Comments for Toxicological Review of Ethyl Tertiary Butyl Ether (External Review Draft)-2

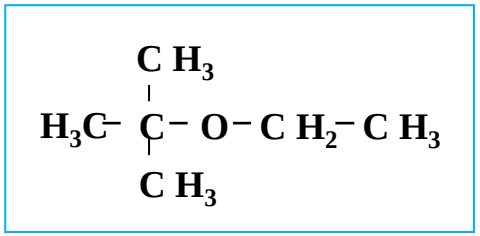
The Public Meeting of the Science Advisory Board Chemical Assessment Advisory Committee Augmented for the Review of EPA's Draft Ethyl Tertiary Butyl Ether(ETBE) and tert-Butyl Alcohol(tert-butanol;TBA) Assessments

August 15-17, 2017
The Residence Inn Arlington Crystal View
Arlington VA, USA

Mode of Action (MOA) of ETBE Hepatotumorigenicity in Rats:
MOA which induces centrilobular hypertrophy of hepatocyte and tumor of the liver

Test Material

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

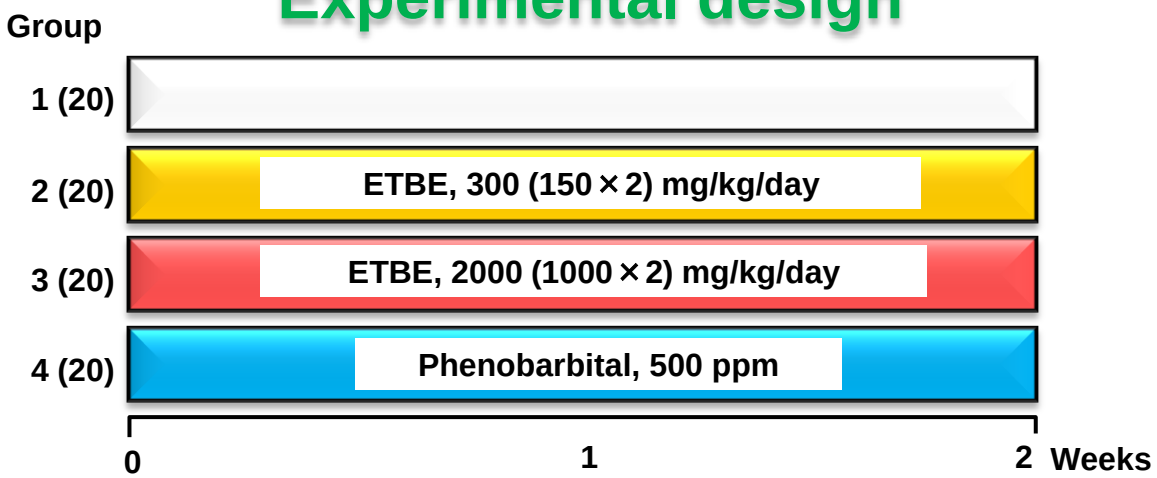


Objectives of the study

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

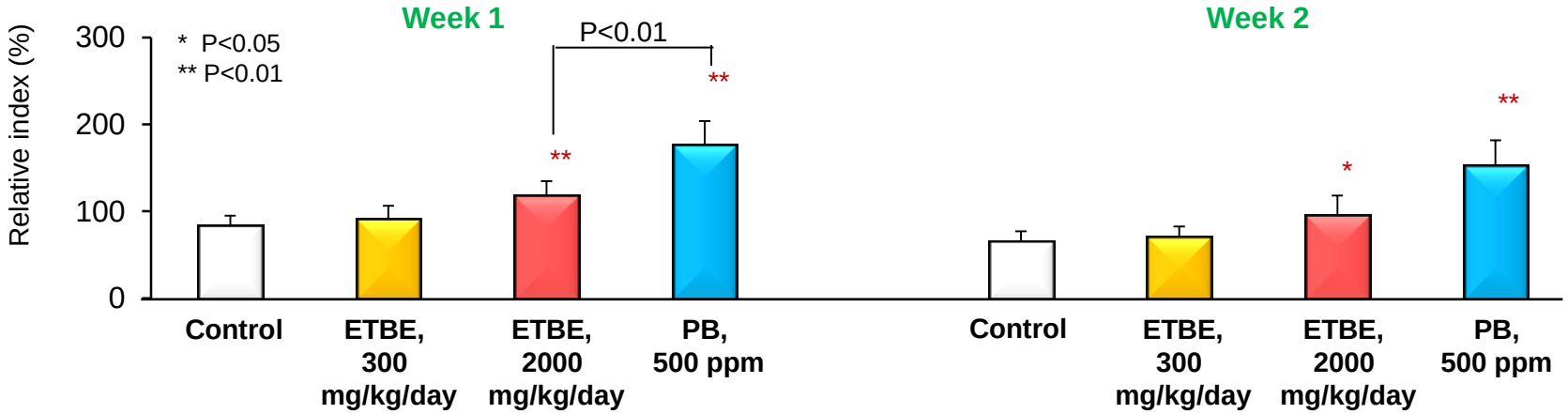
- Formation of oxidative stress (OH• , P450, 8-OHdG)
- DNA repair
- Alteration to protein expression,
- Changes in cell ultrastructure,
- Alteration to apoptosis and cellular proliferation

