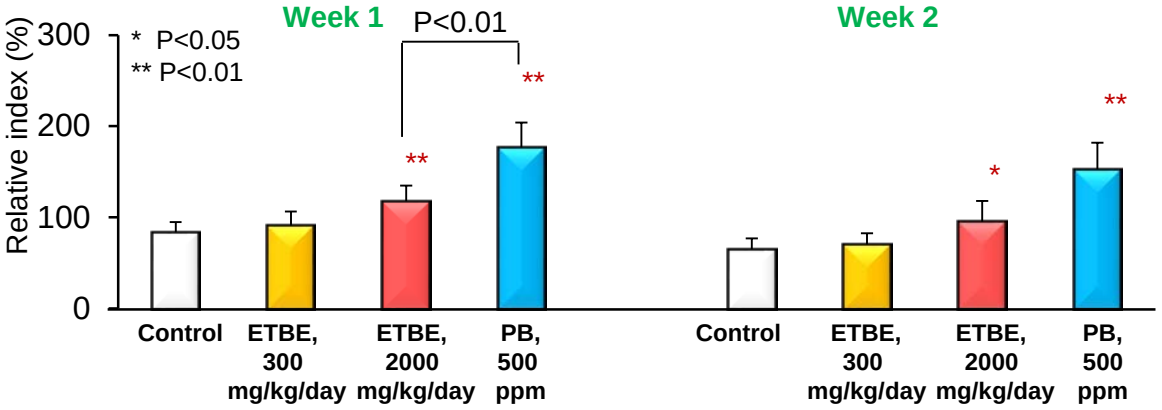
- **Formation of oxidative stress ($OH^{\bullet}$, P450, 8-OHdG)**
- **DNA repair**
- **Alteration to protein expression,**
- **Changes in cell ultrastructure,**
- **Alteration to apoptosis and cellular proliferation**

Experimental design

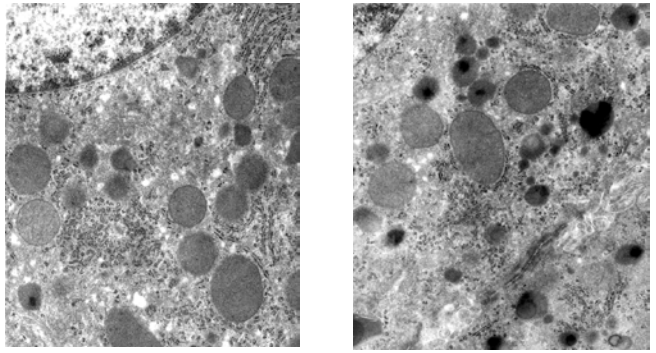


Animals: 6-week-old F344 male rats;
ETBE was administered at dose levels of 0 (control), 300 and 2000 mg/kg/day by gavage in olive oil for 1 and 2 weeks. Animals in group 4 were fed diet containing 500 ppm phenobarbital sodium (PB) . Liver perfusion was performed in all rats.

Generation of hydroxyl radicals in the liver (ESR)



