Hormesis in Carcinogenesis: Evidence for Threshold in Carcinogenicity of Non-genotoxic Environmental Carcinogens

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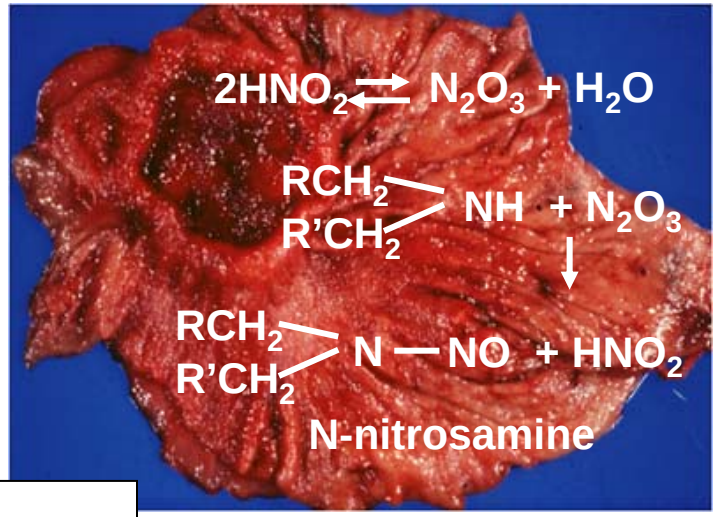
genotoxic



genotoxic

Environmental carcinogens

- genotoxic or non-genotoxic
- natural or synthetic
- cooking process, contamination, or synthesis in the body
- avoidable or unavoidable
- human intake,1.5 g/day (B. Ames)

genotoxic

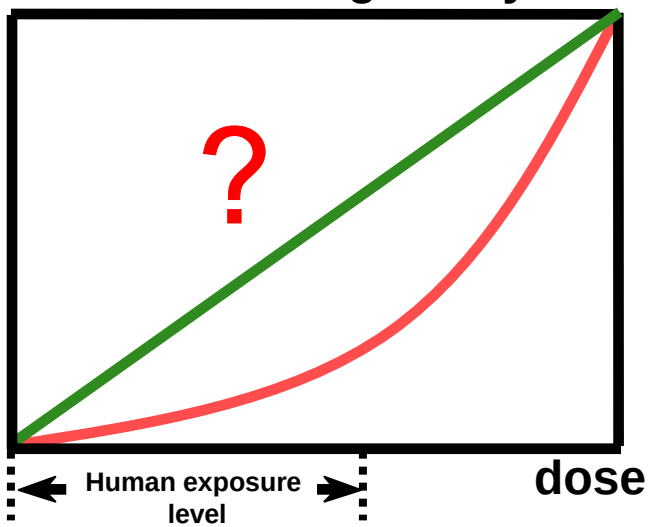
non-genotoxic

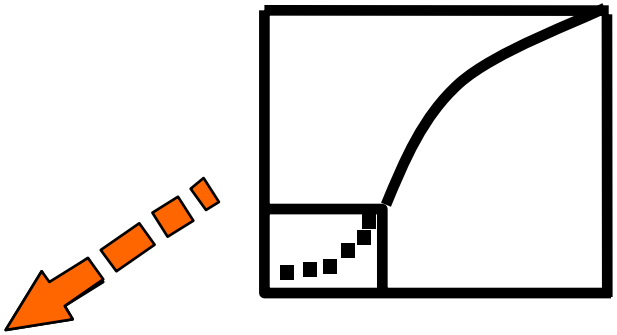
Basic concept in cancer risk assessment

It is generally considered that genotoxic carcinogens have no threshold in carcinogenic potential. This hypothesis has led to acceptance of linear curve that approach zero at low doses for risk assessment. On the other hand, it has been accepted that non-genotoxic carcinogens have threshold. There are, however, limited date available for these hypothesis. Therefore, it is important to resolve this question from the view point of cancer risk assessment and management.

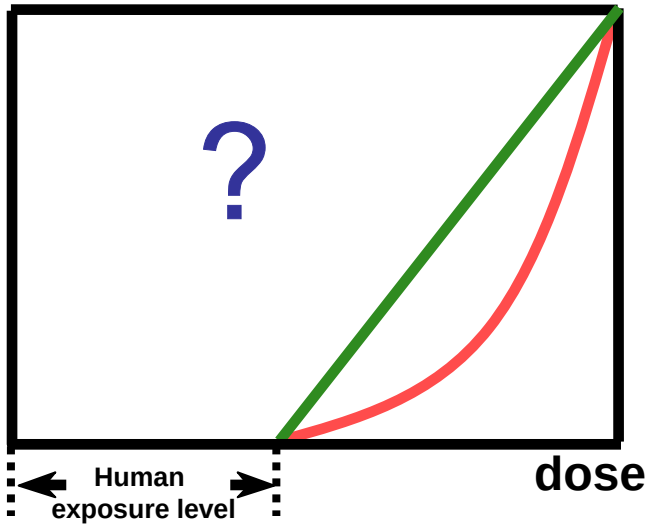
Low-dose carcinogenicity curve: Extrapolation from high to low doses

Genotoxic carcinogenicity





Non-genotoxic carcinogenicity



genotoxic carcinogens: mutagenic

act through interaction with DNA

→ irreversible change

unclear carcinogenicity at low lose

non-genotoxic carcinogens: non-mutagenic

no interaction with DNA

→ reversible change

Threshold in carcinogenicity

A natural question is whether a threshold exists for observed effects of carcinogens. Recently the concepts of "practical" and "perfect" thresholds for genotoxic and non-genotoxic carcinogens have been proposed. In these cases, the carcinogens are associated with a no-observed effect level (NOEL). To answer this question, we examined low dose carcinogenicity of the carcinogens using medium-term bioassay for carcinogens.

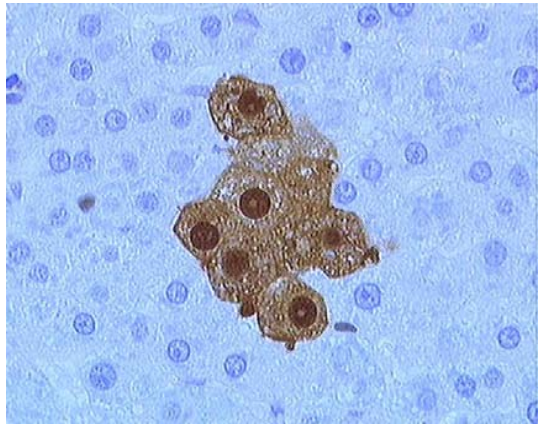
Merit of a medium-term bioassay for carcinogens

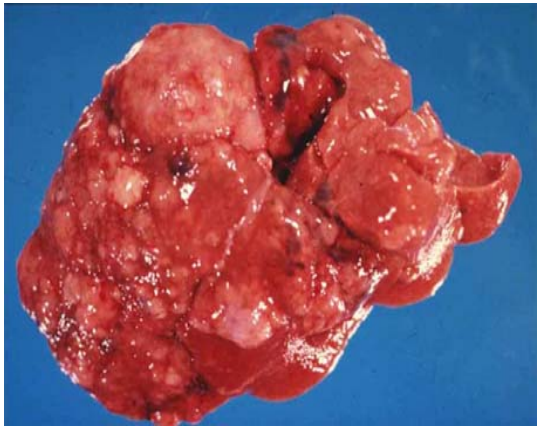
Normal tissue → Preneolastic lesion → Benign tumor → Cancer

Liver medium-term bioassay

Carcinogenicity test

Liver





Number-Area / unit of glutathione *S*-transferase placental form (GST-P) positive foci

Incidence of tumors

Low-dose hepatocarcinogenicity of environmental carcinogens

1. Non-genotoxic carcinogens

   Phenobarbital (PB)
   α-Isomer of benzene hexachloride (α-BHC)
   1,1-Bis(p-chlorophenyl)- 2,2,2-trichloroethane (DDT)
   Ethanol : as a promoter

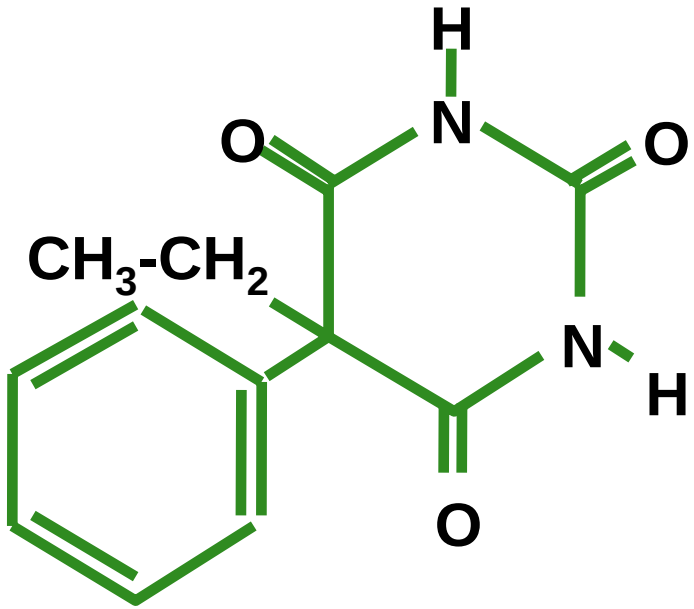
2. Genotoxic carcinogens

   2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)
   Diethylnitrosamine (DEN)
   Dimethylnitrosamine (DMN)

Non-genotoxic carcinogenicity

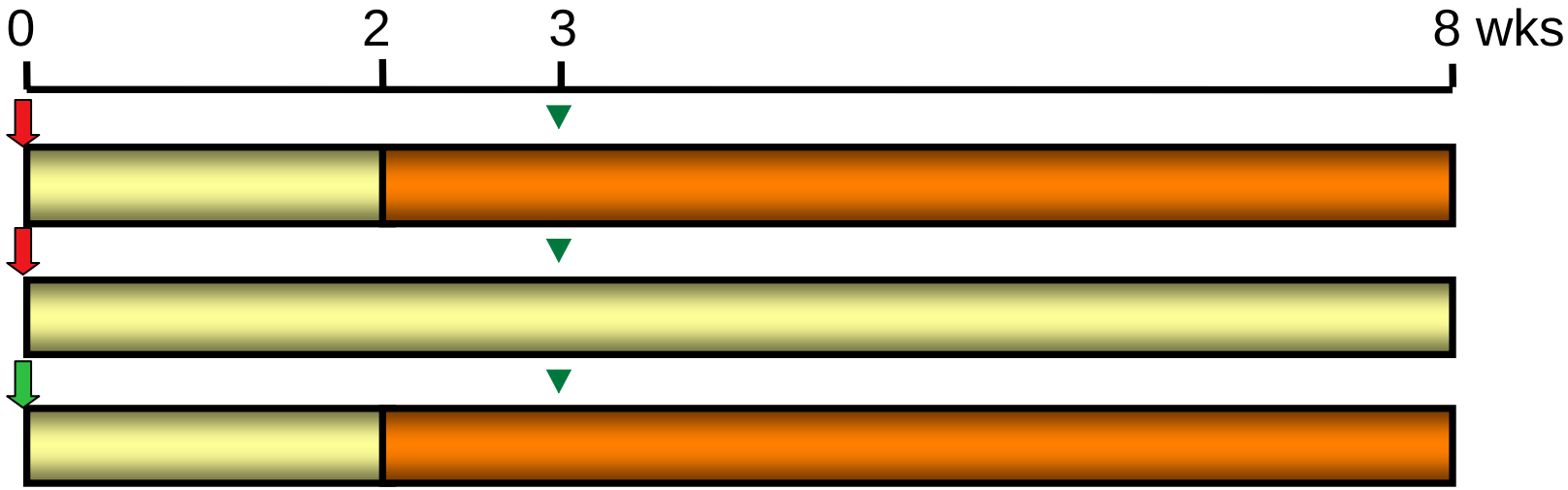
Most of chemicals involved are non-genotoxic chemicals, acting as P-450 inducers at high doses and exhibiting promoting effects on hepatocarcinogenesis, and the existence of threshold was postulated for the substances acting via epigenetic mechanism.

Phenobarbital (PB)



- Drug, sedative and anticonvulsant
- Mutagenicity: negative
- Hepatocarcinogen

Effect of phenobarbital (PB) at different doses on rat hepatocarcinogenesis (Ito test)



Animals: 180 male F344 rats , 6-week-old

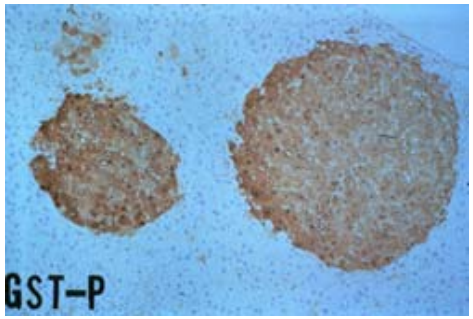
Diethylnitrosamine (DEN, 200mg/kg, i.p.)

Saline , i.p.

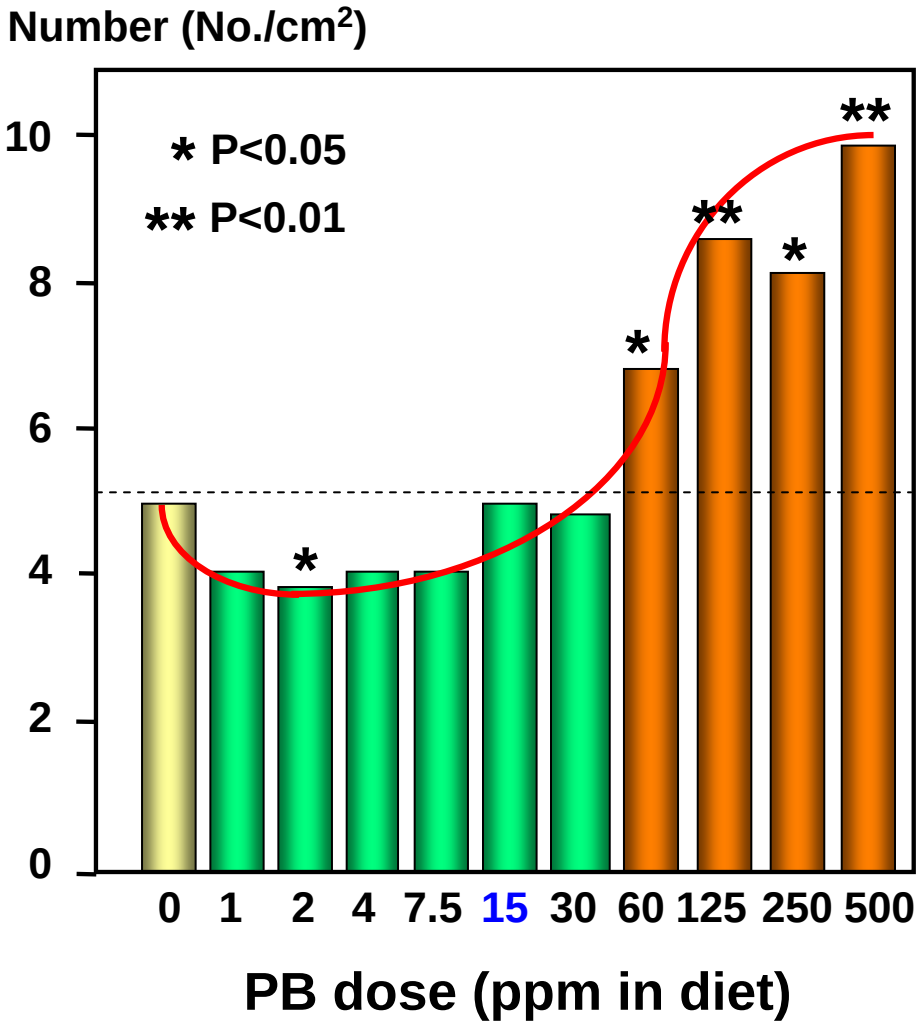
PB: 1, 2, 4, 7.5, 15, 30, 60, 125, 250 and 500 ppm (dose in diet)