Experimental design

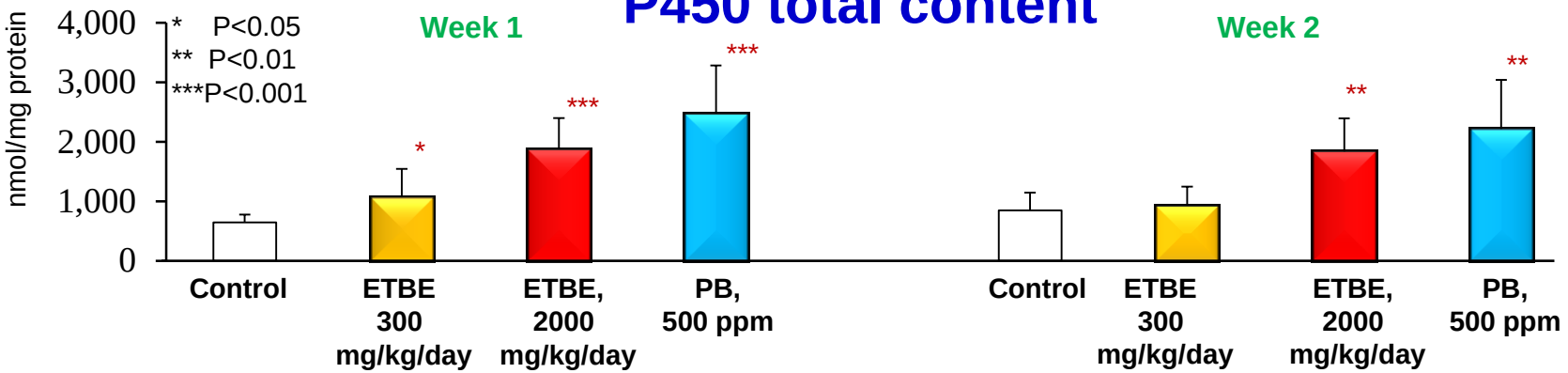


Animals: 6-week-old F344 male rats;
ETBE was administered at dose levels of 0 (control), 300 and 2000 mg/kg/day by gavage in olive oil for 1 and 2 weeks. Animals in group 4 were fed diet containing 500 ppm phenobarbital sodium (PB) . Liver perfusion was performed in all rats.

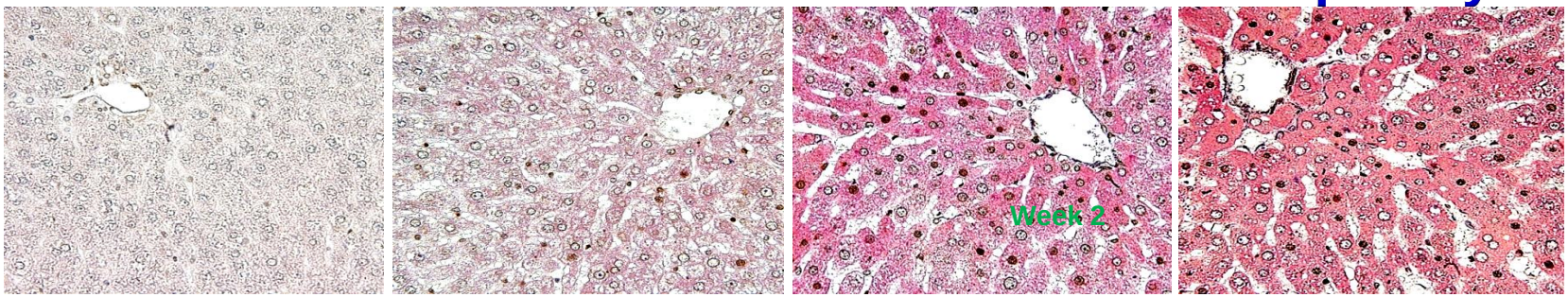
Generation of hydroxyl radicals in the liver (ESR)