Induction of peroxisome proliferation in hepatocytes Week 2

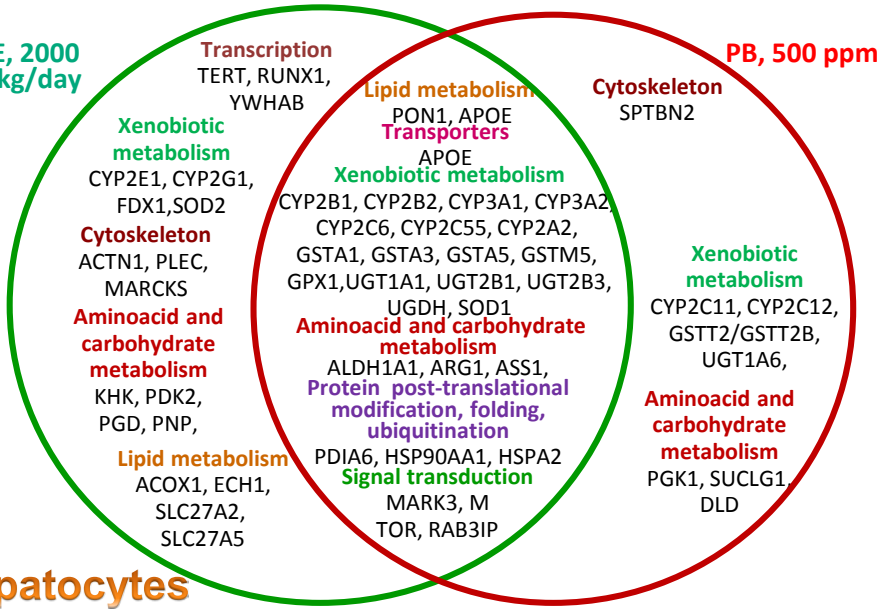


Control

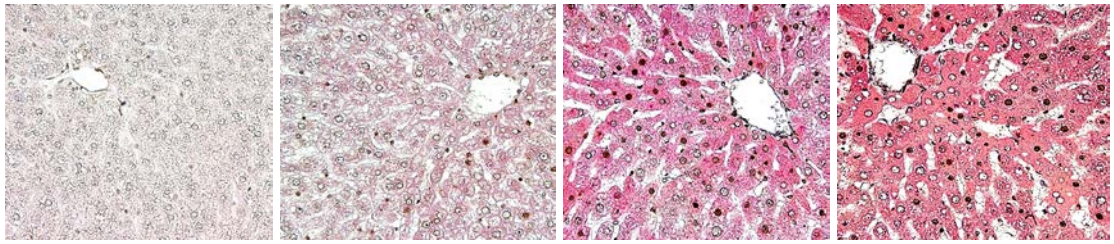
ETBE, 2000 mg/kg/day

Proteome analysis

Week 2



CYP2B1/2 and 8-OHdG coordinated elevation in hepatocytes



Week 2

Control

ETBE, 300 mg/kg/day

ETBE, 2000 mg/kg/day

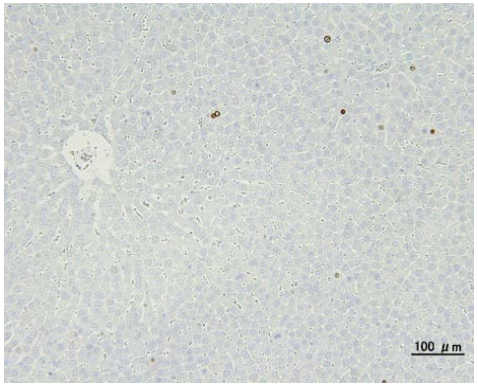
PB, 500 ppm

Immunohistochemical BrdU labeling indices in hepatocytes

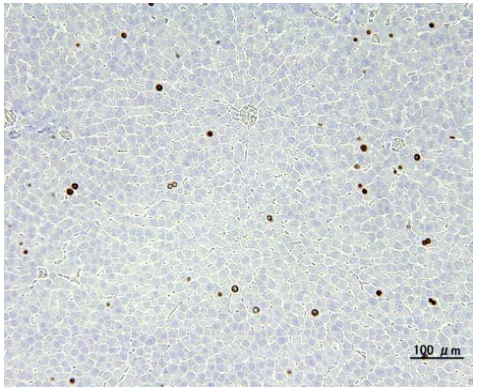
Groups	Treatment duration (days)	No. of rats	BrdU labeling index in hepatocytes (%)
Control	3	3	16.94±1.51
ETBE	3	3	23.82±3.05*
Control	10	5	4.08±2.38
ETBE	10	5	5.48±3.14
Control	17	5	2.12±1.68
ETBE	17	5	3.03±1.79
Control	28	5	0.89±0.33
ETBE	28	5	2.21±0.99*

Rats were administered ETBE by gavage, 1000 mg/kg b.w. twice a day with a 6-hour interval.
*P<0.05 vs control group