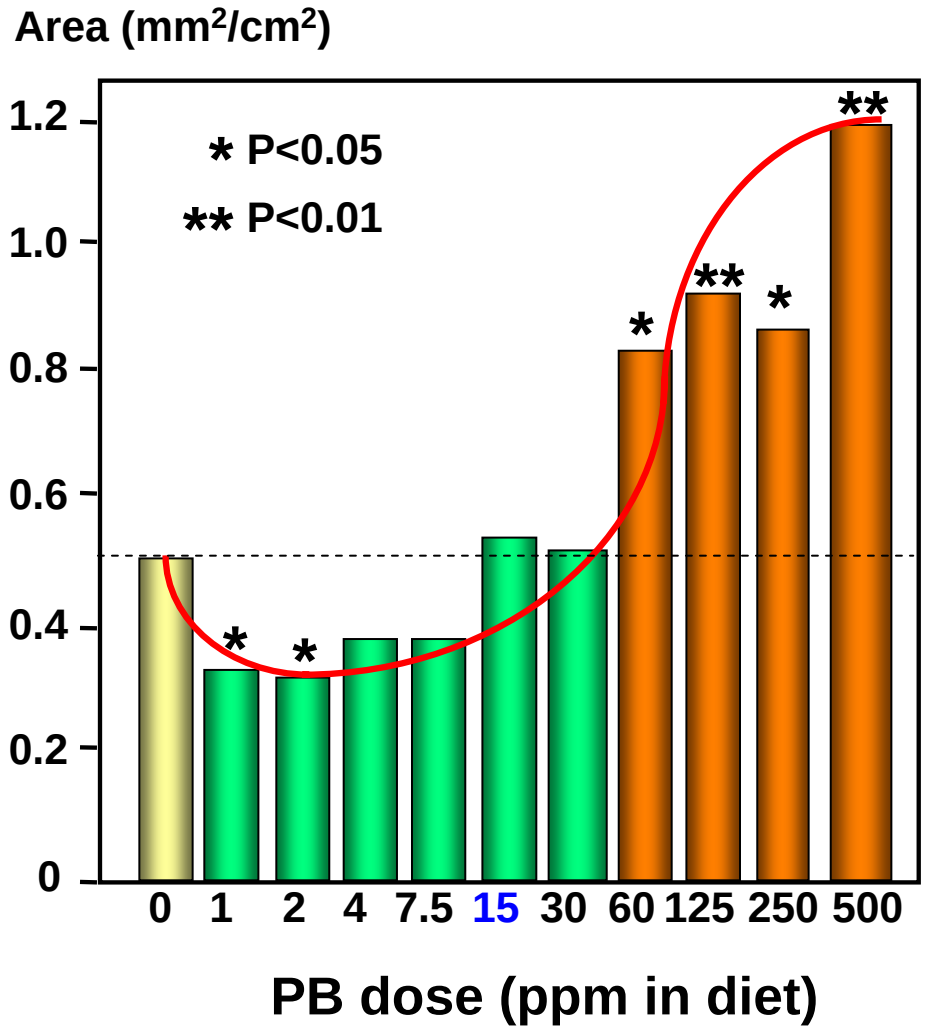
Control diet ▼ 2/3 partial hepatectomy

Endpoint: Liver GST-P positive foci

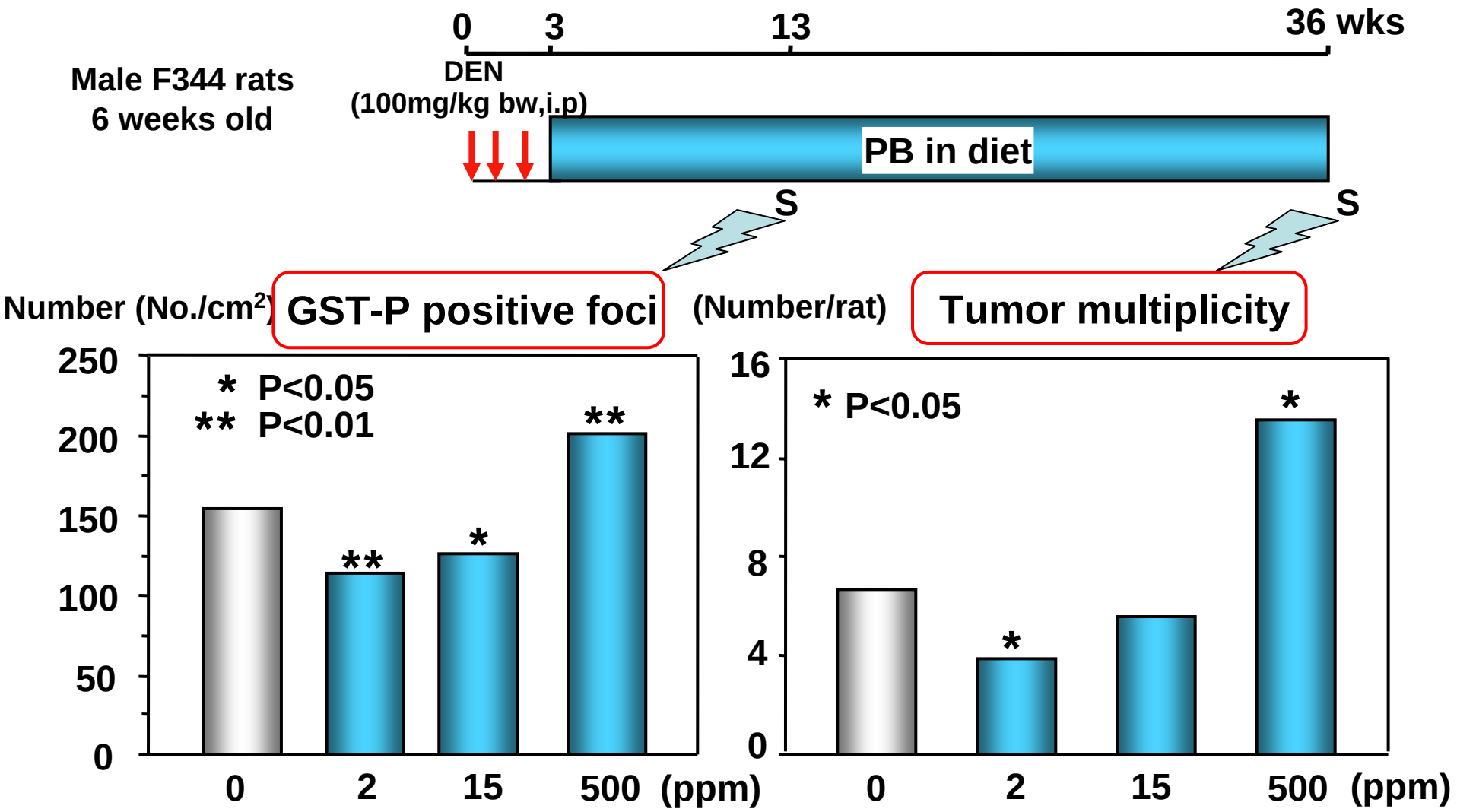


Development of GST-P positive foci in the liver of rats induced by PB treatment

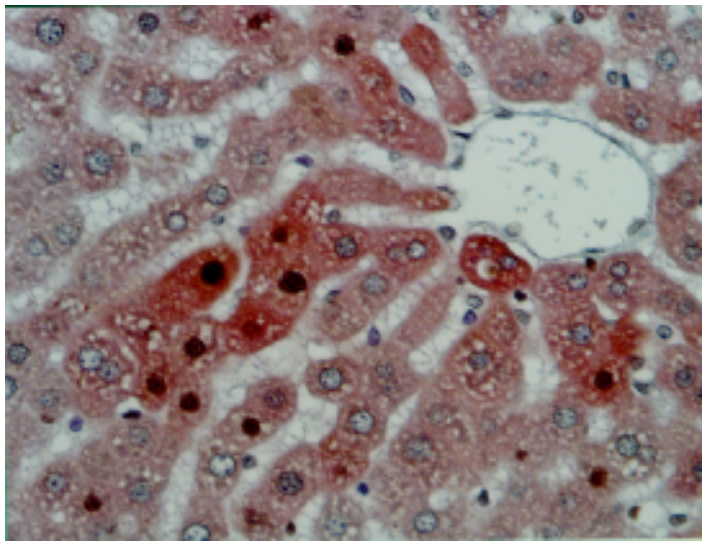




Hepatocarcinogenicity of PB in the rat liver: GST-P positive foci and tumor developments (DEN→PB)

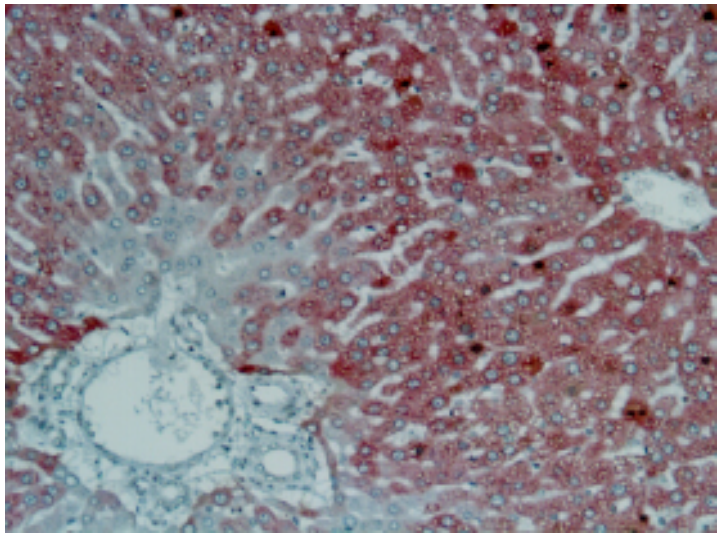


8-OHdG and P-450 in rat liver treated with PB at 500 ppm for 8 days

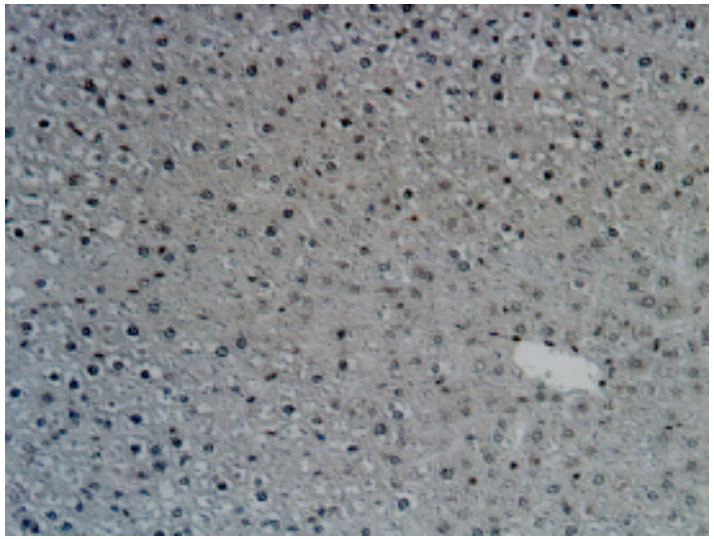


8-OHdG and CYP3A2

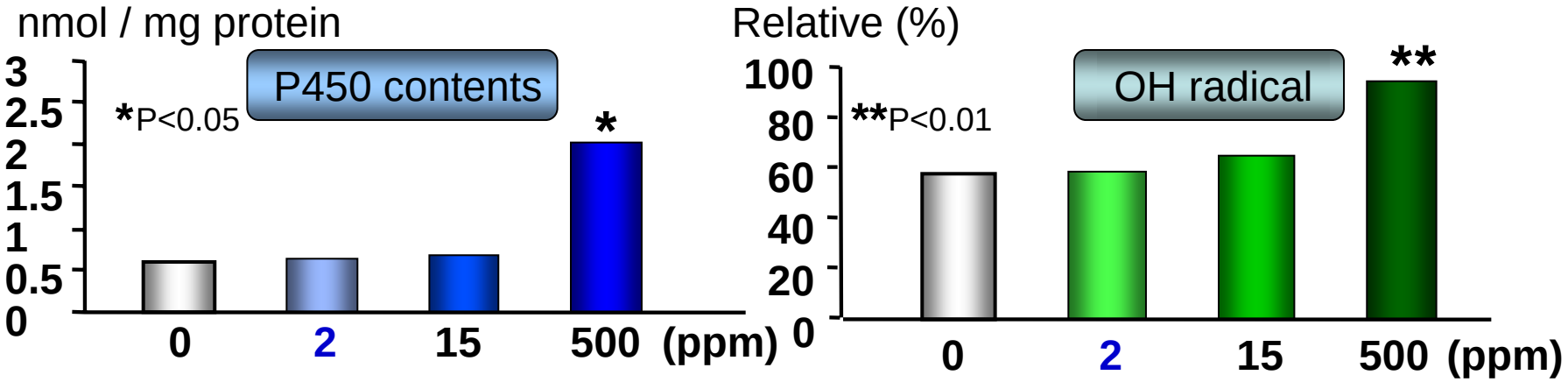
8-OHdG and CYP2B1



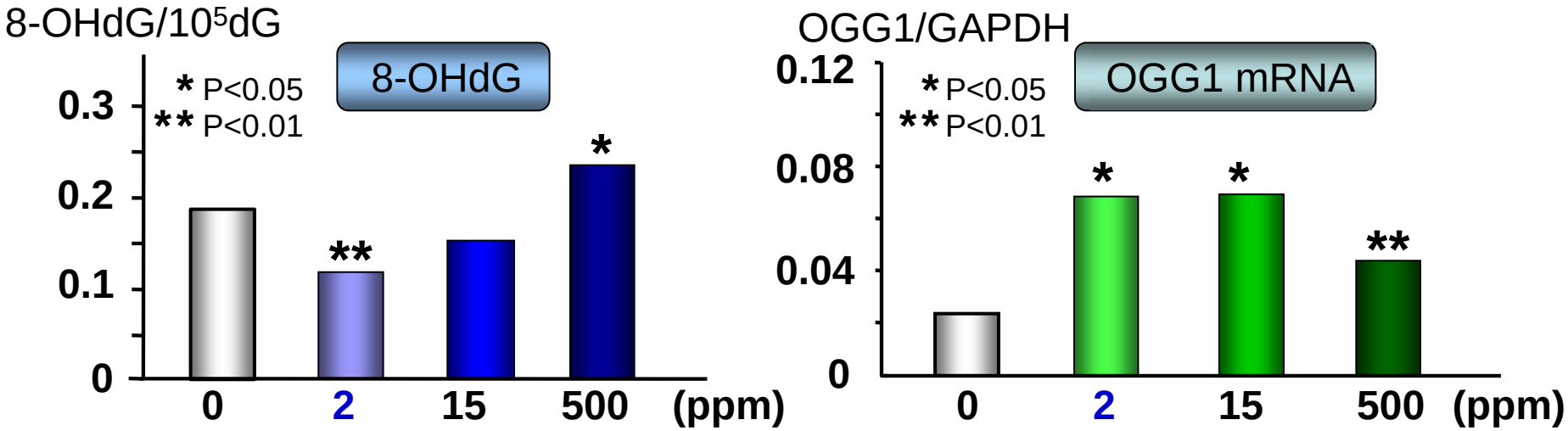
control



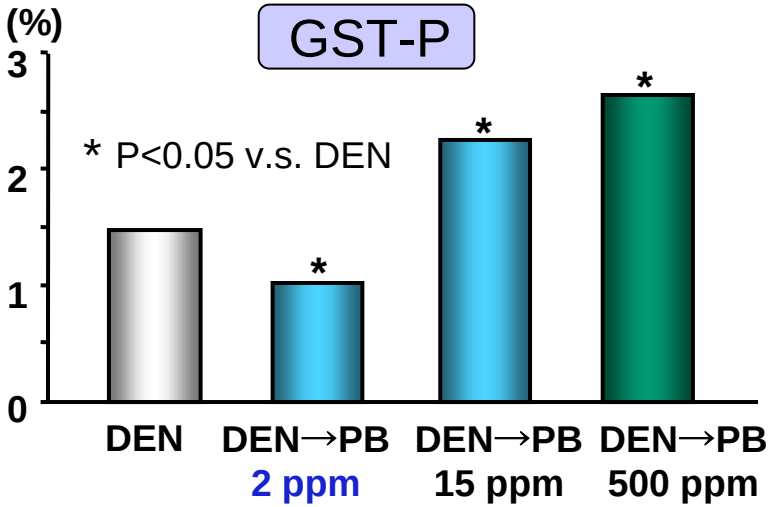
P-450 total contents and OH radicals generation in the rat liver induced by DEN→PB treatment

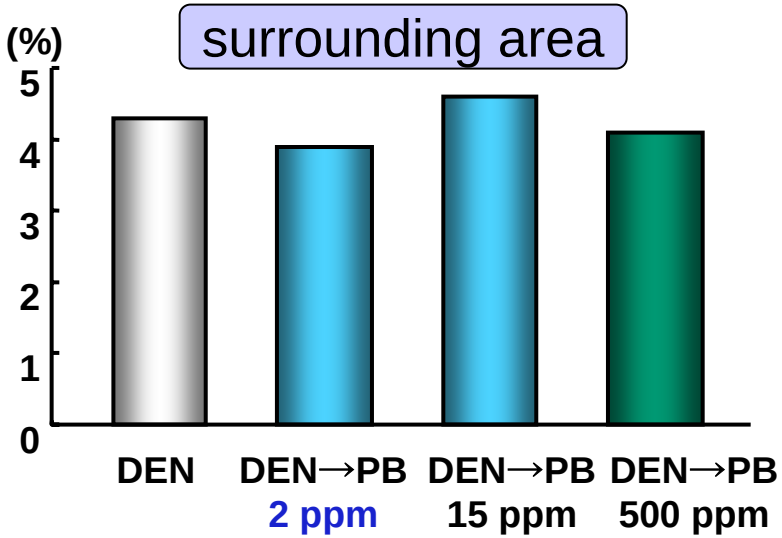


8-OHdG and OGG1 mRNA expression levels induced in the rat liver by DEN→PB administration

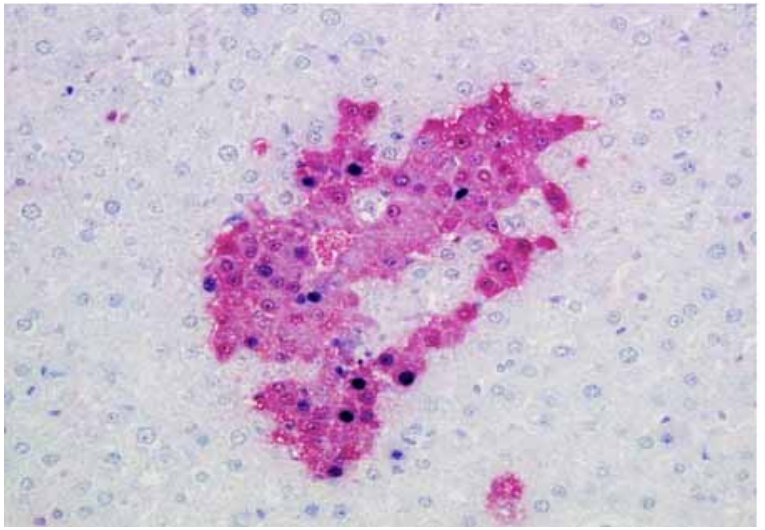


PCNA positive index within and surrounding area of GST-P positive foci in rat liver



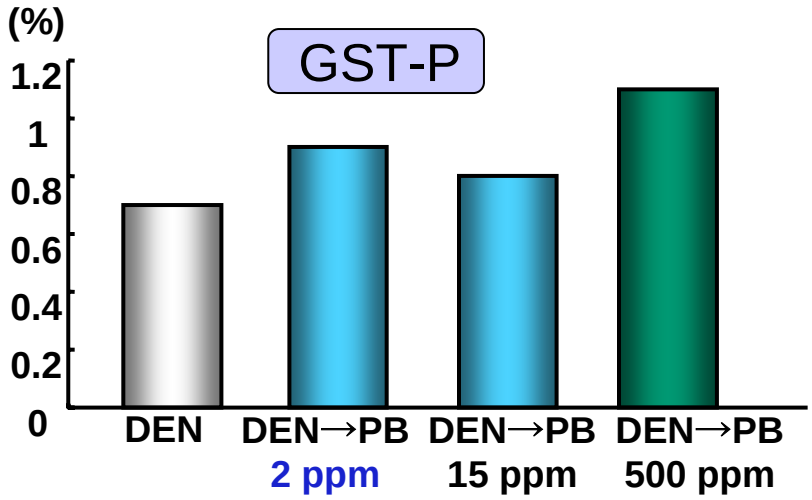


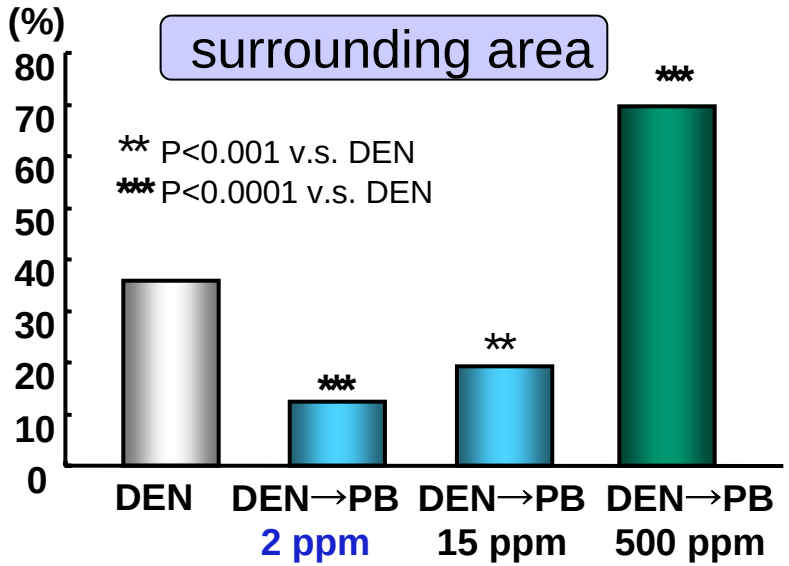
Immunohistochemistry

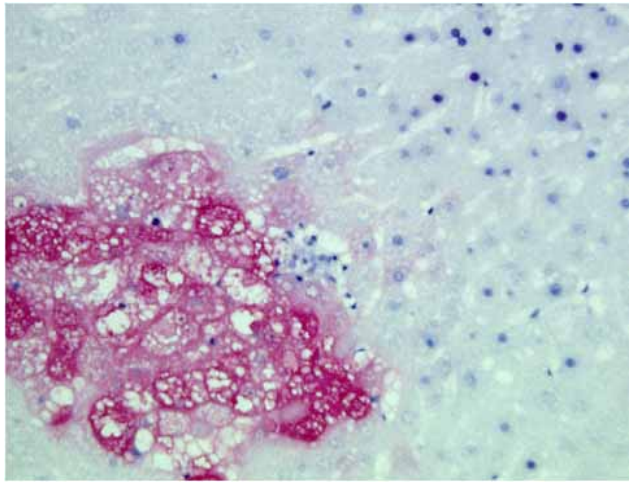


PCNA (blue) and GST-P (red) double staining

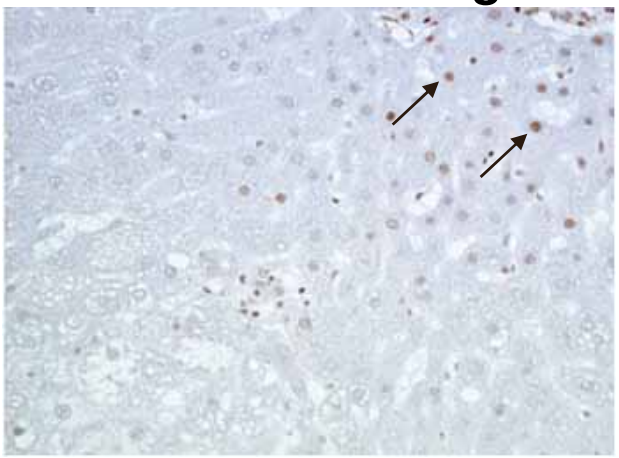
Apoptotic index within and surrounding area of GST-P positive foci in rat liver