P450 total content

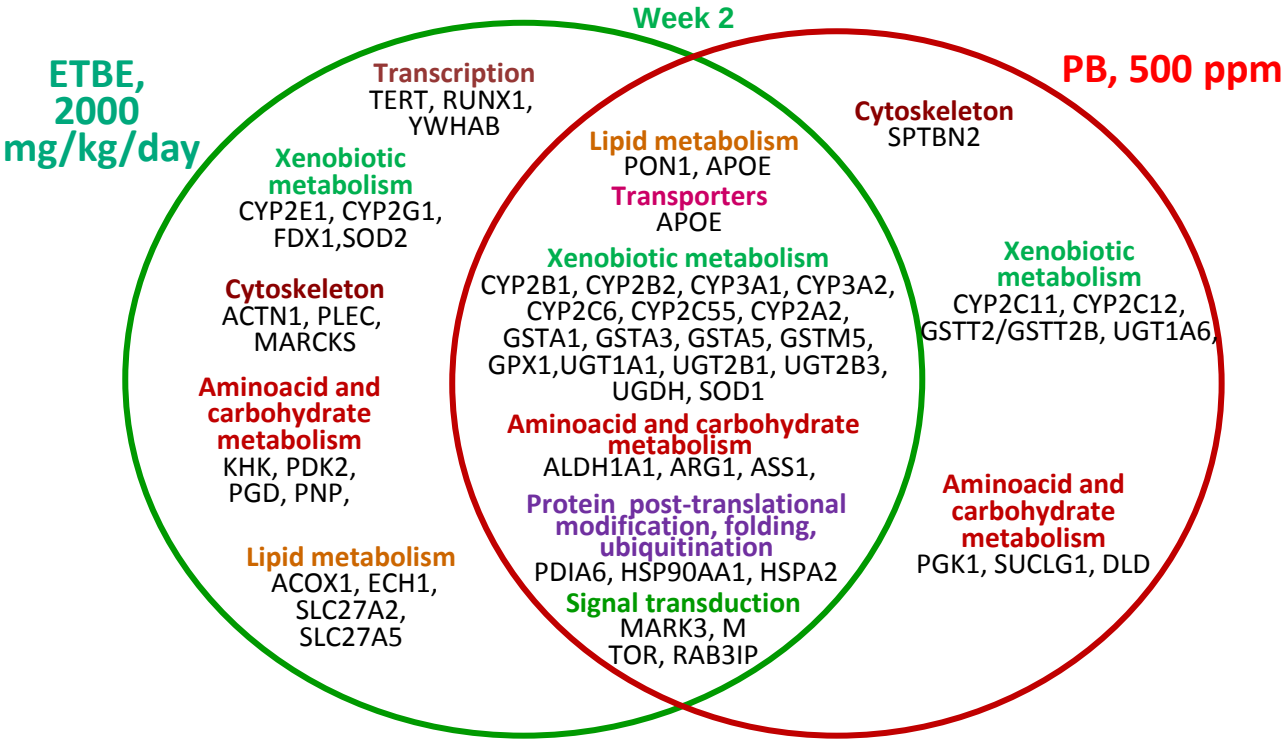


CYP2B1/2 and 8-OHdG coordinated elevation in hepatocytes



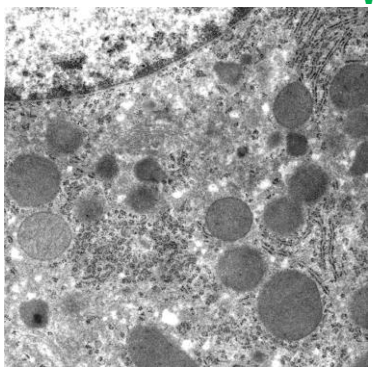
Control | ETBE, 300 mg/kg/day | ETBE, 2000 mg/kg/day | PB, 500 ppm

Proteome analysis

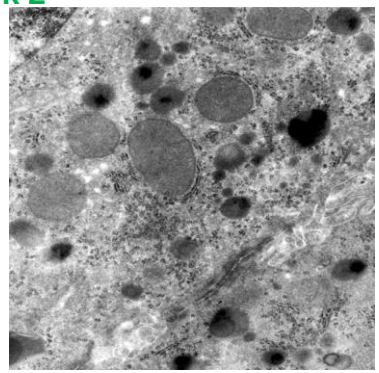


Induction of peroxisome proliferation in hepatocytes

Week 2



Control



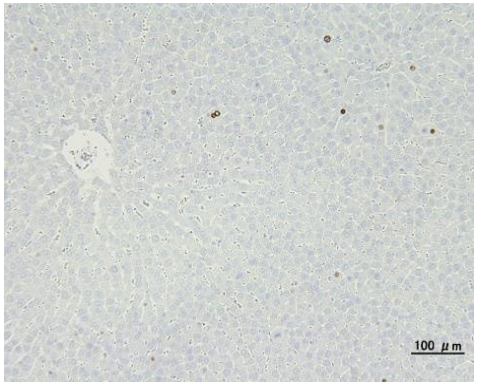
ETBE, 2000 mg/kg/day

Immunohistochemical BrdU labeling indices in hepatocytes

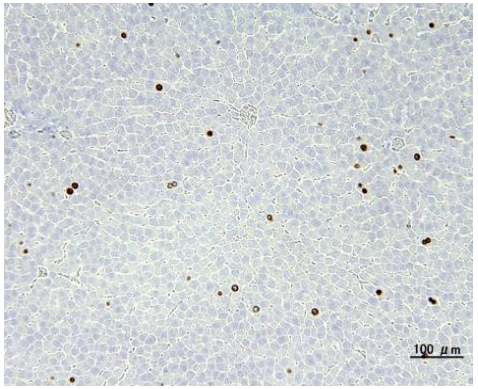
Groups	Treatment duration (days)	No. of rats	BrdU labeling index in hepatocytes (%)
Control	3	3	16.94±1.51
ETBE	3	3	23.82±3.05*
Control	10	5	4.08±2.38
ETBE	10	5	5.48±3.14
Control	17	5	2.12±1.68
ETBE	17	5	3.03±1.79
Control	28	5	0.89±0.33
ETBE	28	5	2.21±0.99*