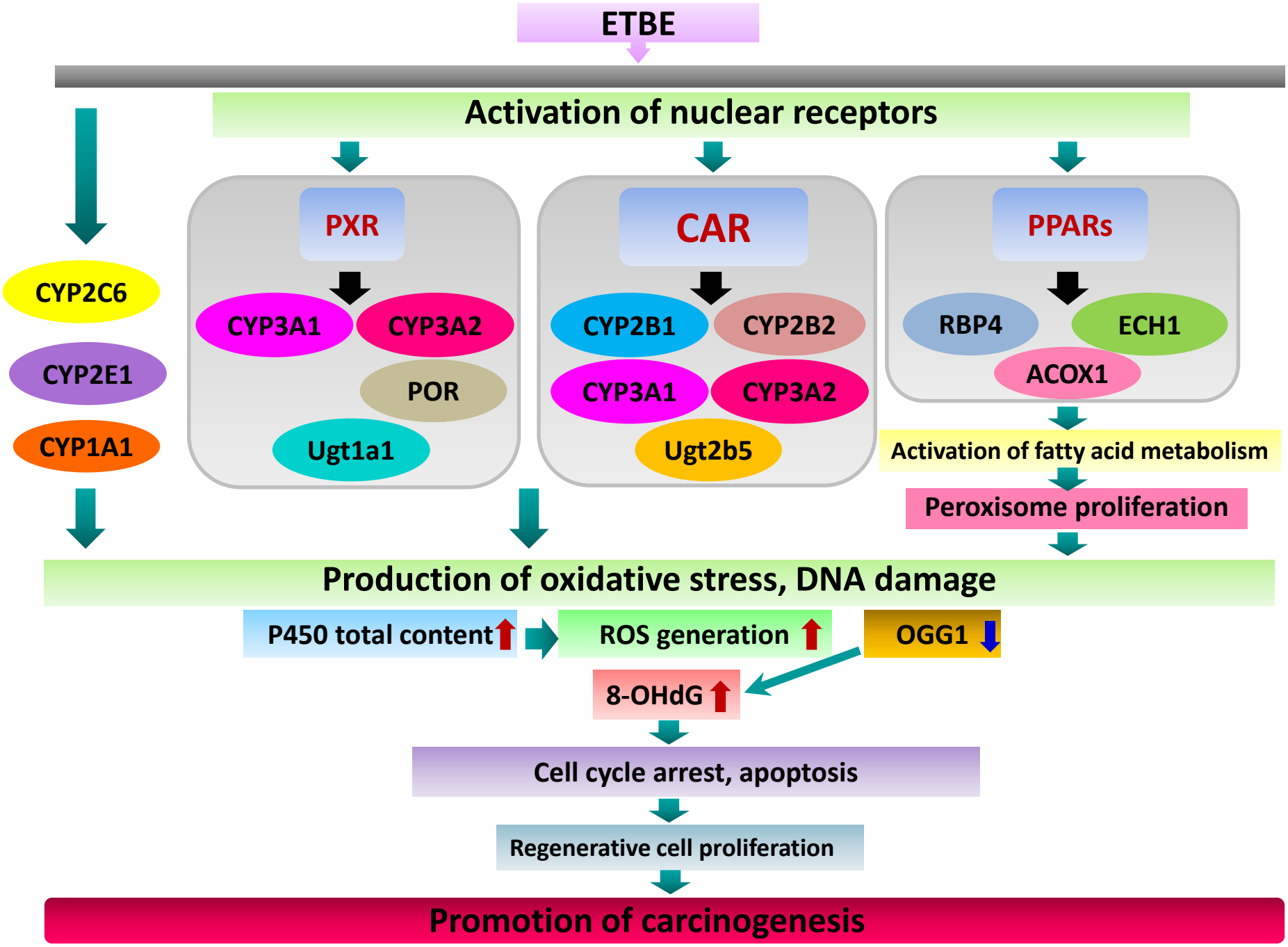
Day 3



Control



ETBE,
2000 mg/kg/day



Graphically illustrated MOA for ETBE hepatotumorigenicity in rats