Apoptosis (blue) and GST-P (red) double staining

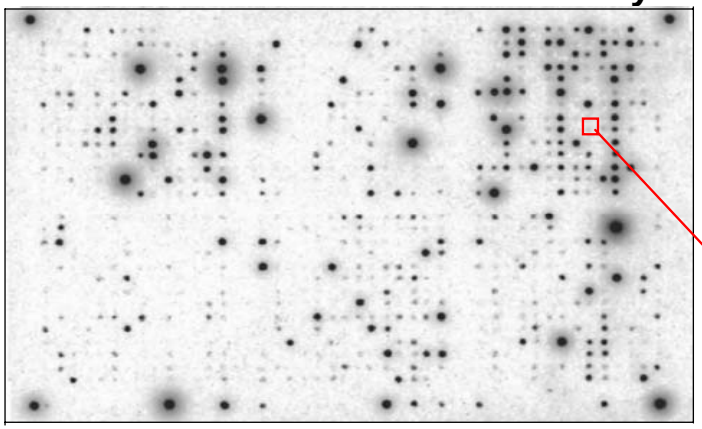


8-OHdG

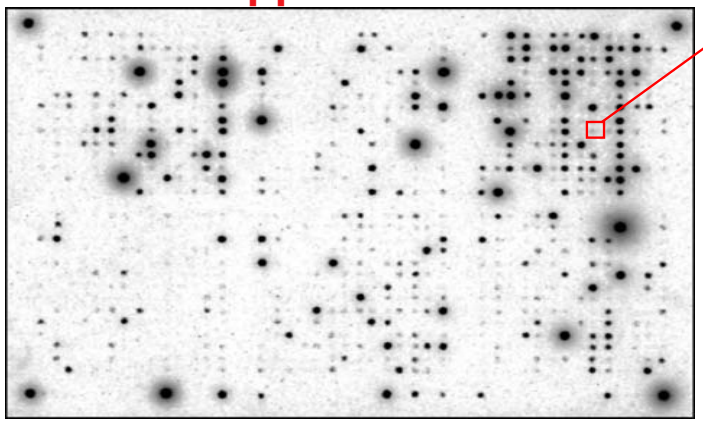
Differentially expressed genes in the rat liver after DEN →PB treatment detected by cDNA microarray analysis

DEN

Atlas 1.2 Rat Array



DEN→PB 2 ppm



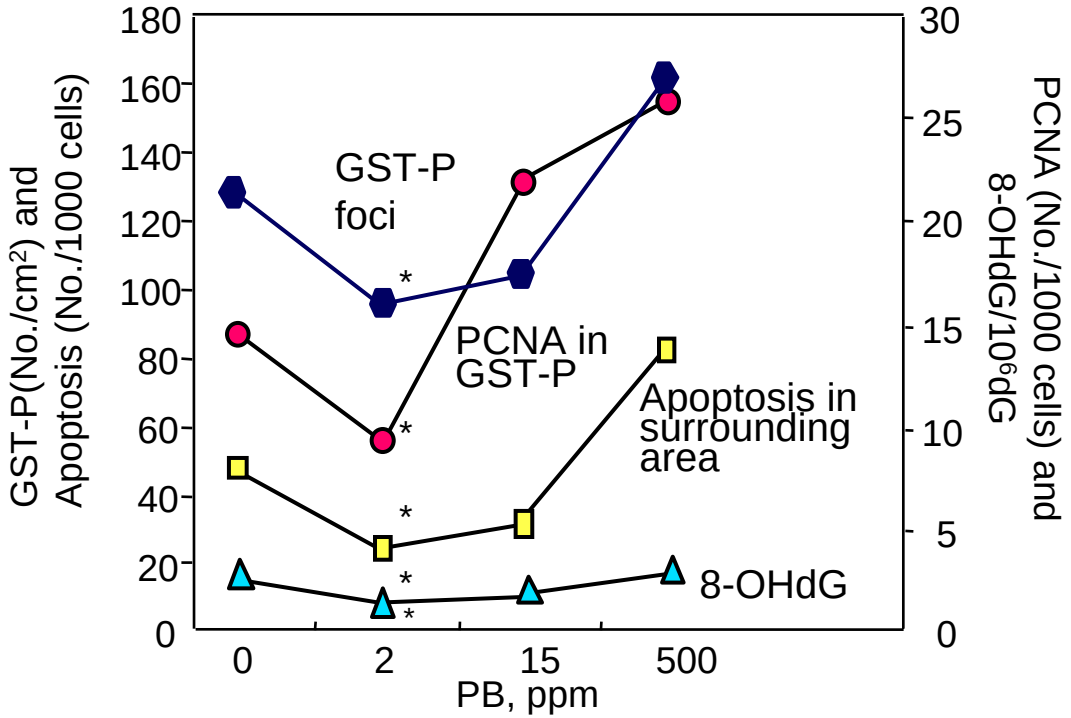
Treatment	Gene	Alteration
[a]DEN	Glutathione-S-transferase	↑
	Cytochrome P450 3A	↑
	G protein α subunit	↑
	Glutamic acid decarboxylase	↓
	GABA receptor beta sububit	↓
[b]DEN → PB, 2 ppm	Glutamic acid decarboxylase (GAD65)	↑
	Chloride channel ClC-7	↑
	Phospholipase C	↑
	MAP kinase p38	↓
	Ca-CAM dependent PK	↓
[b]DEN → PB, 15 ppm	Cytochrome P450 3A	↑
	Nuclear tyrosine phosphatase	↑
	Glutathione S-transferase	↑
[b]DEN → PB, 500 ppm	Cytochrome P450 3A, 2C, 4A	↑
	DOPA decarboxylase	↑
	Nuclear tyrosine phosphatase	↑
	NADPH-P450 reductase	↑
	G protein α subunit	↑
	VEGFR 1	↑

[a] VS. non-treated control [b] VS. DEN

Summary of PB study

1. PB at low doses inhibited the development of GST-P positive foci and tumor formation in the rat liver, whereas PB at high doses exhibited promoting effect of hepatocarcinogenesis (J-shape curve).
2. Inhibition of DNA 8-OHdG formation, decrease of cell proliferation within the GST-P positive foci and induction of apoptosis in the surrounding liver tissue were found in the low dose group.
3. This hormetic phenomenon of PB carcinogenicity supports the concept discussed recently by E. Calabrese and E. A. Baldwin.

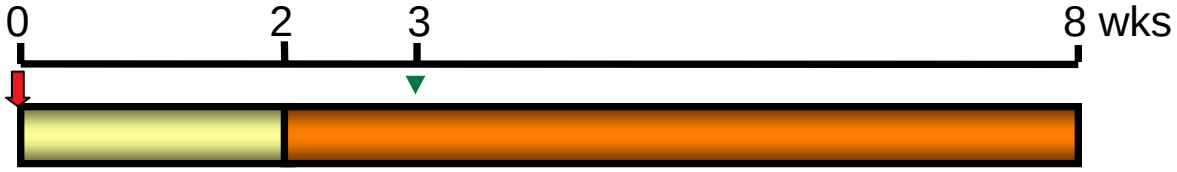
Hormetic effect of PB



α-Isomer of benzene hexachloride (α-BHC)

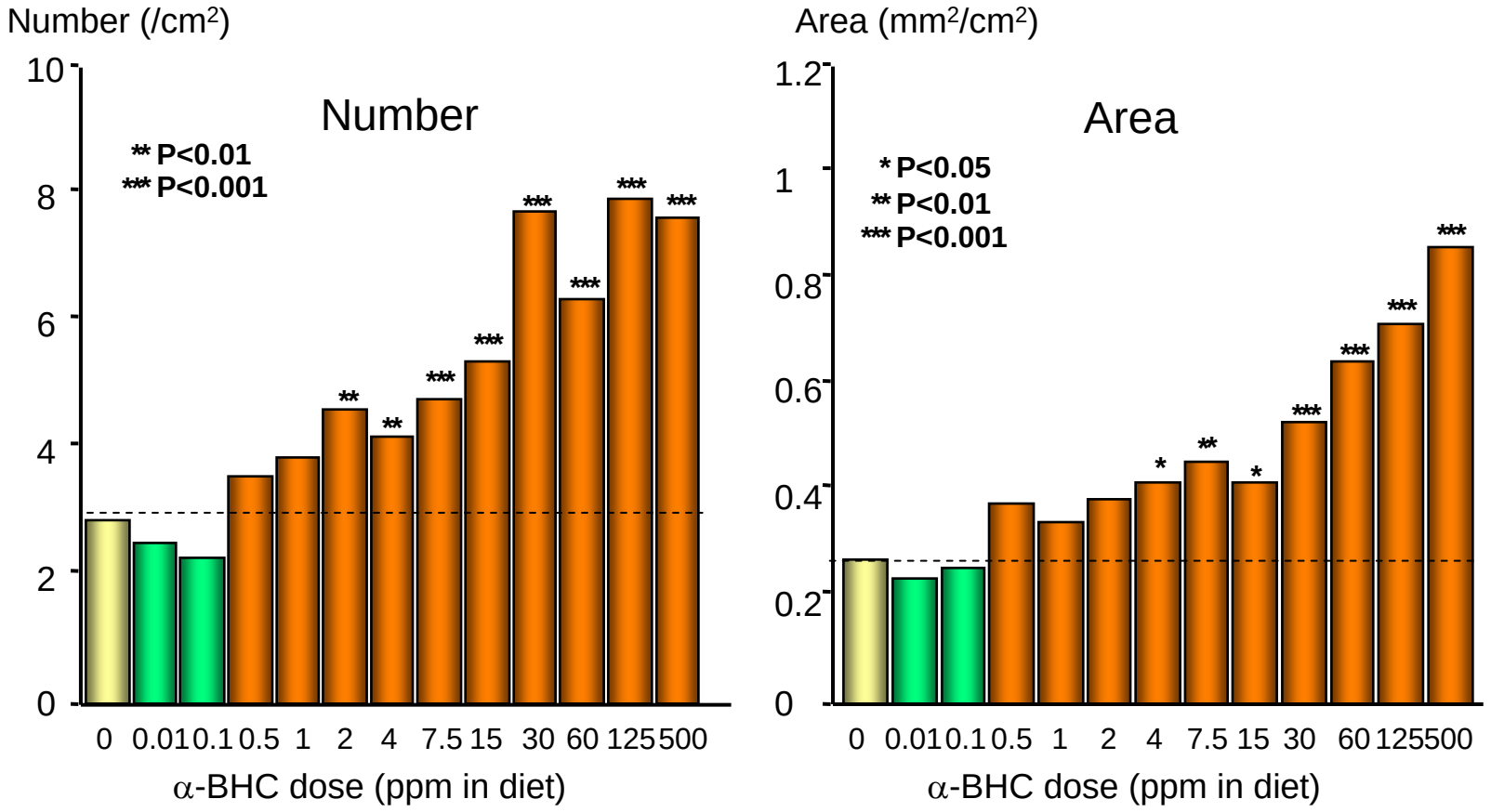
- Agricultural chemical
- A major organochlorine byproduct in the manufacture of lindane
- Mutagenicity: negative
- Hepatocarcinogen

Development of GST-P positive foci in the liver of rats induced by α-isomer of benzene hexachloride (α-BHC) (Ito test)



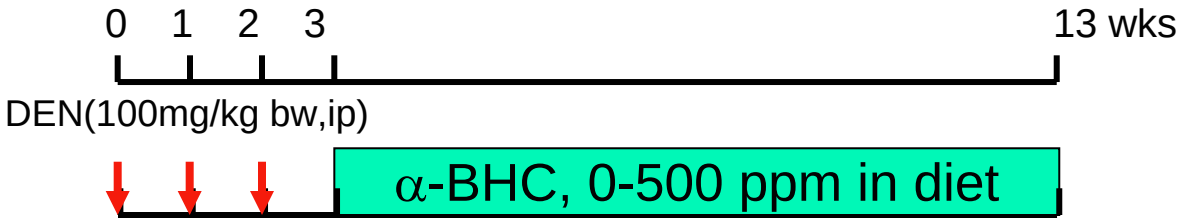
Animals: 180 male F344 rats , 6-week-old

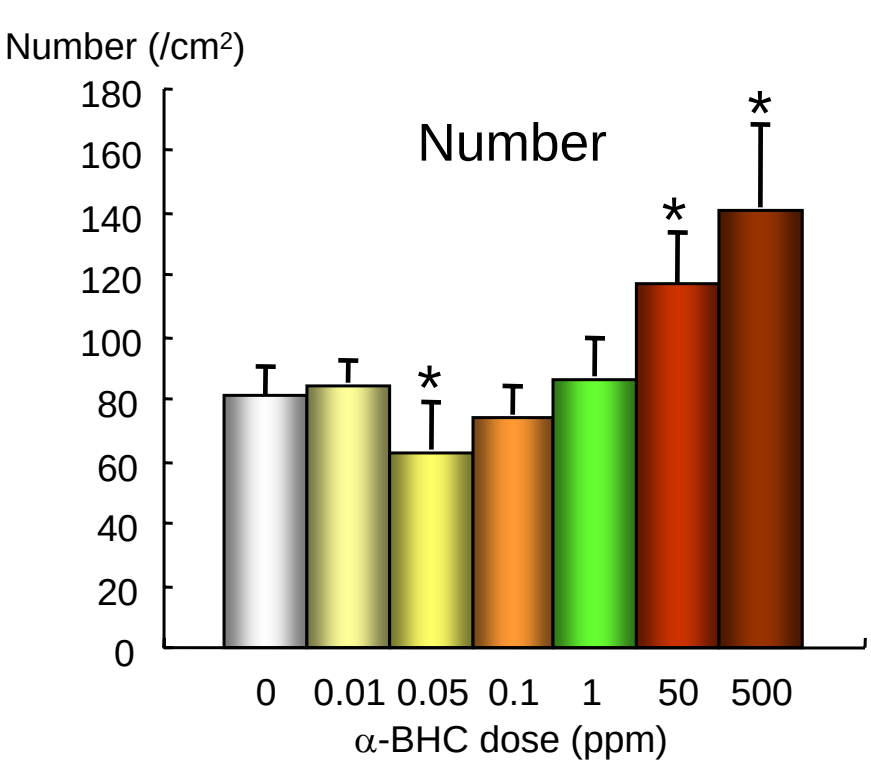
Diethylnitrosamine (DEN, 200mg/kg, i.p.); 2/3 partial hepatectomy