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

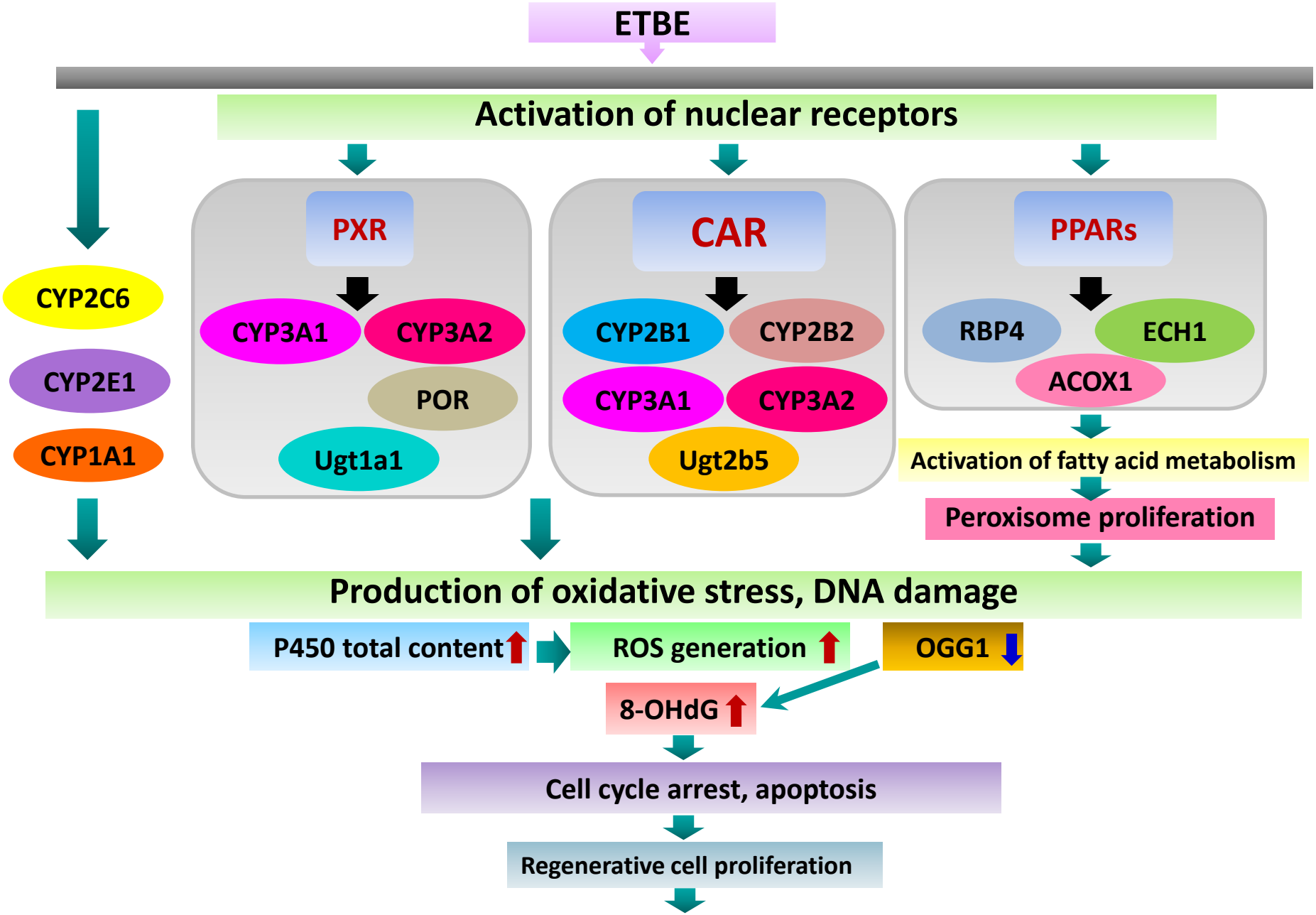
Day 3



Control



ETBE,
2000 mg/kg/day



Graphically illustrated MOA for ETBE hepatotumorigenicity in rats

Conclusion and Discussion (1)

Short-term treatment of ETBE:

1. Activate CAR and PXR nuclear receptor and PPAR
2. Induce centrilobular hypertrophy of hepatocytes
3. Led to increase in cell proliferation activity
4. Induce regenerative cell proliferation, which contributes to hepatotumorigenicity
5. The MOA data are adequate for ETBE tumorigenicity

Conclusion and Discussion (2)

1. In general, centrilobular hypertrophy develops in early stage, but not in late stage of 2-year carcinogenicity study
2. This is adaptive response (not adverse response)
3. MOA due to activation of CAR and PXR, and PPAR is accepted for hepatocarcinogenesis of Phenobarbital and PPAR proliferators in rodent (not relevant to human)
4. Phenobarbital and PPAR proliferators do not induce tumor in human
5. Therefore, liver tumor induced by ETBE is not relevant to human

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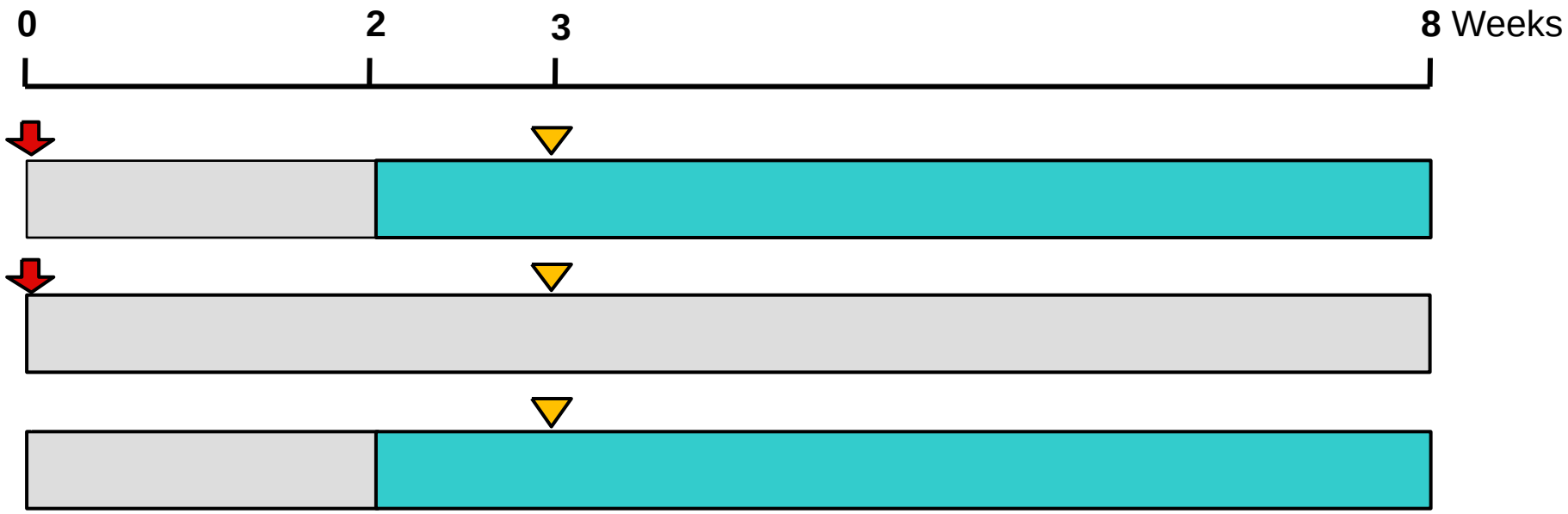
A Rat Medium-term Liver Bioassay for Carcinogens

Liver as a Target Organ for Carcinogenicity

Data source	No. of Chemicals evaluated	Carcinogenic chemicals	
		Total	Liver
IARC	587	147	87 (59%)
NCI/NTP	224	149	80 (54%)