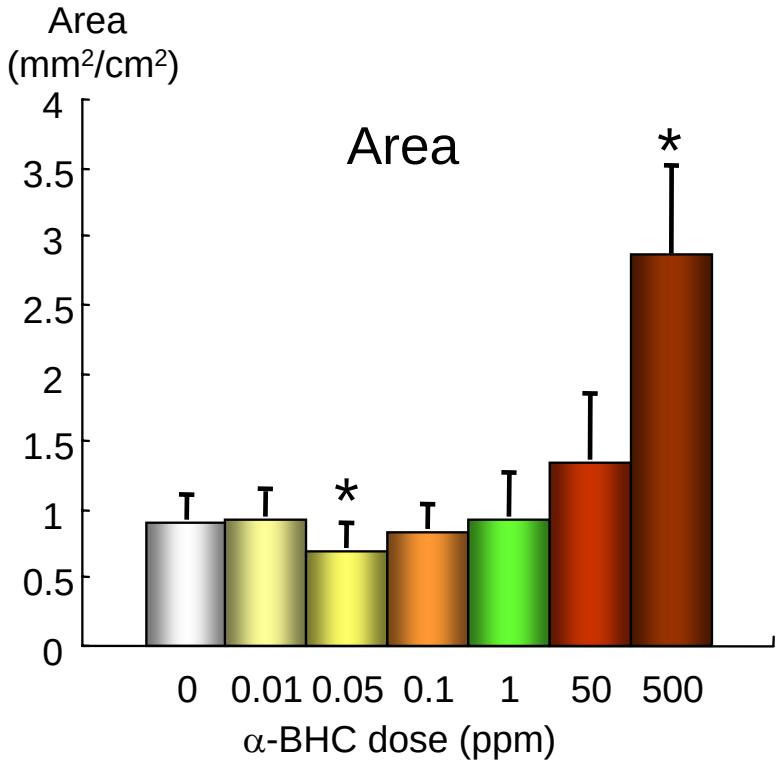


GST-P positive foci in the rat liver initiated by DEN followed by α-BHC

Male F344 rat,
6 weeks old

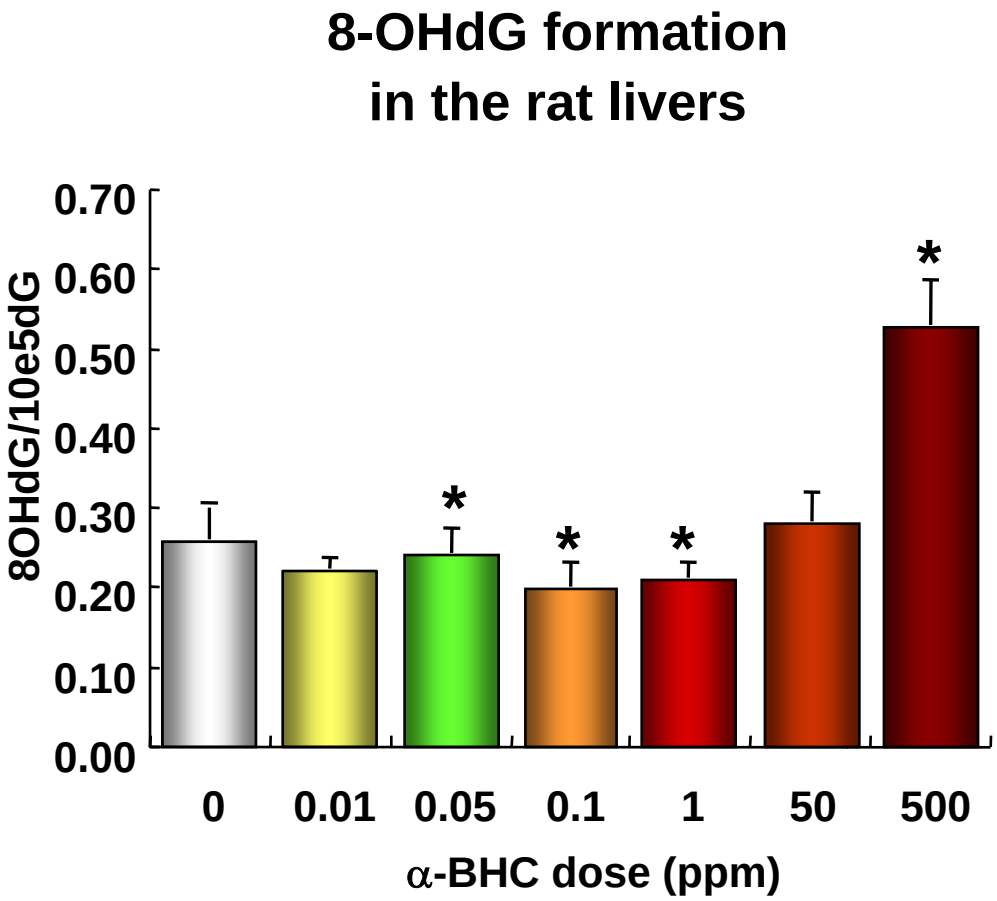


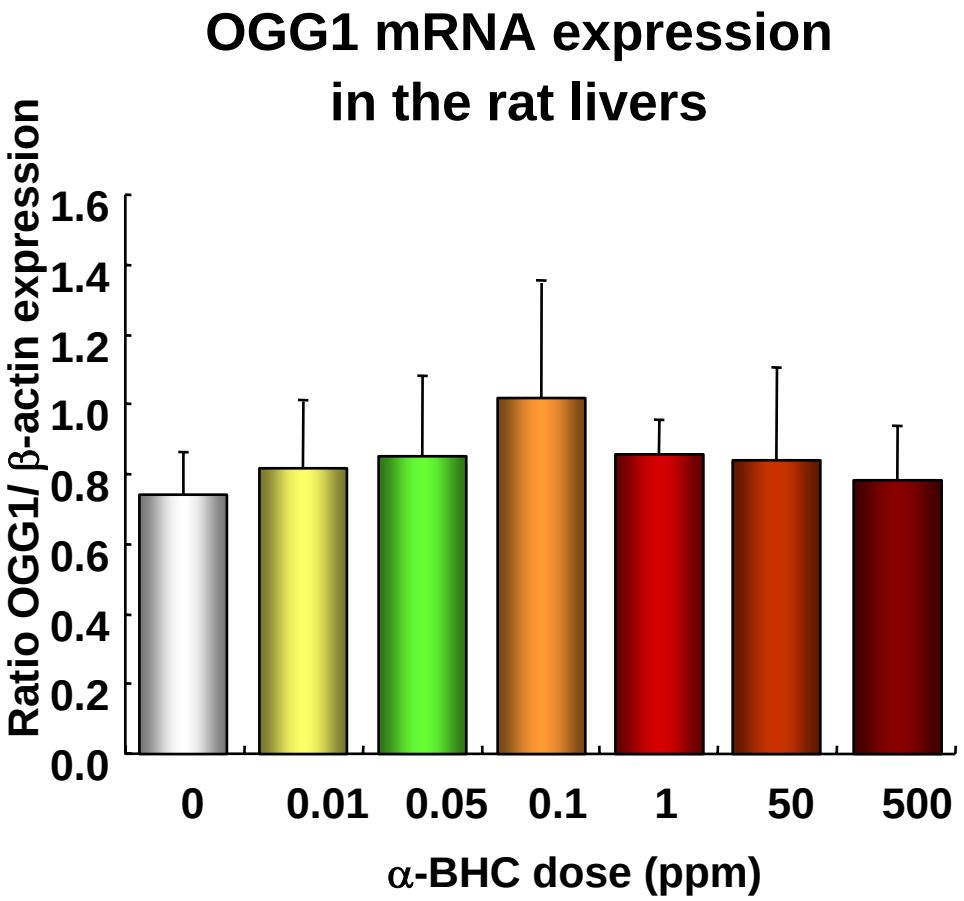




* P < 0.05 when compared with DEN alone group

Effect of α-BHC on oxidative stress

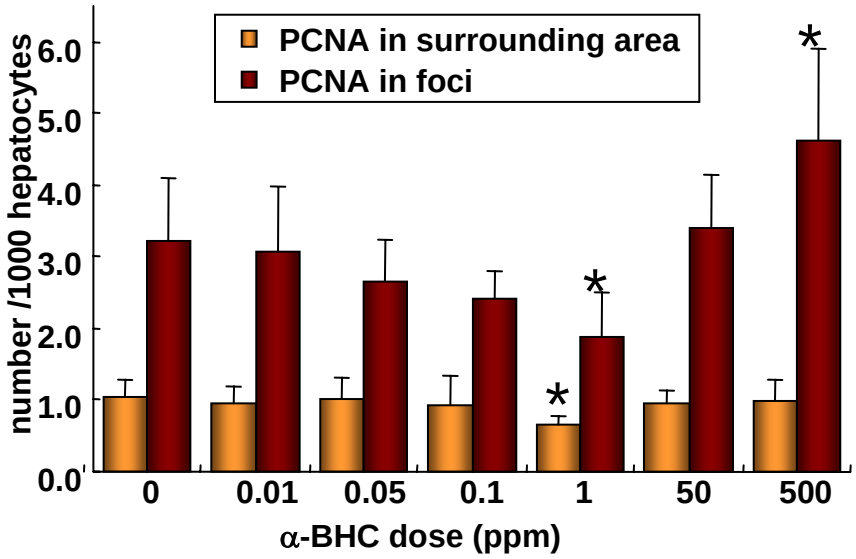




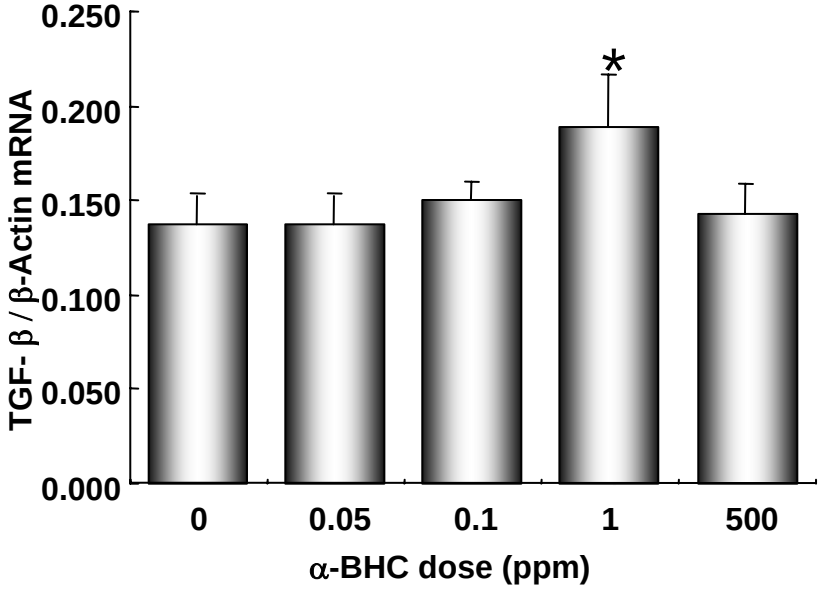
* P<0.05 when compared with DEN alone group

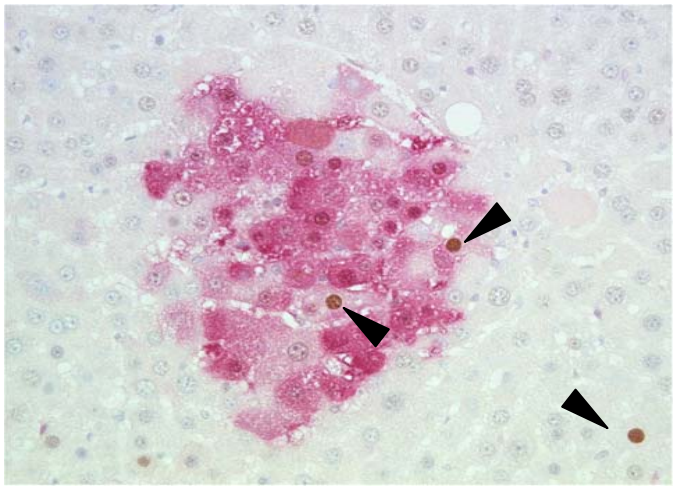
Effect of α-BHC on cell proliferation

PCNA in GST-P positive foci and their surrounding area



TGF-β mRNA expression

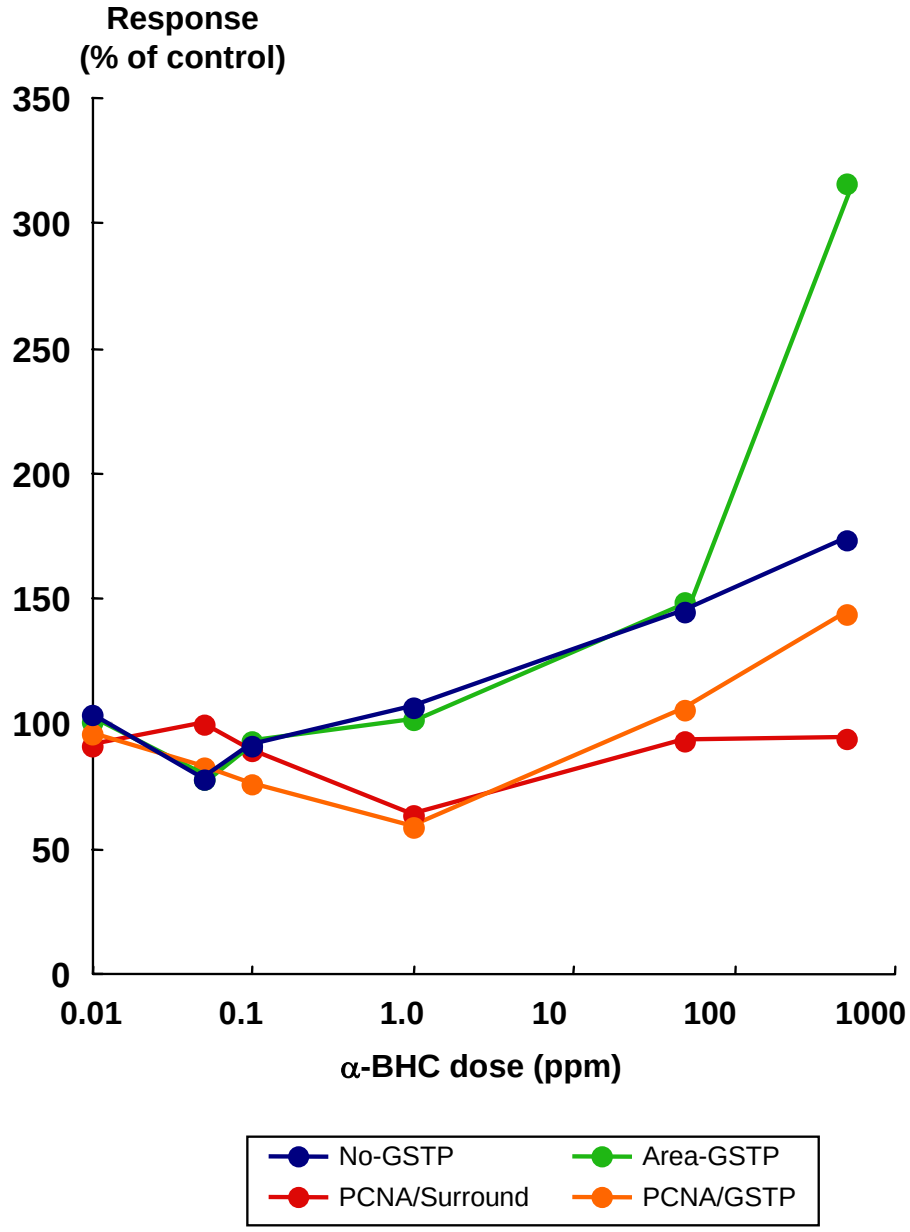


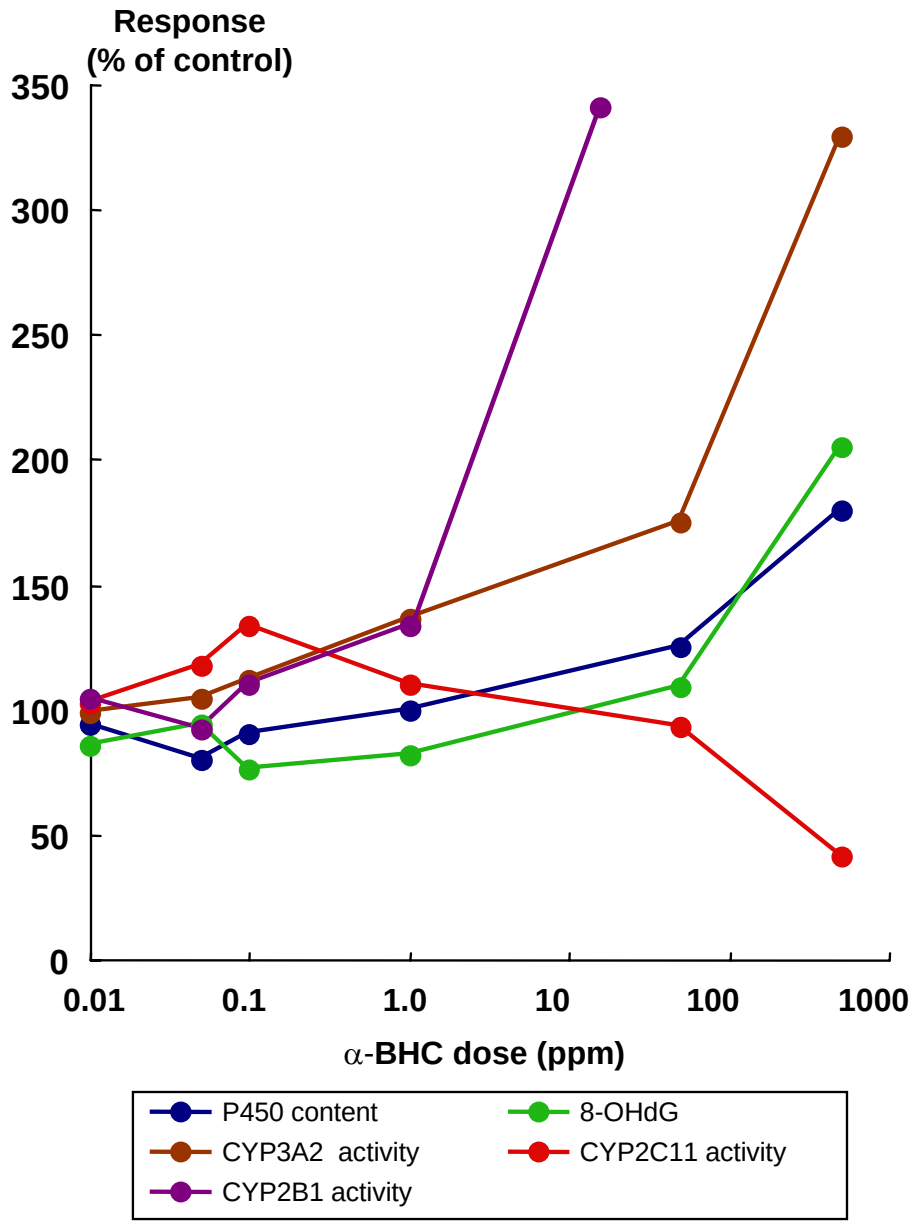


∗ P<0.05 when compared with DEN alone group

Double immunostaining of GST-P and PCNA

Correlation of some biological markers in α-BHC treated rats





Summary of α-BHC study

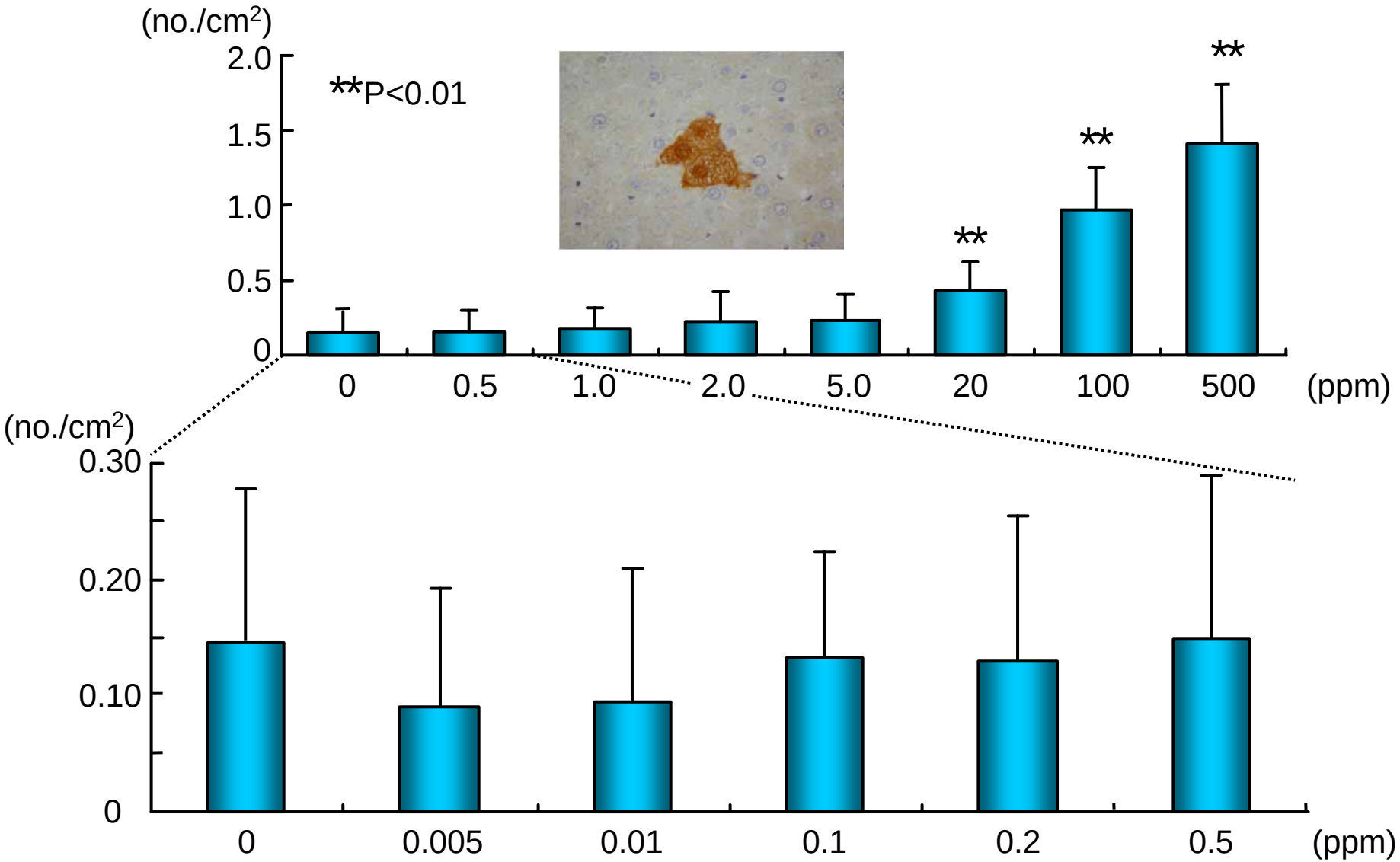
1. **Using GST-P positive foci as the endpoint marker, α-BHC showed hormetic phenomenon in DEN-initiated hepatocarcinogenesis. (J-shape curve).**

2. **The possible mechanism of hormesis might involve alterations in xenobiotic metabolism, cytochrome P450 oxidoreductase system, that produce free radicals followed by oxidative stress and consequently pathological change in the liver.**

1,1-Bis(p-chlorophenyl)- 2,2,2-trichloroethane (DDT)

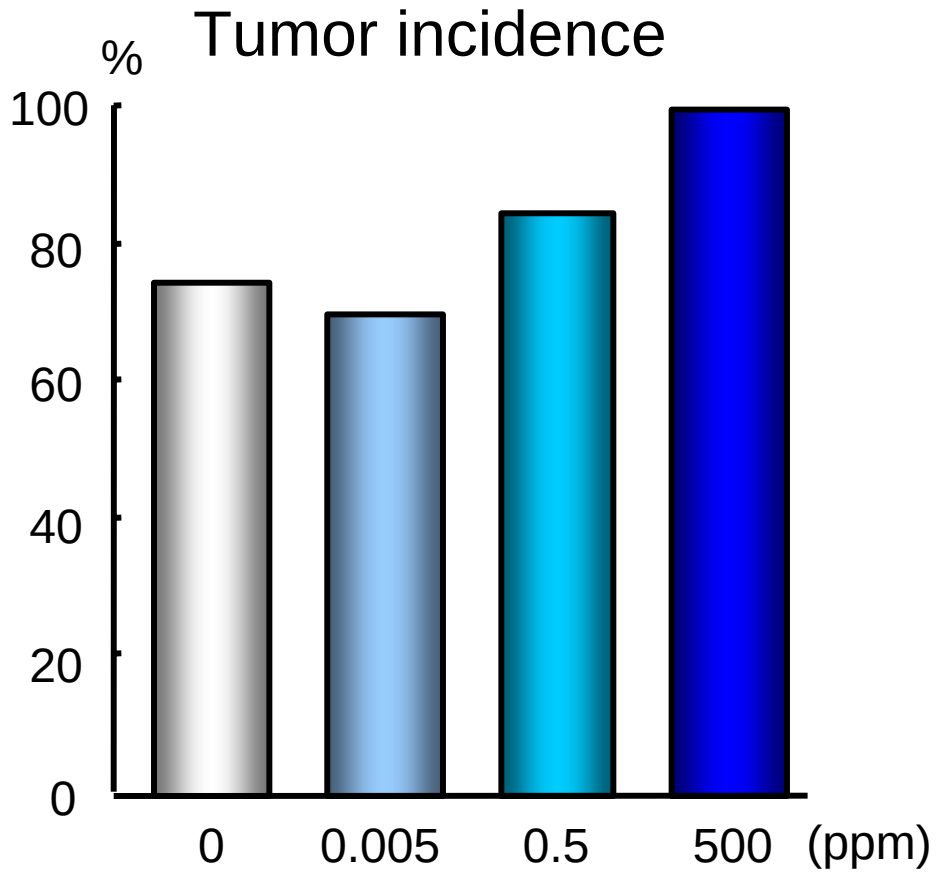
- Pesticide
- Mutagenicity: negative
- Hepatocarcinogen

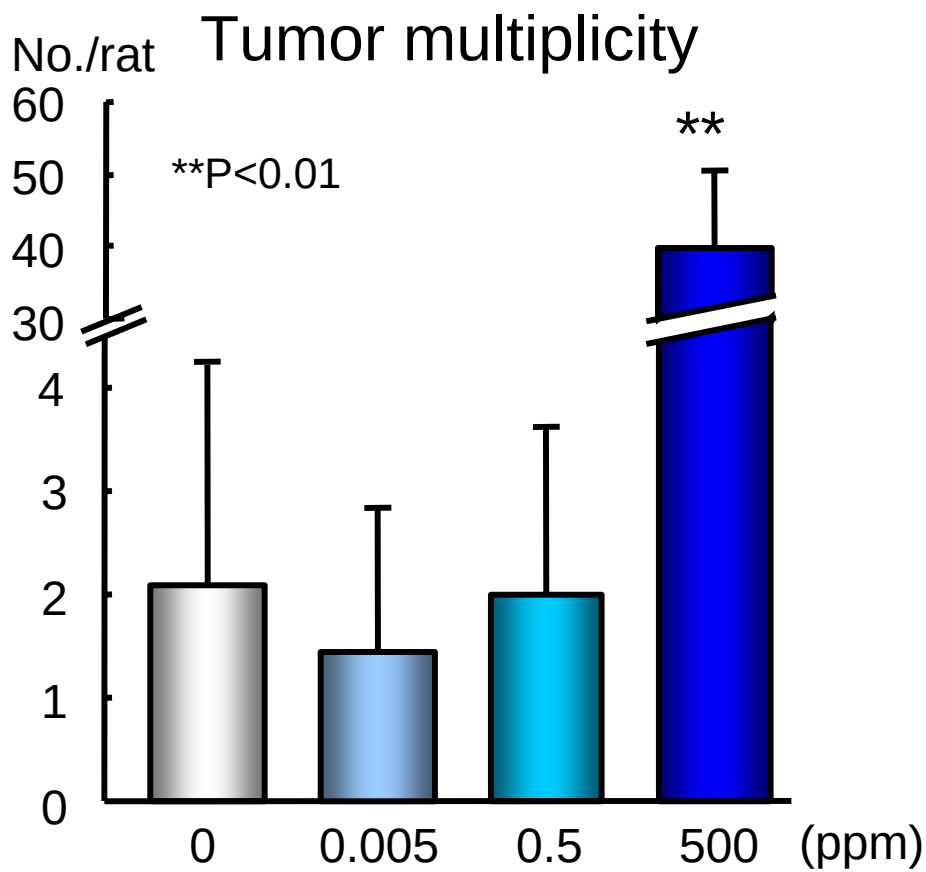
Low dose carcinogenicity of DDT in rat liver
(Male F344, 21-day-old, 16 wks study)



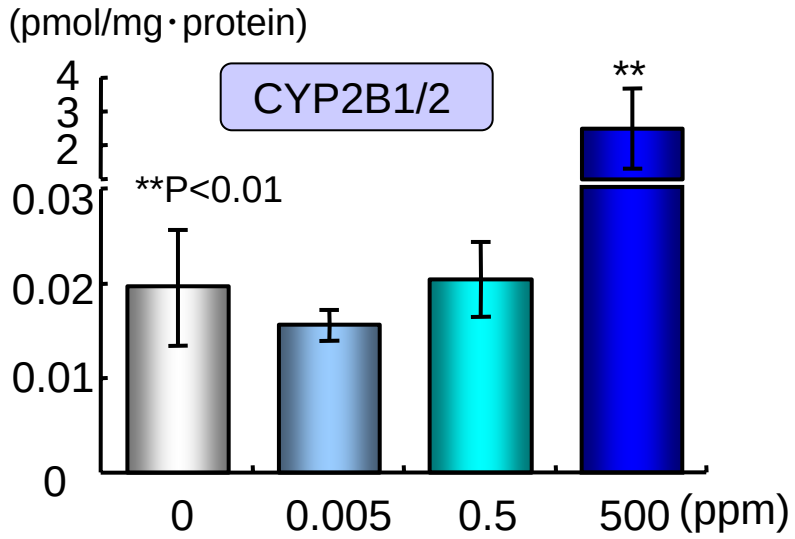
Hepatocarcinogenicity of DDT in the rat liver

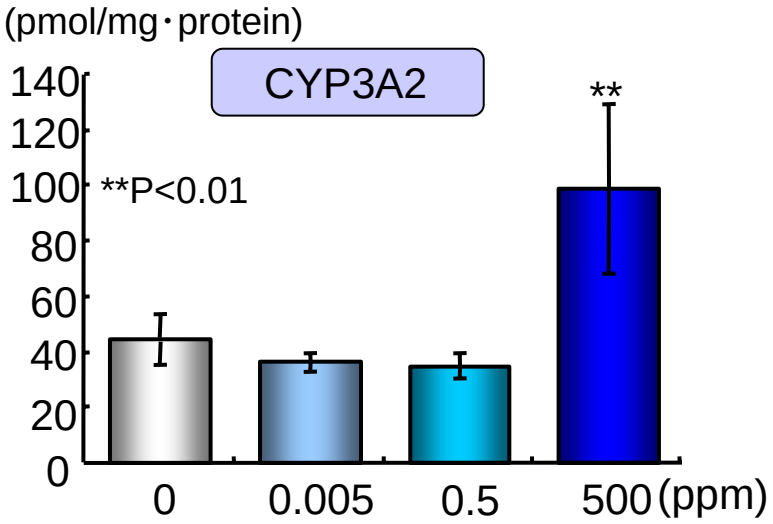
Male F344 rats
6 weeks old



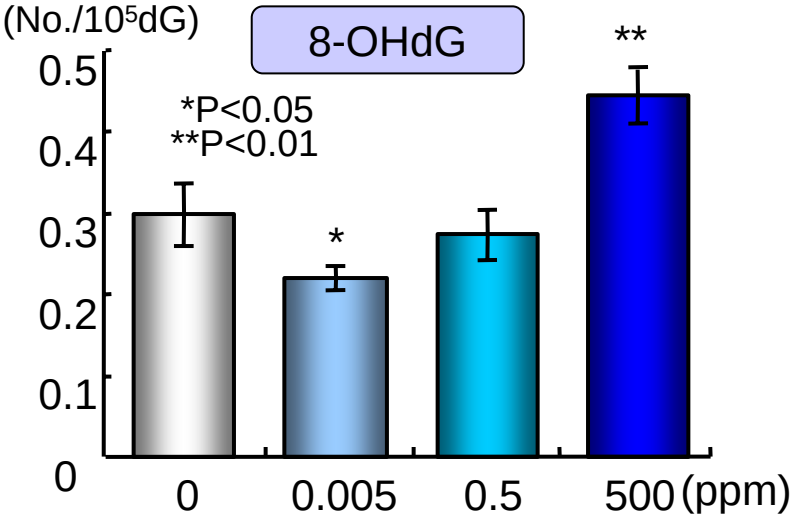


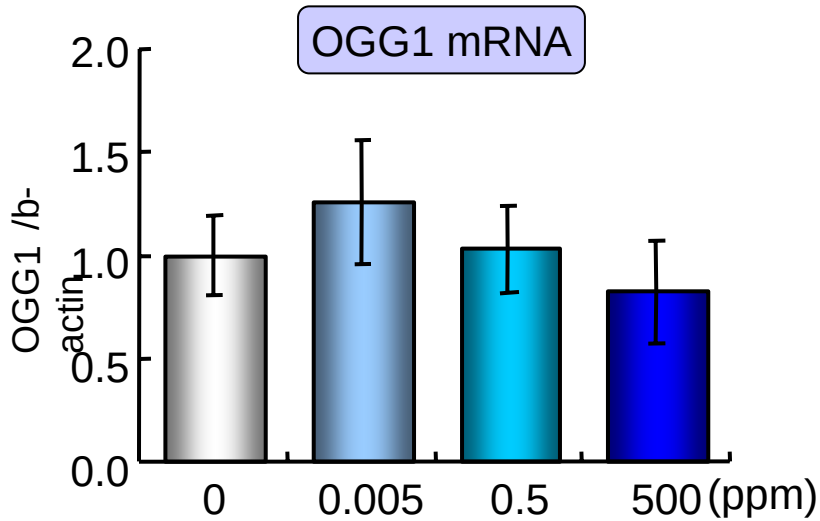
P-450 contents in the rat liver induced by DEN→DDT



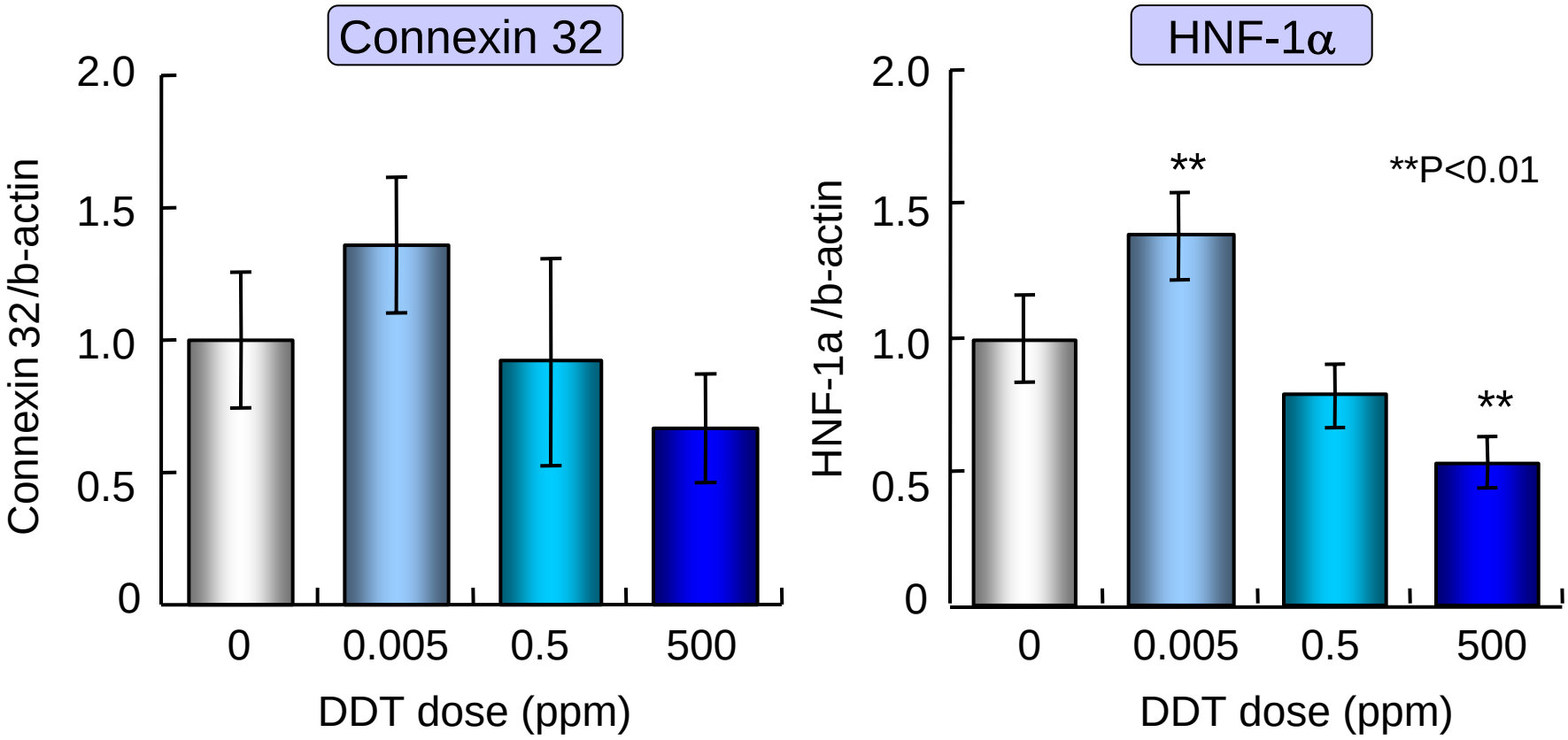


8-OHdG formation and OGG1 mRNA expression in the rat liver induced by DEN→DDT





Alteration of gap junctional intercellular communication (Cx32) and its regulator gene (HNF-1α) in the liver



Summary of DDT study

1. **Inductions of GST-P positive foci and tumors tended to be inhibited by DDT at low dose, whereas DDT at high doses increased GST-P positive foci (J-shape curve, hormesis).**

2. **OGG1, connexin 32 and HNF-1α expressions showed inverted U-shape curve.**

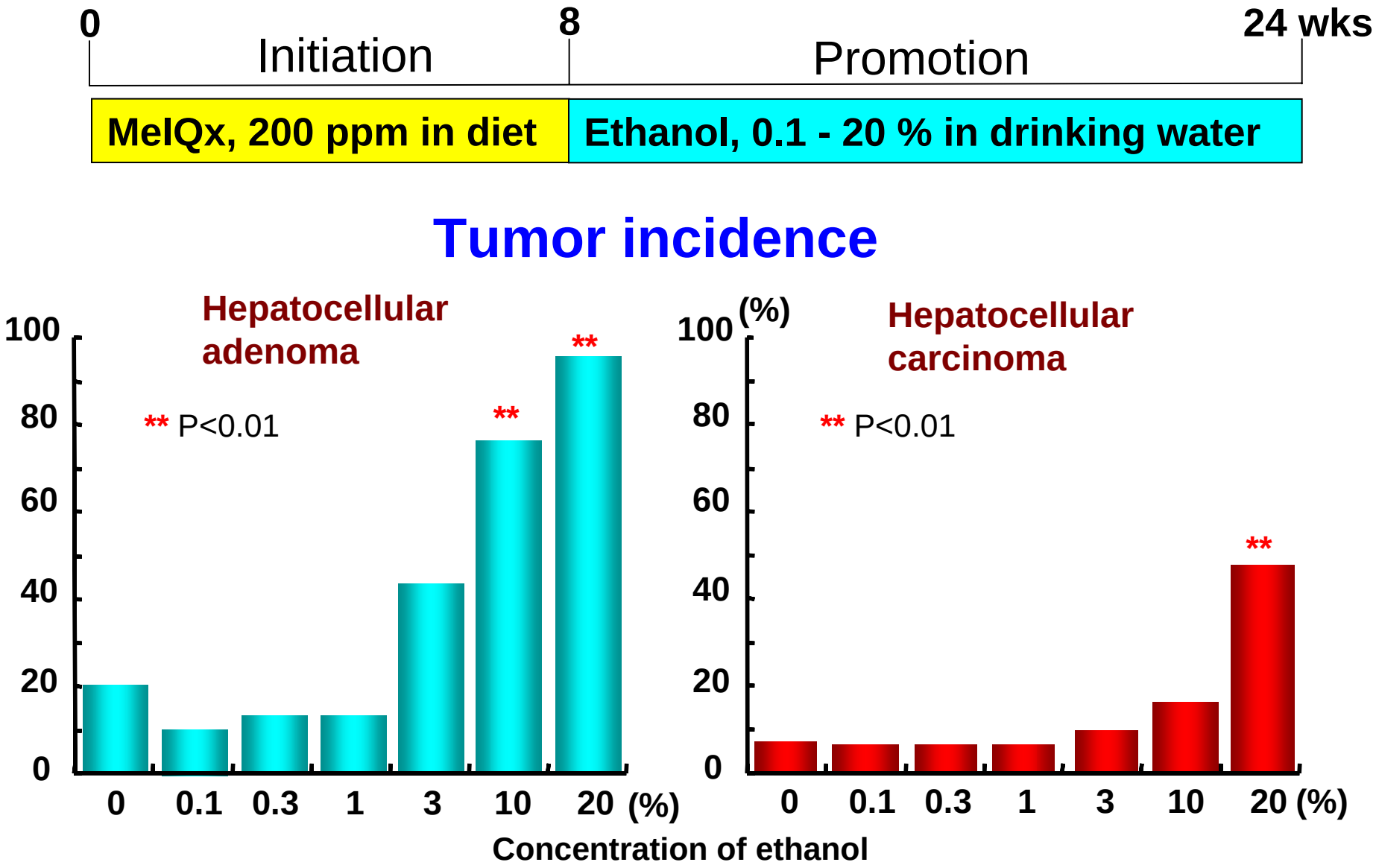
Proposal of a flow scheme toward dose-effect relations, risk assessment and mechanisms of hormesis of non-genotoxic chemical carcinogens

Non-genotoxic hepatocarcinogen

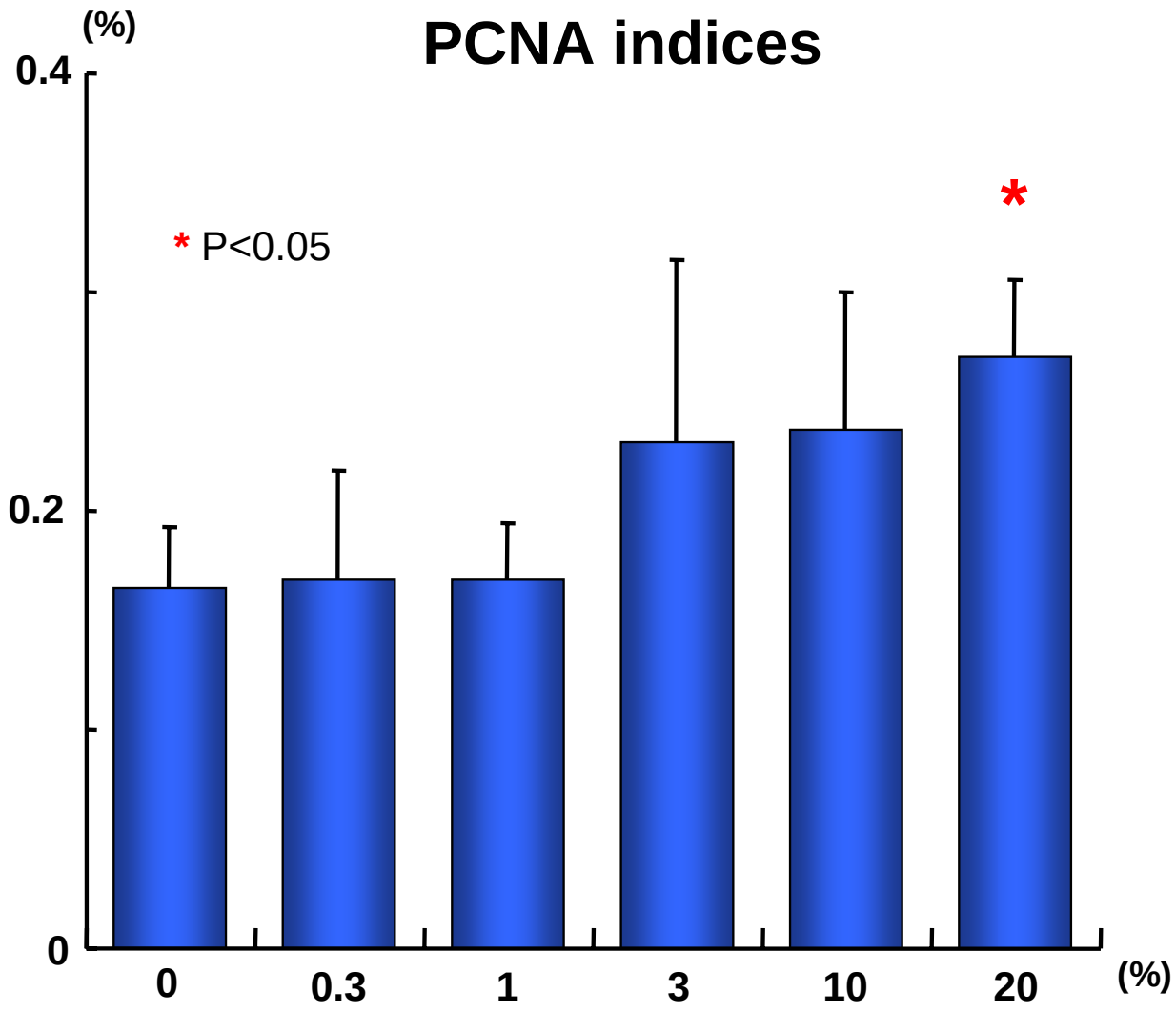
Low dose	High dose
Inhibition of oxidative stress (CYP3A2, 8-OHdG)	Induction of oxidative stress (P-450, ROS, 8-OHdG)
Increase of DNA repair	Induction of DNA repair
Inhibition of cell proliferation in the areas of GST-P positive foci	Increase of cell proliferation in the areas of GST-P positive foci
Suppression of apoptosis in normal-appearing liver cell area	Induction of apoptosis in normal-appearing liver cell area
Protection of GJIC	Inhibition of GJIC
Activation of liver detoxification system (CYP2C11, P-450 NADPH oxidoreductase)	Inhibition of CYP2C11
Cell signaling (GABA ↑ , MAP kinases signaling pathways ↑)	
Inhibition of carcinogenesis	Promotion of carcinogenesis