IARC Monographs, Supplement 7, 1987
E. Zeiger, Cancer Res., 1987



Animals : **6-week-old, F344 male rats**

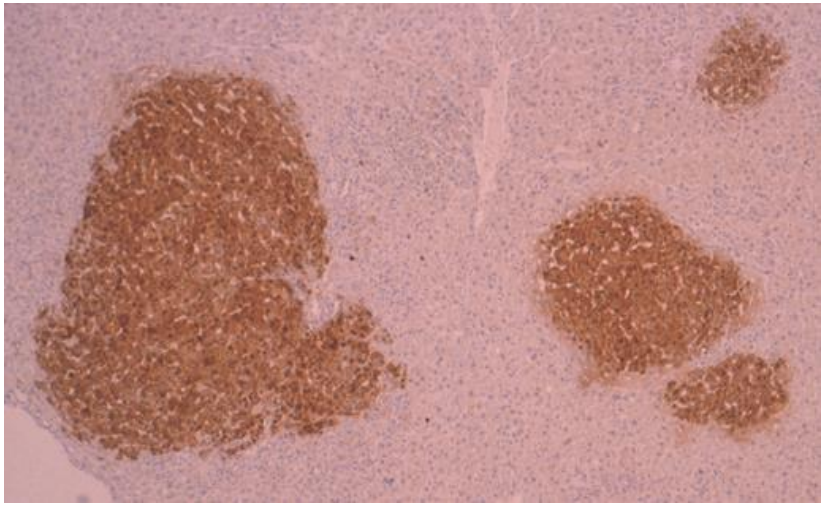
: **DEN, 200mg/kg, i.p.**

: **Saline, i.p.**

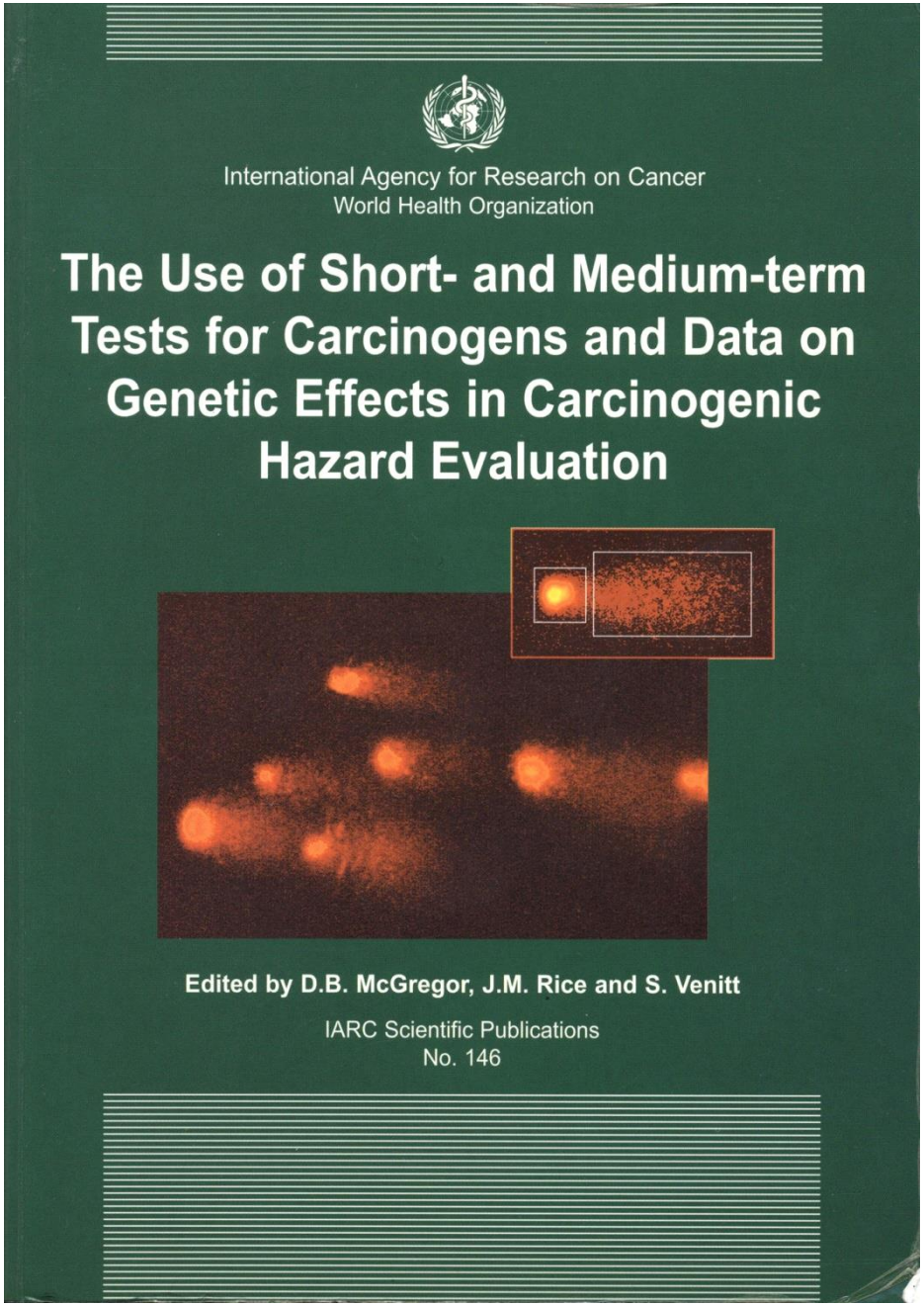
: **2/3 Partial Hepatectomy**

: **Basal diet**

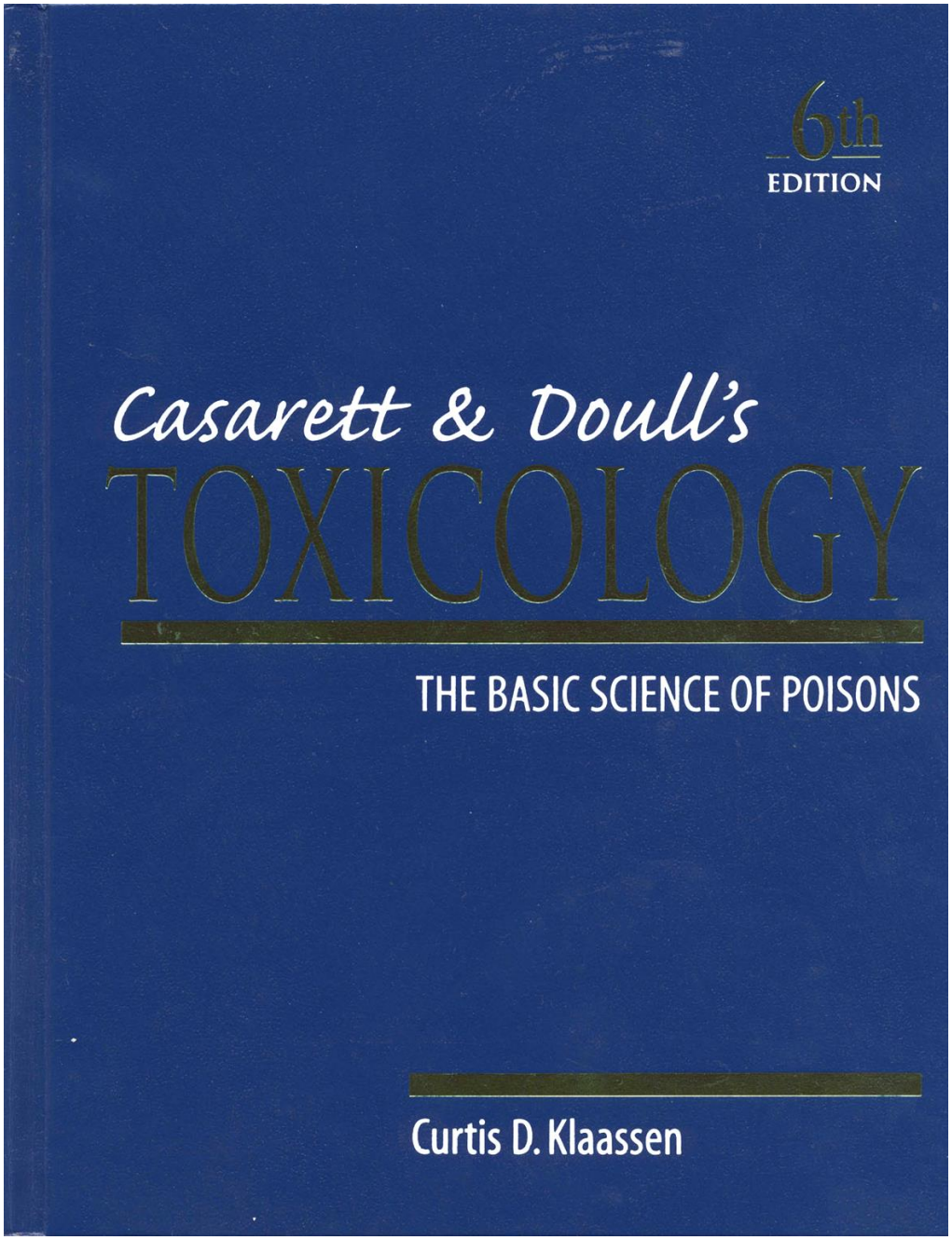
: **Test compounds**



Rat Medium-term Liver Bioassay for Carcinogens



IARC Scientific Publication
No. 146
1999



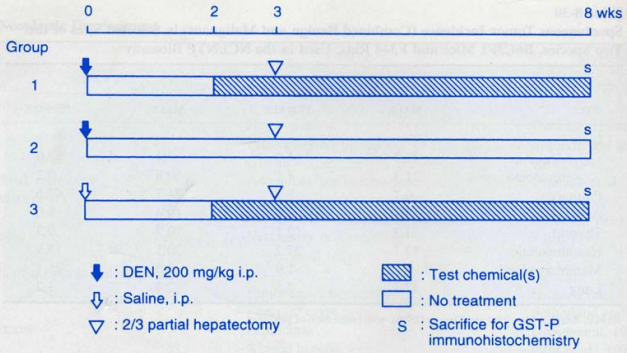


Figure 8-30. The medium-term liver bioassay protocol for identification of potentially carcinogenic agents.

DEN, diethylnitrosamine; GST-P, glutathione *S*-transferase–π. [Reproduced from Shirai (1997), with permission of author and publisher.]

dose of diethylnitrosamine poses some problems in that this dose by itself is carcinogenic, but only after a year or more. Furthermore, this high dose is also clastogenic to rat hepatocytes in vivo (Sargent et al., 1989). However, these authors and their colleagues have demonstrated a significant degree of correlation between long- and medium-term results, indicating the usefulness of this assay as a potential surrogate for the chronic bioassay (Ogiso et al., 1990). More recently these authors have used a slightly modified protocol in which five potent carcinogenic agents are administered for a 4-week period, followed by administration of the test chemical for a subsequent 24- to 32-week period (Ito et al., 1996). Unlike the assay depicted in Fig. 8-29, this more complicated procedure may allow the detection of promoting and progressor agents as well as complete carcinogens in a variety of different tissues. However, outside of Japan these assay procedures have not been generally utilized.

The newborn mouse model of chemical carcinogenesis was initially described by Shubik and his colleagues (Pietra et al., 1959) and later used extensively in studies of mouse hepatocarcinogenesis by Vesselinovitch and his colleagues (1978). More recently, Fujii (1991) has utilized this procedure in the determination of the carcinogenic potential of 45 different chemicals with quite reasonable results. The endpoint of neoplasms in a variety of different tissues, including lung, liver, lymphoid and hematopoietic tissues, is determined within a 1-year period. The assay is relatively inexpensive, utilizing small amounts of the test materials. As yet, however, this assay has not found general usefulness in the determination of carcinogenic potential by regulatory agencies.

Multistage Models of Neoplastic Development

As we have previously noted, the original studies on multistage models of carcinogenesis were developed with the epidermis of the mouse. It was not until some 40 years after those initial experiments that there was some attempt at standardization of the multistage model of carcinogenesis in mouse skin for the analysis of the carcinogenic potential of specific chemicals (Pereira, 1982). The format for such assays was essentially that depicted in Fig. 8-18. Few refinements in the procedure were added with the exception of the use of a genetically susceptible strain of mice, the SENCAR strain, which is now utilized in such tests (Slaga, 1986). This system may also be extended to the potential analysis of progressor agents (Hennings et al., 1993; Warren et al., 1993).

Considerably later than the initial reports of the mouse skin system, Hicks et al. (1975) demonstrated the cocarcinogenic or promoting action of several agents in the development of bladder cancer in the rat. Subsequently, other promoting agents have been demonstrated with this or a related assay, some of which appear to be relatively unique to this tissue for both anatomical and chemical reasons (Cohen and Lawson, 1995; Ito and Fukushima, 1989). At about the same time as the initial report of the multistage bladder model of carcinogenesis, Peraino and associates (1977) reported a multistage model of carcinogenesis in the rat liver. This finding has led to the development of a number of models of multistage carcinogenesis in the rat liver. Solt and Farber (1976) reported a model somewhat analogous to that of Ito and his colleagues, but with an aim directed primarily at studying mechanisms of hepatocarcinogenesis rather than utilizing it as an assay system for potential carcinogens. Shortly thereafter, Pitot et al. (1978) developed a model wherein initiation was performed with a nonnecrogenic dose of the initiating agent, subsequently followed by chronic administration of a promoting agent. The format of these two assay systems are noted in Fig. 8-31. The endpoint of these systems is the quantitative analysis of altered hepatic foci measured by one of several enzymatic markers, the most sensitive being the expression of GSTP (Hendrich et al., 1987). Several studies have investigated the potential for such analyses in the detection of chemical carcinogens (Pereira and Stoner, 1985; Williams, 1989; Oesterle and Deml, 1990). A similar format has been used to study the preneoplastic aberrant crypt foci in the colon of animals administered potential carcinogens (Ghia et al., 1996). However, as yet all such assays utilizing preneoplastic endpoints have not found general usefulness in the identification of potential carcinogenic agents.

A Medium-term Multi-organ Bioassay

Animals : 6-week-old, F344 male rats

DMBDD treatments

: DEN, 100mg/kg, i.p.

: MNU, 20mg/kg, i.p.

: DMH, 40mg/kg, s.c.

: BBN, 0.05% in drinking water

: DHPN, 0.1% in drinking water, 2 weeks

: Test chemicals

: Basal diet

Rat Multi-Organ Bioassay (DMBDD Methods)

Endpoint Markers Available for the Rat Medium-term Multi-Organ Bioassays for Carcinogens

Organ	Marker Lesions
Nasal cavity	PN hyperplasia, Papilloma, Carcinoma
Lung	Hyperplasia, Adenoma, Carcinoma
Tongue, Esophagus	PN hyperplasia, Adenoma, Carcinoma
Forestomach	Hyperplasia, Adenoma, Carcinoma
Glandular stomach	PAPG, Hyperplasia, Adenoma, Carcinoma
Intestines	ACF, Adenoma, Carcinoma
Liver	GST-P positive foci, Adenoma, Carcinoma
Pancreas	Acinar cell focus, Adenoma, Carcinoma
Kidneys	Altered tubulus, Adenoma, Carcinoma
Urinary bladder	PN hyperplasia, Adenoma, Carcinoma
Thyroid	Adenoma, Carcinoma
Prostate	Dysplasia, Carcinoma

PN, papillary or nodular;
PAPG, pepsinogen 1 altered pyloric glands

CONCLUSIONS

The rat medium-term liver bioassays for carcinogens and the rat multi-organ bioassays for carcinogens are useful and reliable methods to detect carcinogenic or modifying potentials for screening of chemicals.

Positive Results of Medium-Term Bioassay

Based on initiation-promotion carcinogenesis theory,

Positive results ➔ promotion (promoter or carcinogen)

In a case of ETBE:

The bioassay ➔ liver, forestomach, colon, kidney, urinary bladder, thyroid

***Two-year carcinogenicity study ➔ liver**

Conclusion: **Carcinogenicity in liver**

Promoting activity in forestomach, colon, kidney, urinary bladder, thyroid