Perfect threshold in carcinogenicity

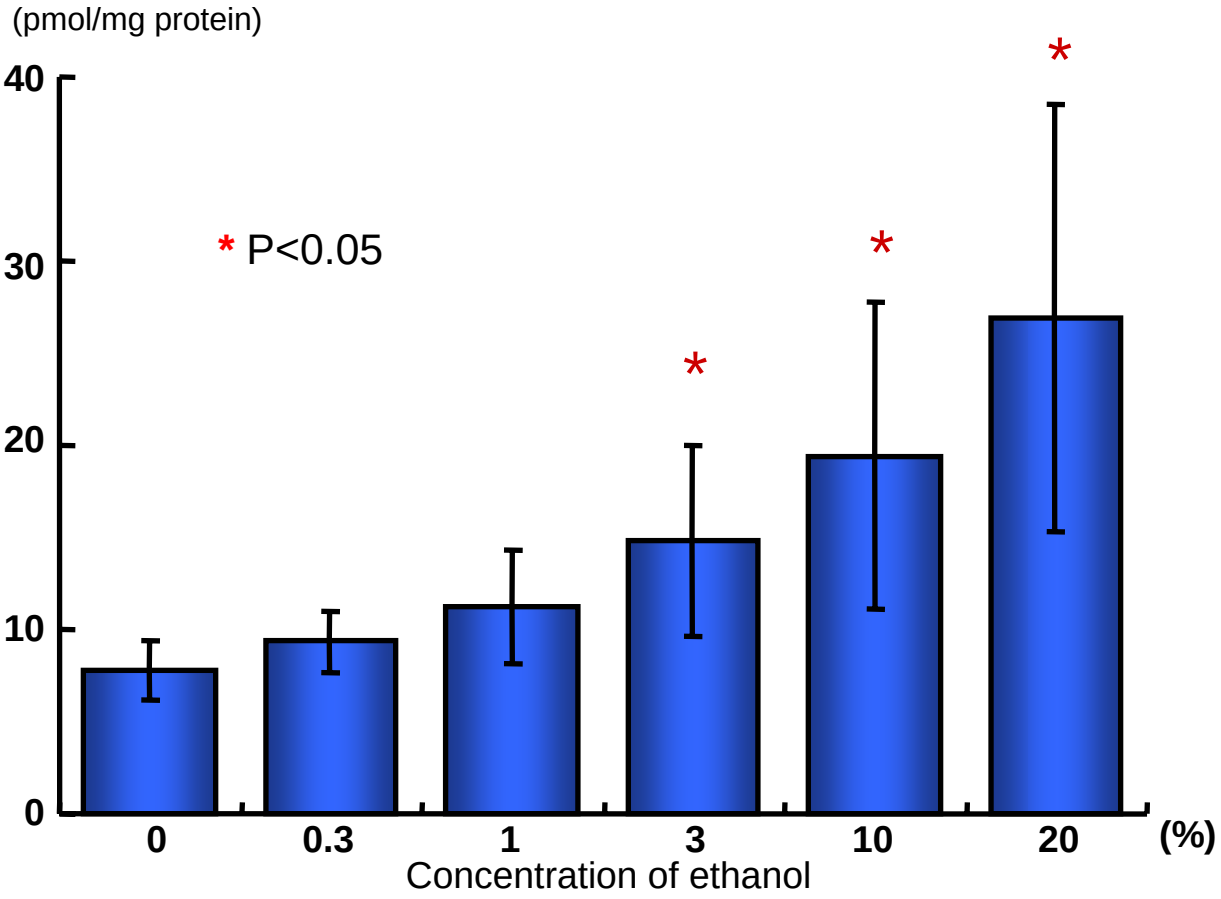
Dose-dependence of promotion by ethanol on rat hepatocarcinogenesis

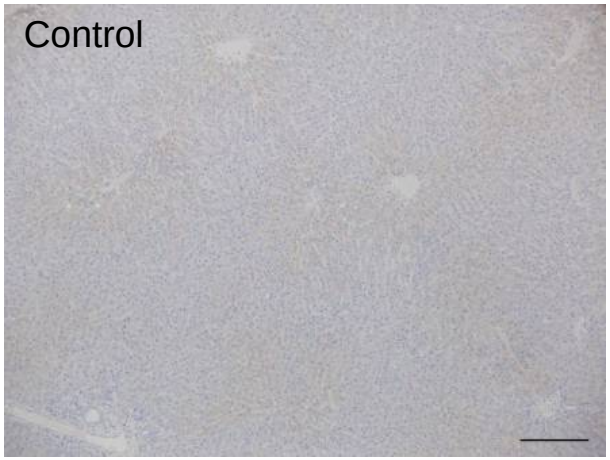


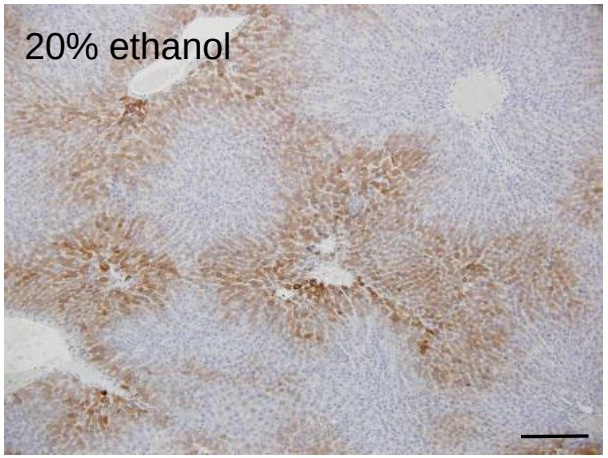
Dose-dependent liver tumor induction by ethanol



Liver cell proliferation with dose-dependence: PCNA indices







Protein expression

Increase of CYP2E1 expression by ethanol

Summary of ethanol study

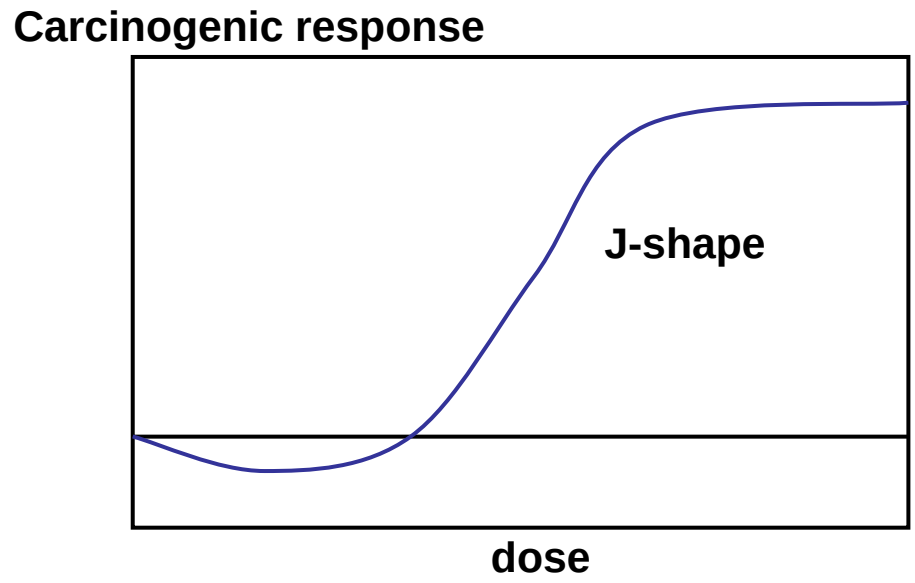
1. **Ethanol dose-dependently promoted hepatocarcinogenesis induced by MeIQx, but with no adverse influence at doses of 1 % and less, comparable to sensible drinking levels in human.**

2. **Cell proliferation and CYP2E1 influenced promotion activities of ethanol, without evidence of increase in 8-OHdG, a oxidative DNA damage marker.**

3. **Hormesis phenomenon was not observed in ethanol-mediated promotion of hepatocarcinogenesis in rats.**

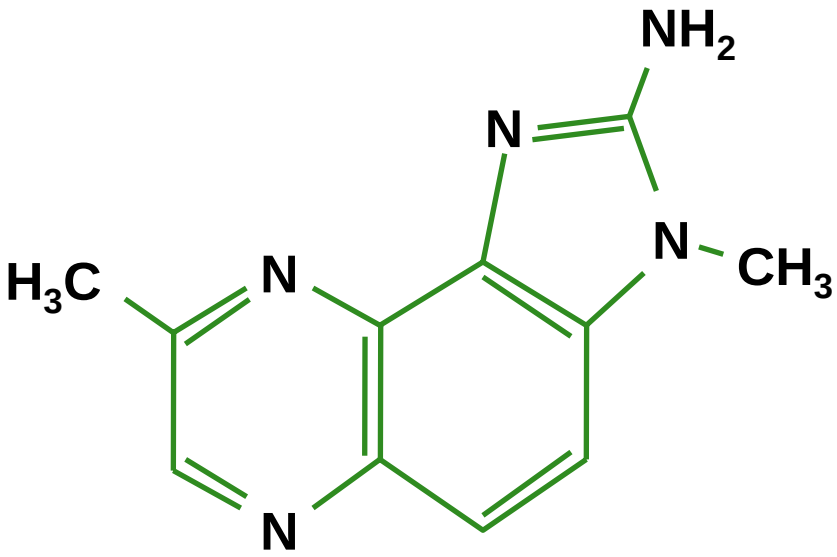
Conclusions

1. **Hepatocarcinogenicity of non-genotoxic carcinogens, such as PB, α-BHC and DDT showed hormetic phenomenon (J-shape curve).**
2. **Alteration to cell proliferation, oxidative damage at high and low doses may have important roles in the hormesis.**
3. **These non-genotoxic carcinogens have perfect threshold for their carcinogenicity.**



Genotoxic carcinogenicity

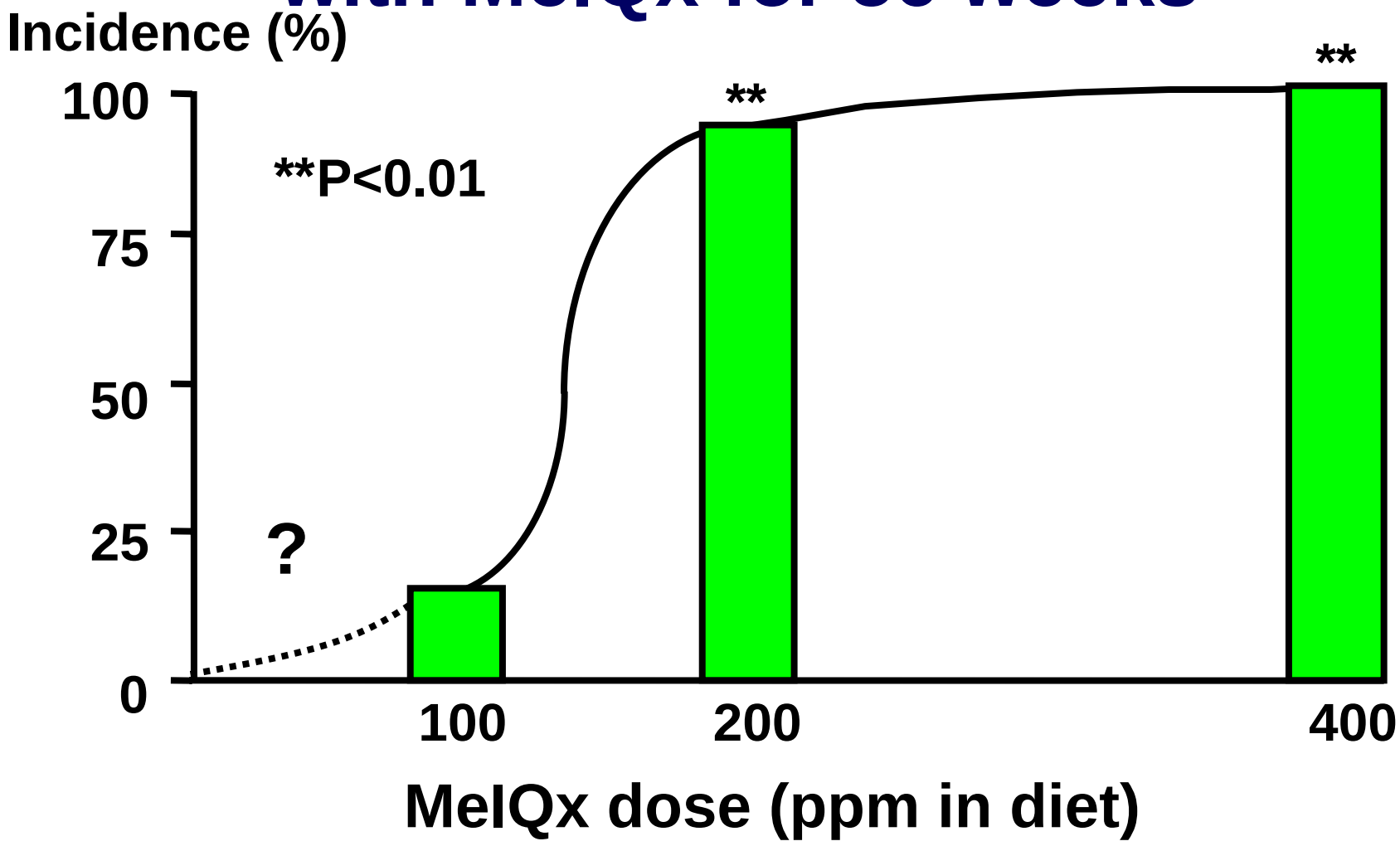
MeIQx



2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline:

- One of heterocyclic amines
- Exists in well-cooked fish and meat
- Mutagenicity: positive
- Hepatocarcinogen
- Human exposure level：0.2-2.6 μg */* day

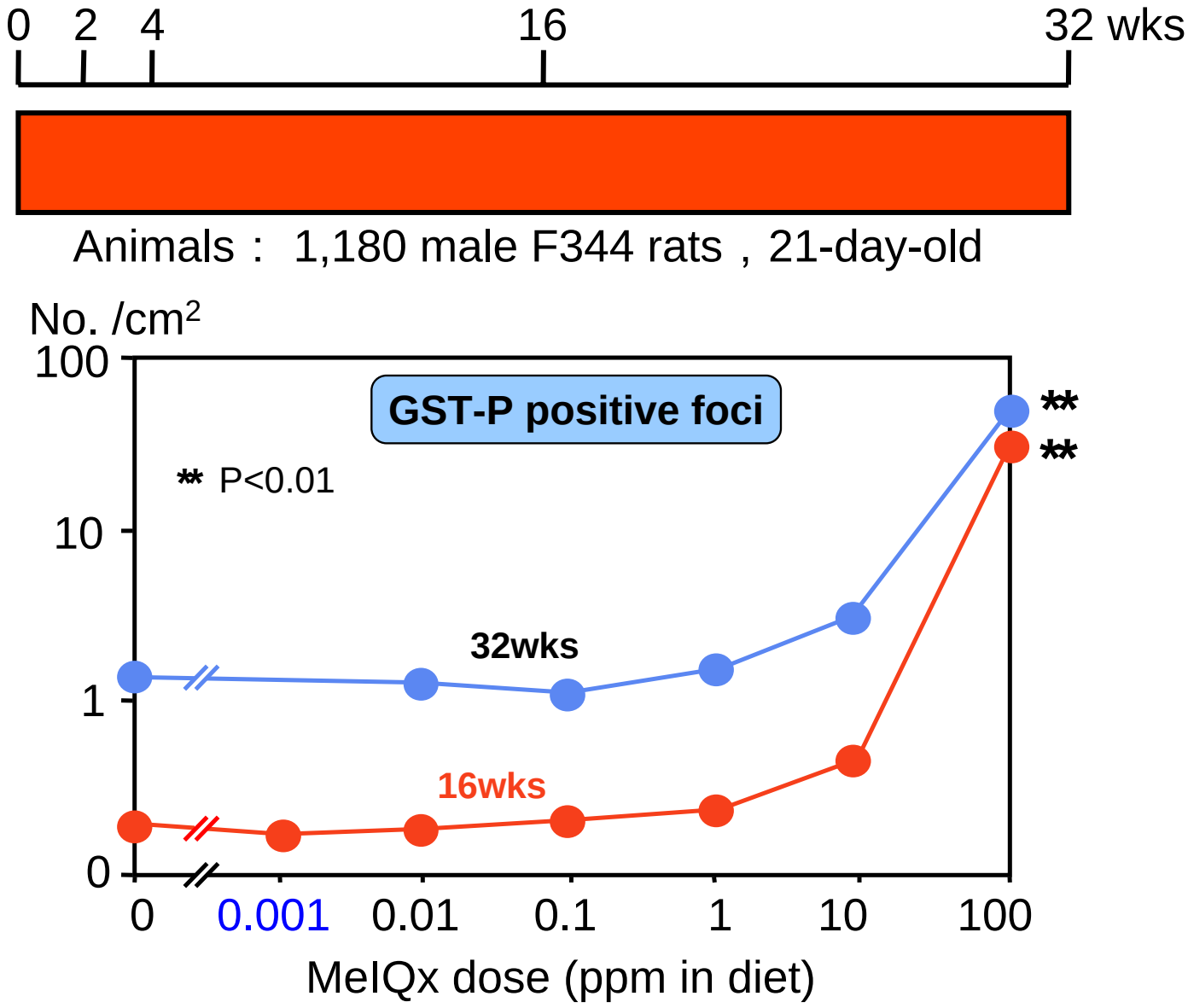
Induction of rat liver cancers treated with MeIQx for 56 weeks



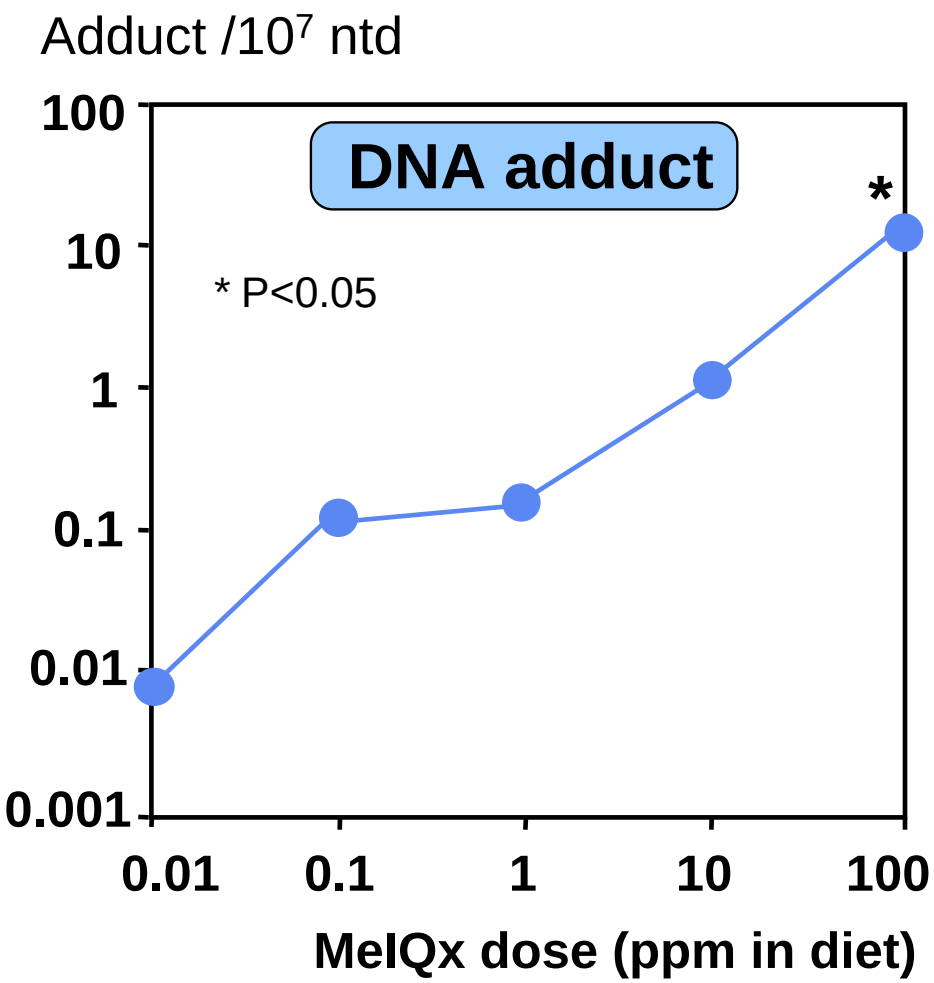
MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline

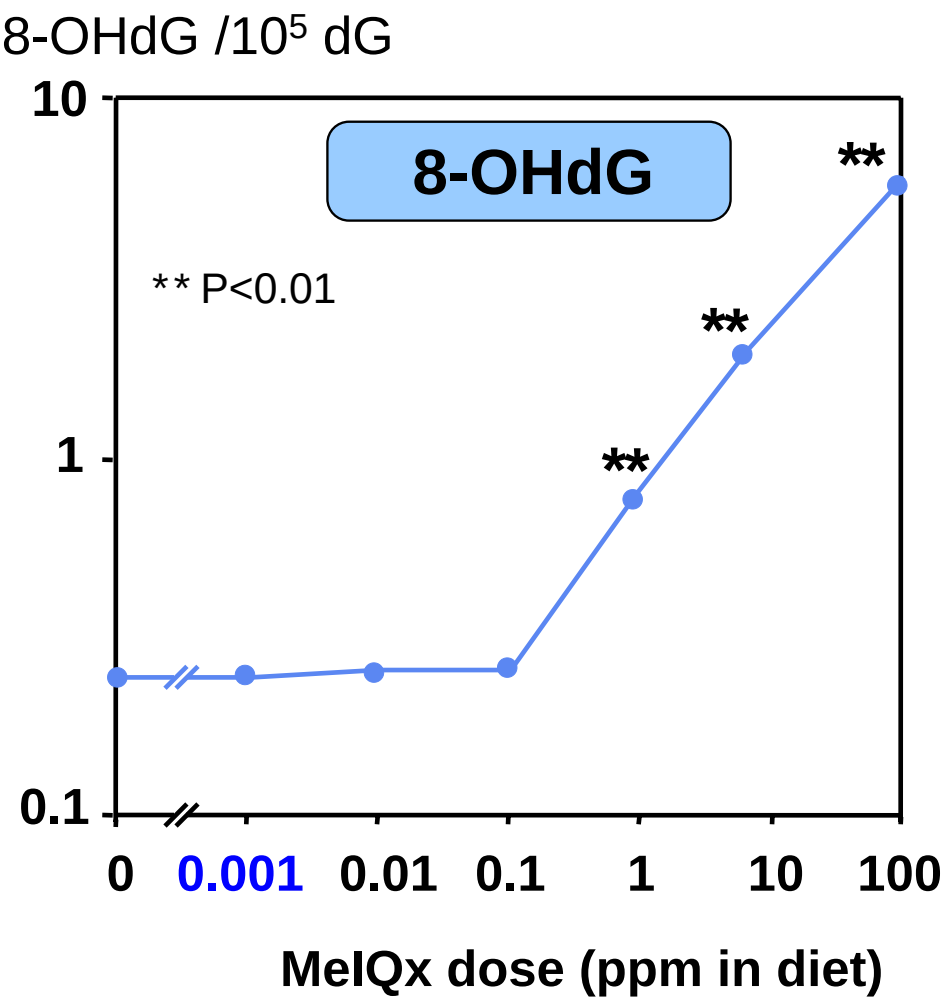
(Wakabayashi et al, 1995)

Rat hepatocarcinogenicity of MeIQx at low doses



Formation of MeIQx-DNA adducts and 8-OHdG in the rat liver treated with MeIQx for 4 weeks





MeIQx

Big Blue rat

Liver

DNA isolation

in vitro packaging

Phage

LacI gene：30 - 40 copies on chromosome 4 in the F344 rat

Mutation in the lacI gene

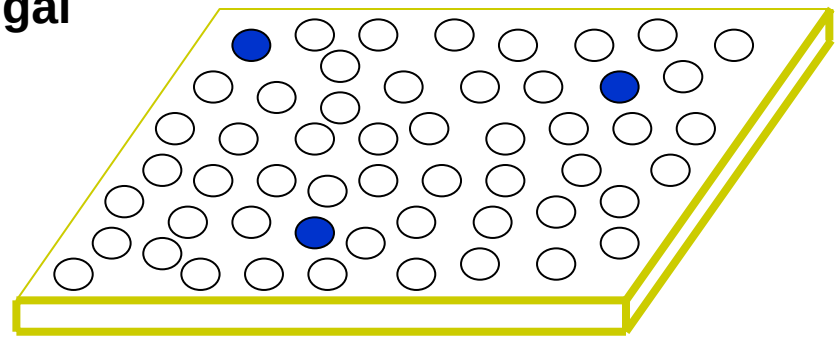
lacI lacO αlacZ

Transcriptional

βgalactosidase

Repressor X-gal Blue

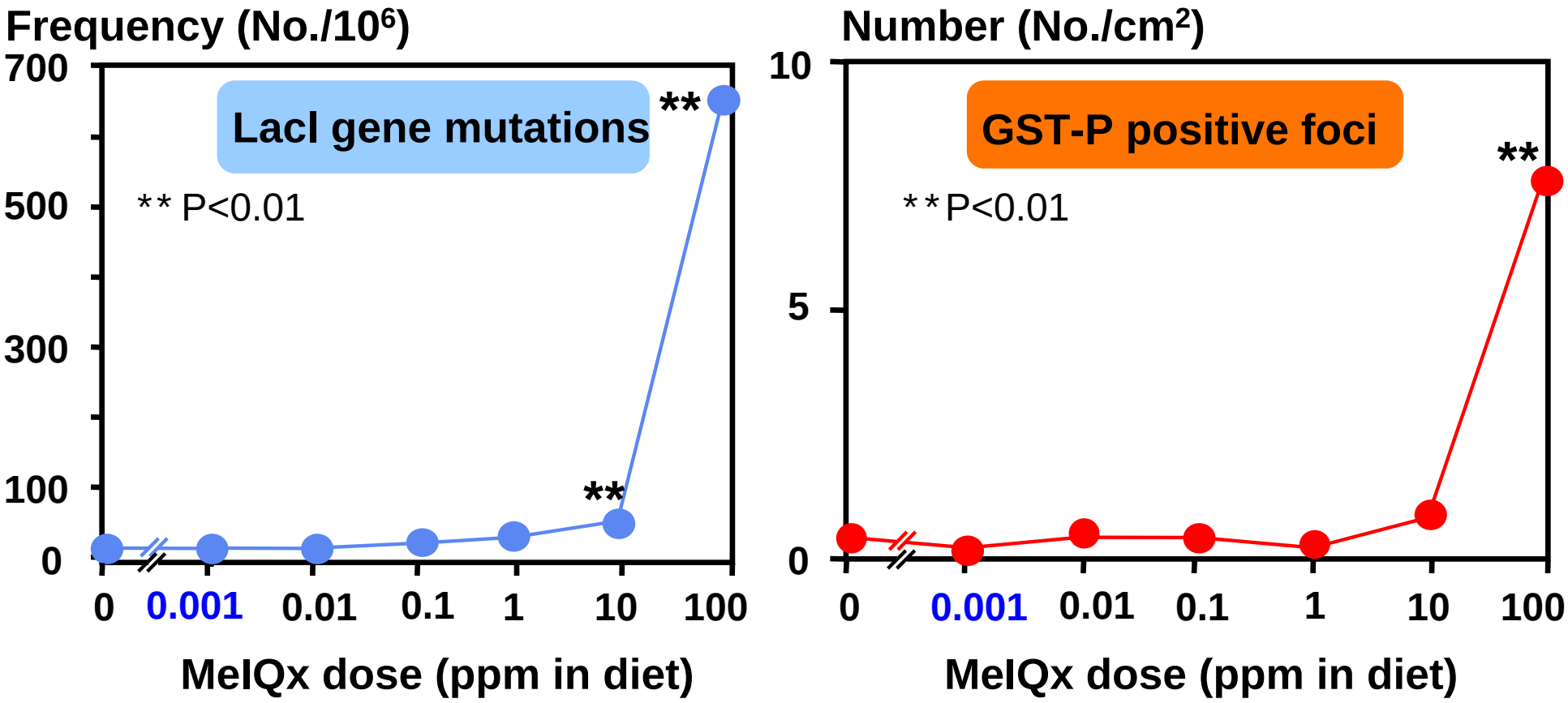
Infection E.coli
Incubation containing X-gal



Blue plaque=Mutation (+)
White plaque=Mutation (-)

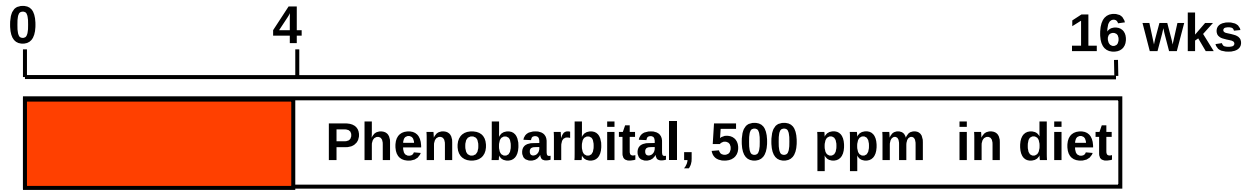
In vivo mutagenicity test in Big Blue rats (Plaque Color Screening Assay)

Frequency of LacI gene mutations and development of GST-P positive foci in the liver of Big Blue rats treated with MeIQx for 16 weeks



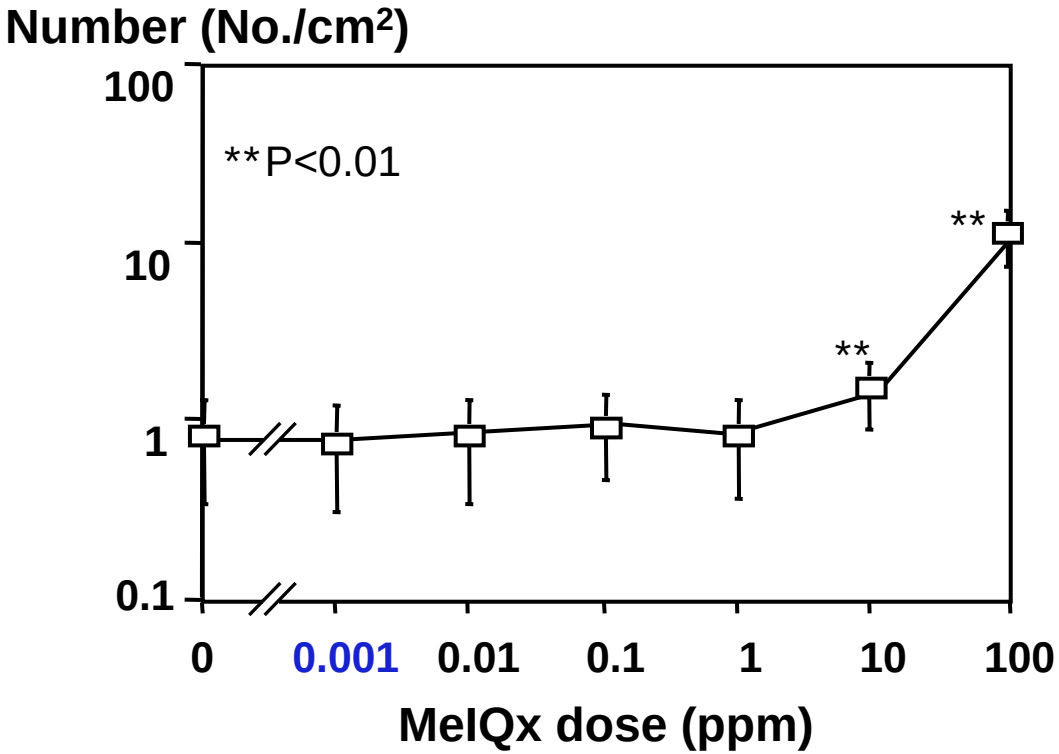
lacI gene: 30 - 40 copies on chromosome 4 in the F344 rat

Initiation activity of MeIQx at low doses in the rat liver

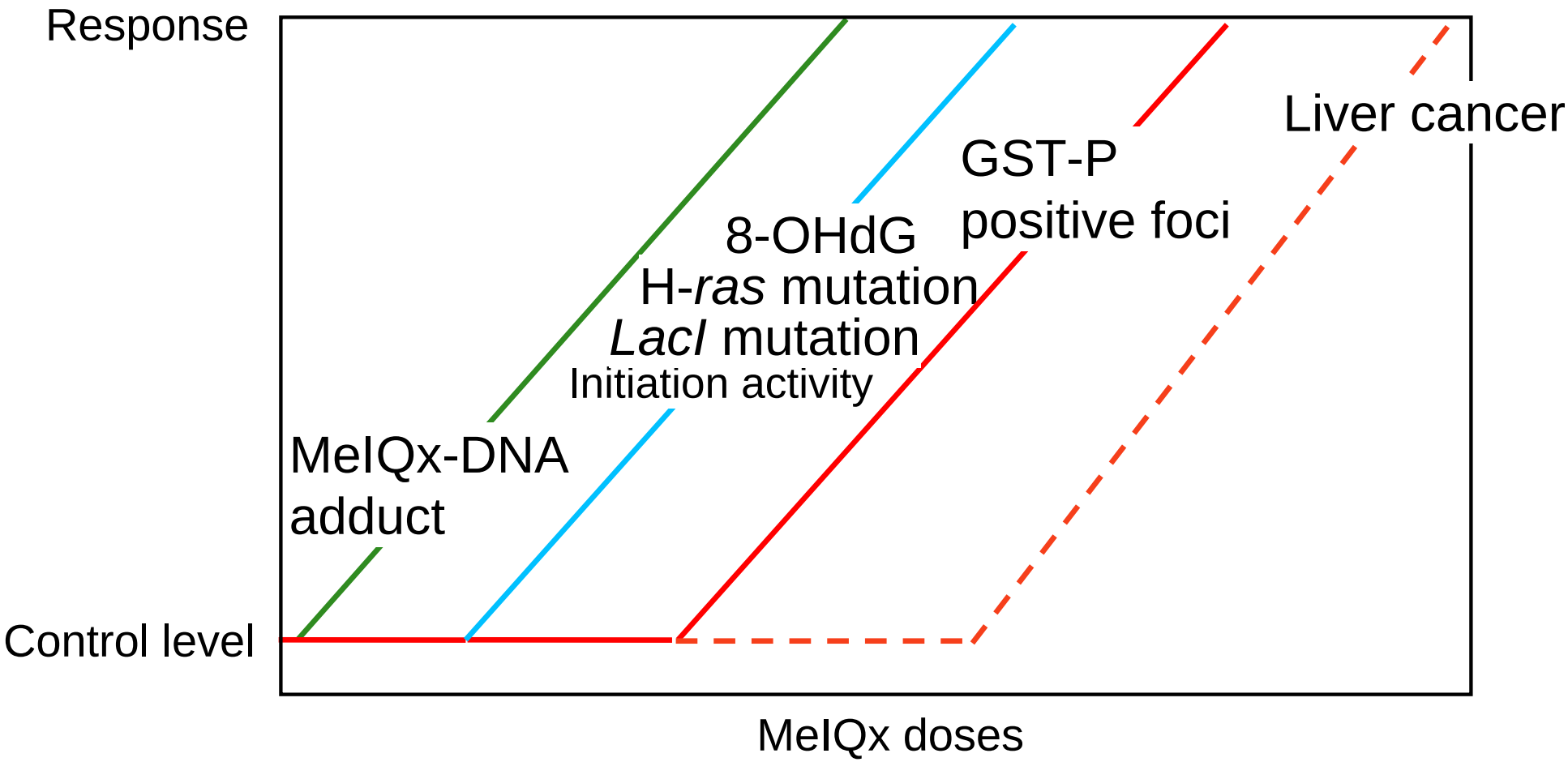


Animals: 850 male F344 rats, 21-day-old

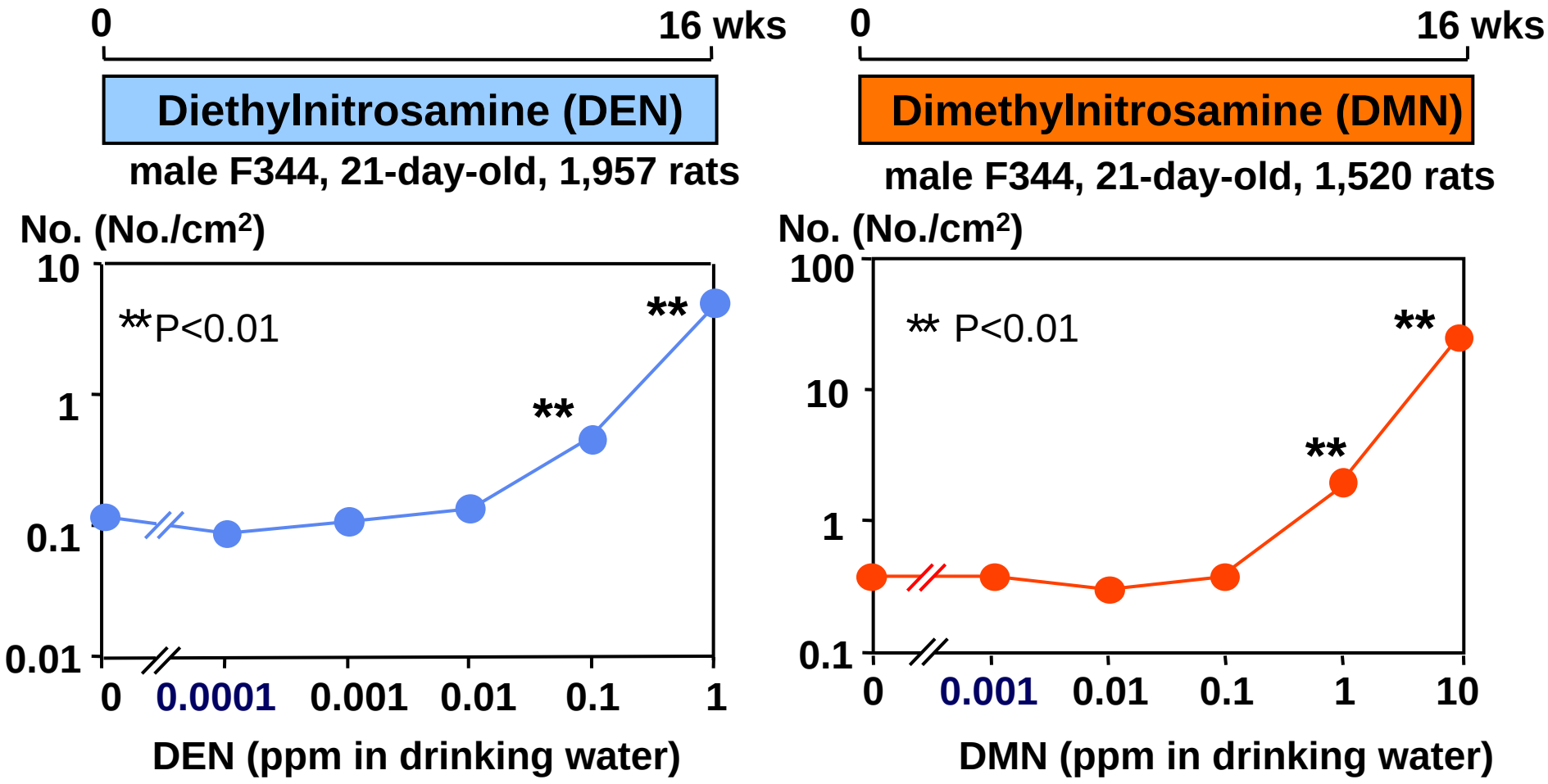
MeIQx; 0, 0.001, 0.01, 0.1, 1, 10, 100 ppm in diet



Risk of liver cancer: Reaction curves for the carcinogenicity markers dependent on the dose of MeIQx

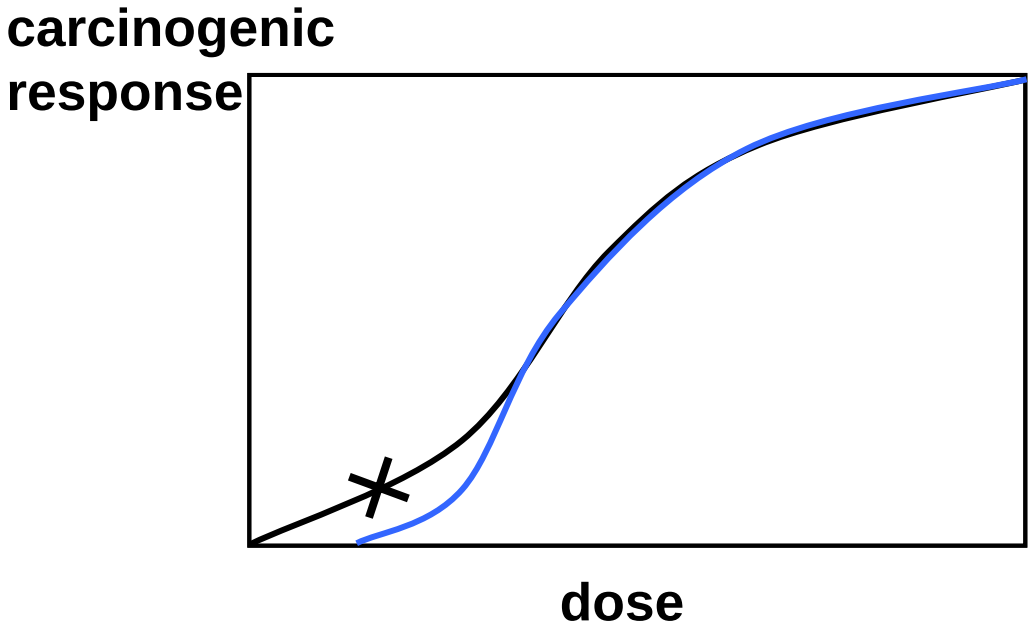


Rat hepatocarcinogenicity of *N*-nitroso compounds: Induction of GST-P positive foci



Conclusion

1. **The carcinogenicity markers showed no-effect levels for their response.**
2. **The genotoxic carcinogens such as MeIQx and DEN have practical threshold for their carcinogenicity.**
3. **Further studies are required for hormetic effect of genotoxic carcinogens.**



Summary

Our data demonstrate that some of non-genotoxic carcinogens have hormesis for their carcinogenicity, showing existence of perfect threshold. Genotoxic carcinogens exhibit threshold, at least practical threshold. These conclusions may introduce new concept for cancer risk assessment and management.

Collaborators

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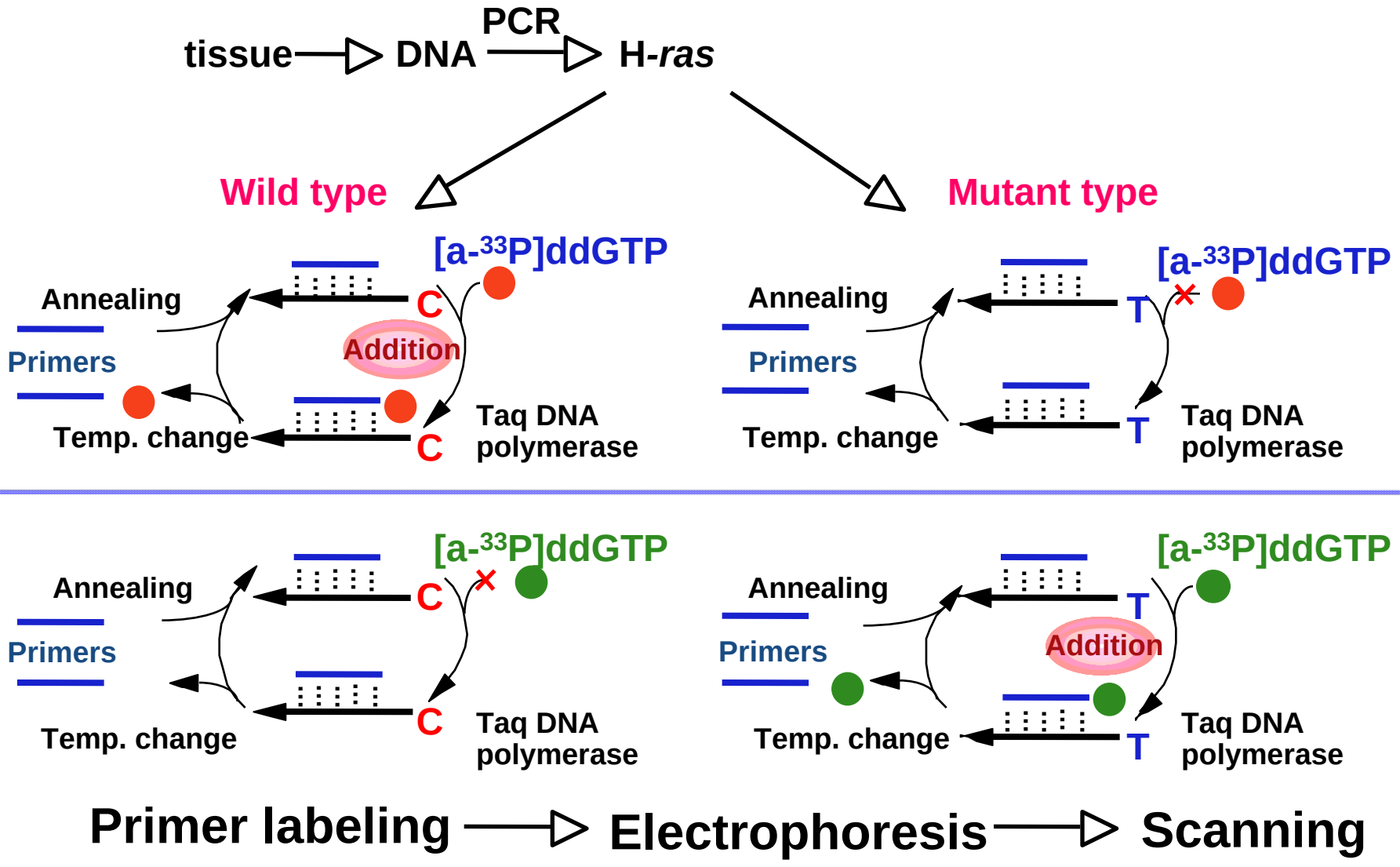
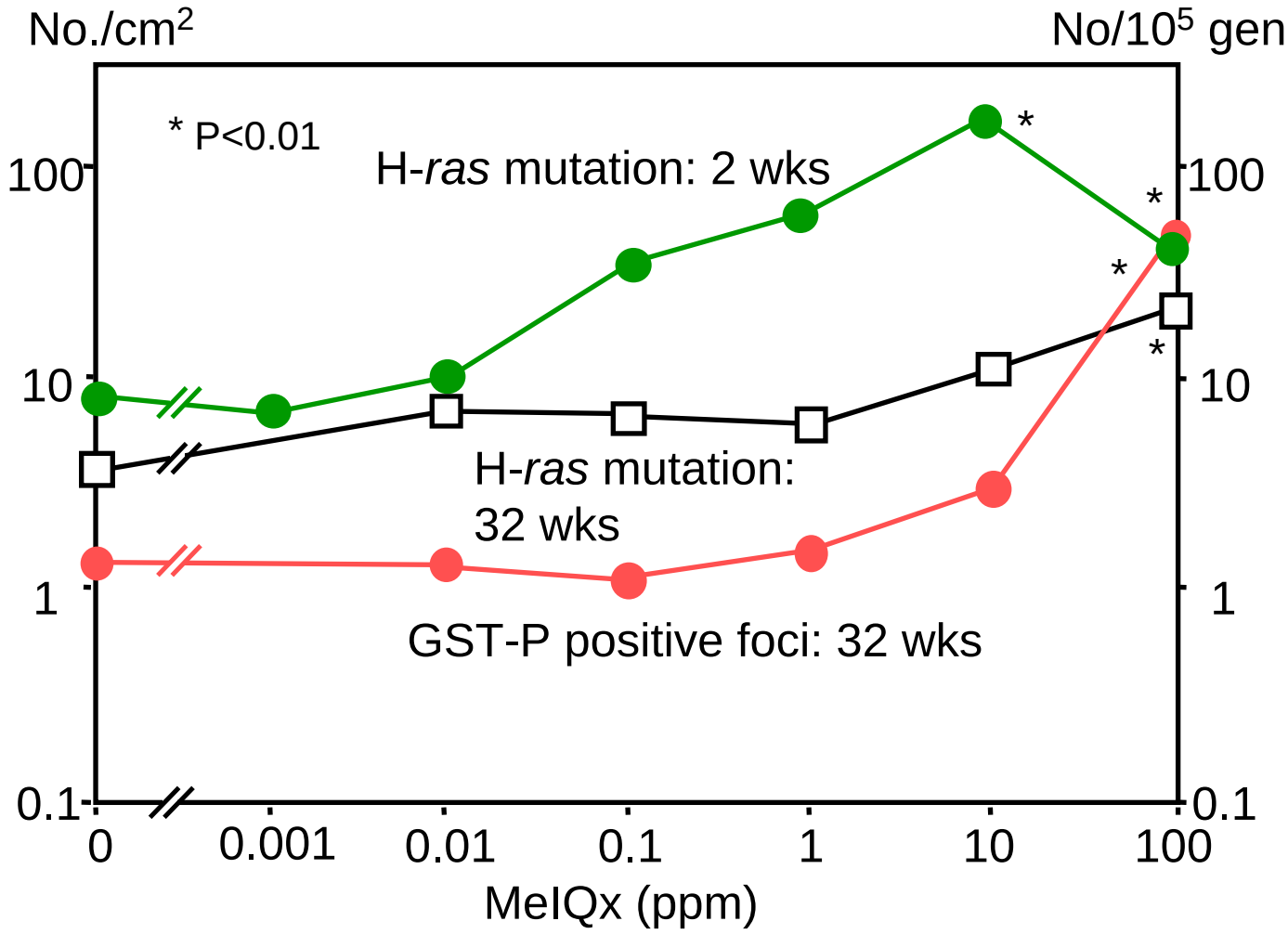
Detection of H-*ras* mutation: Thermosequenase cycle end labeling (TCEL) method



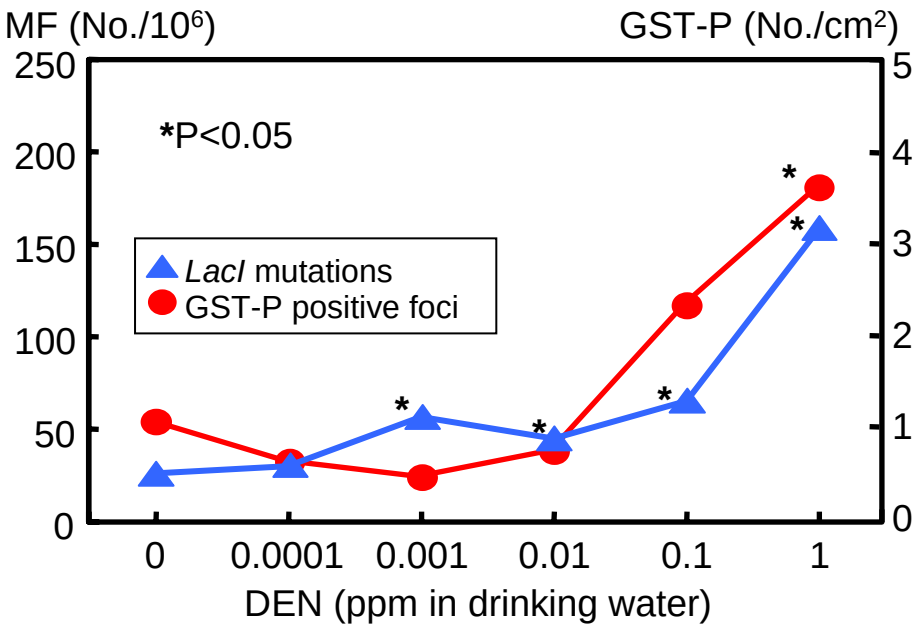
Primer labeling → Electrophoresis → Scanning

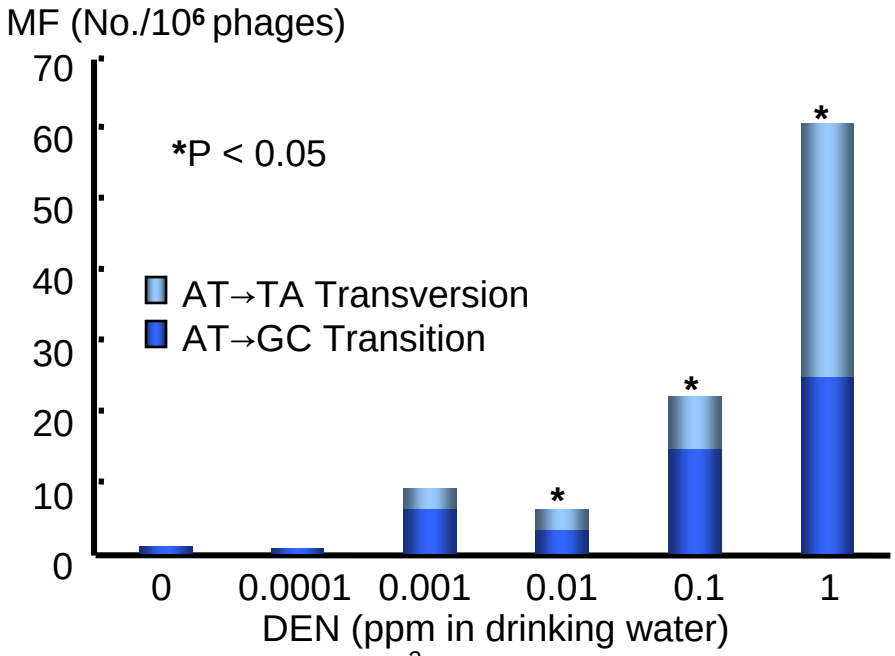
Frequencies of H-*ras* mutation and GST-P positive foci in the liver of rats treated with MeIQx

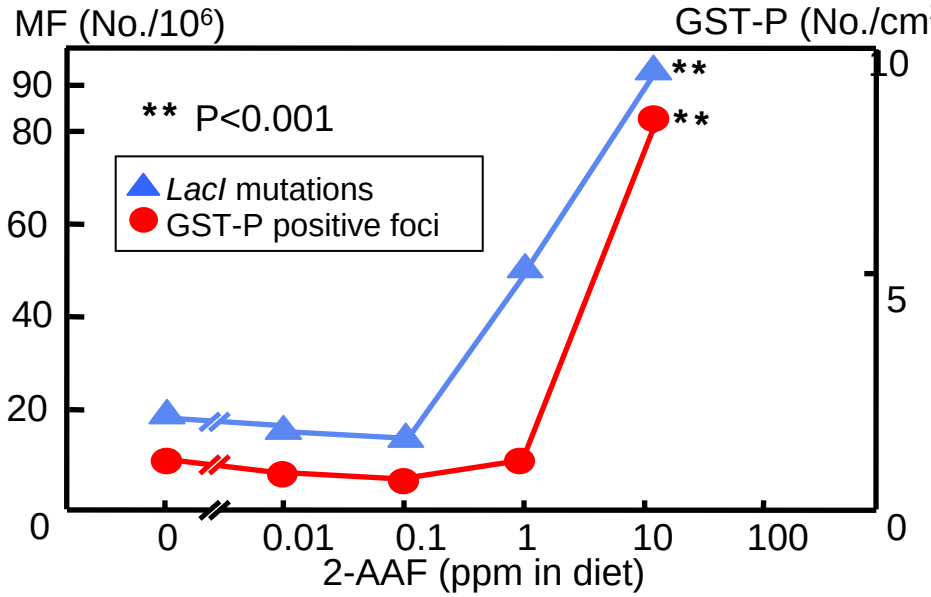


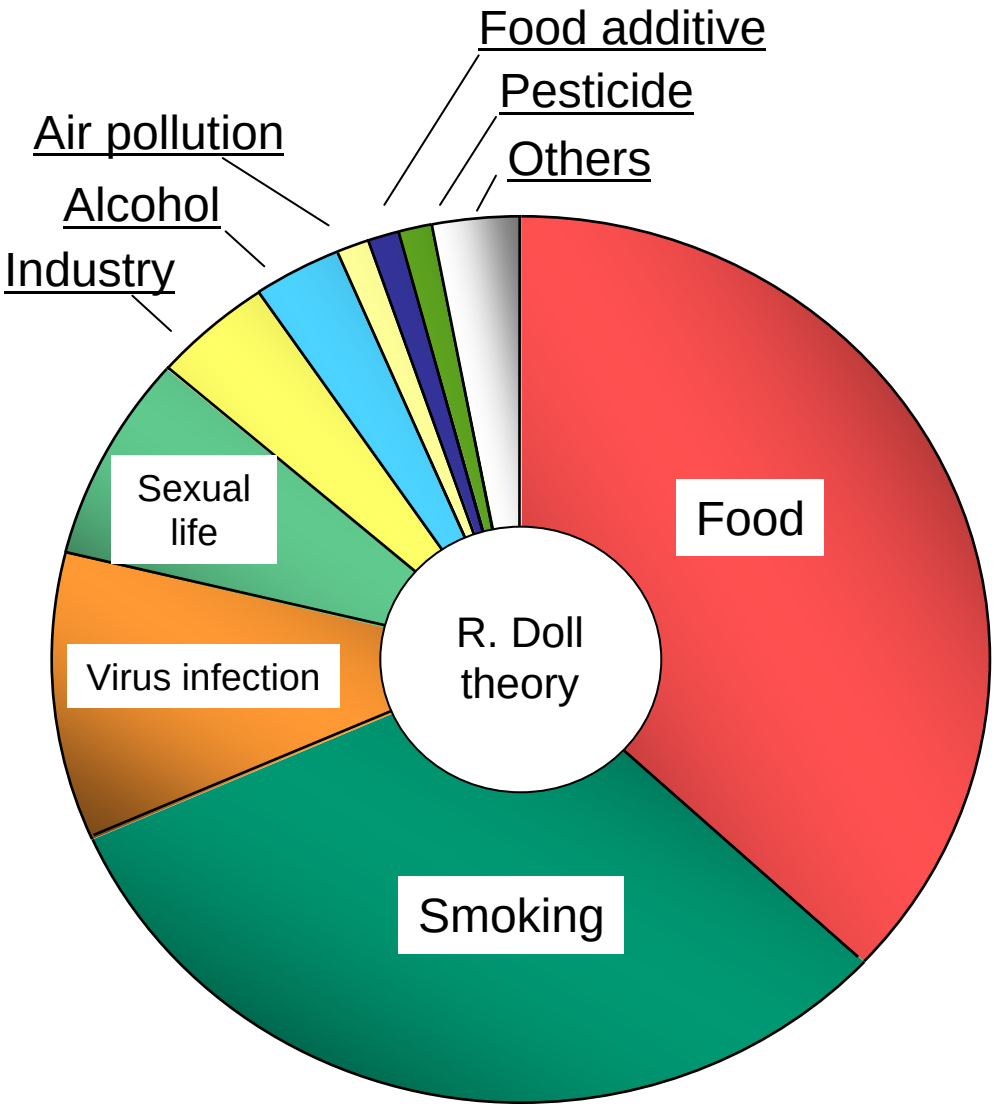
Detection of H-*ras* mutation:
Thermosequenase cycle end labeling (TCEL) method

LacI Mutation frequency and development of GST-P positive foci in the liver of Big Blue rats treated with DEN or 2-acetaminofluorene (2-AAF) for 16 weeks



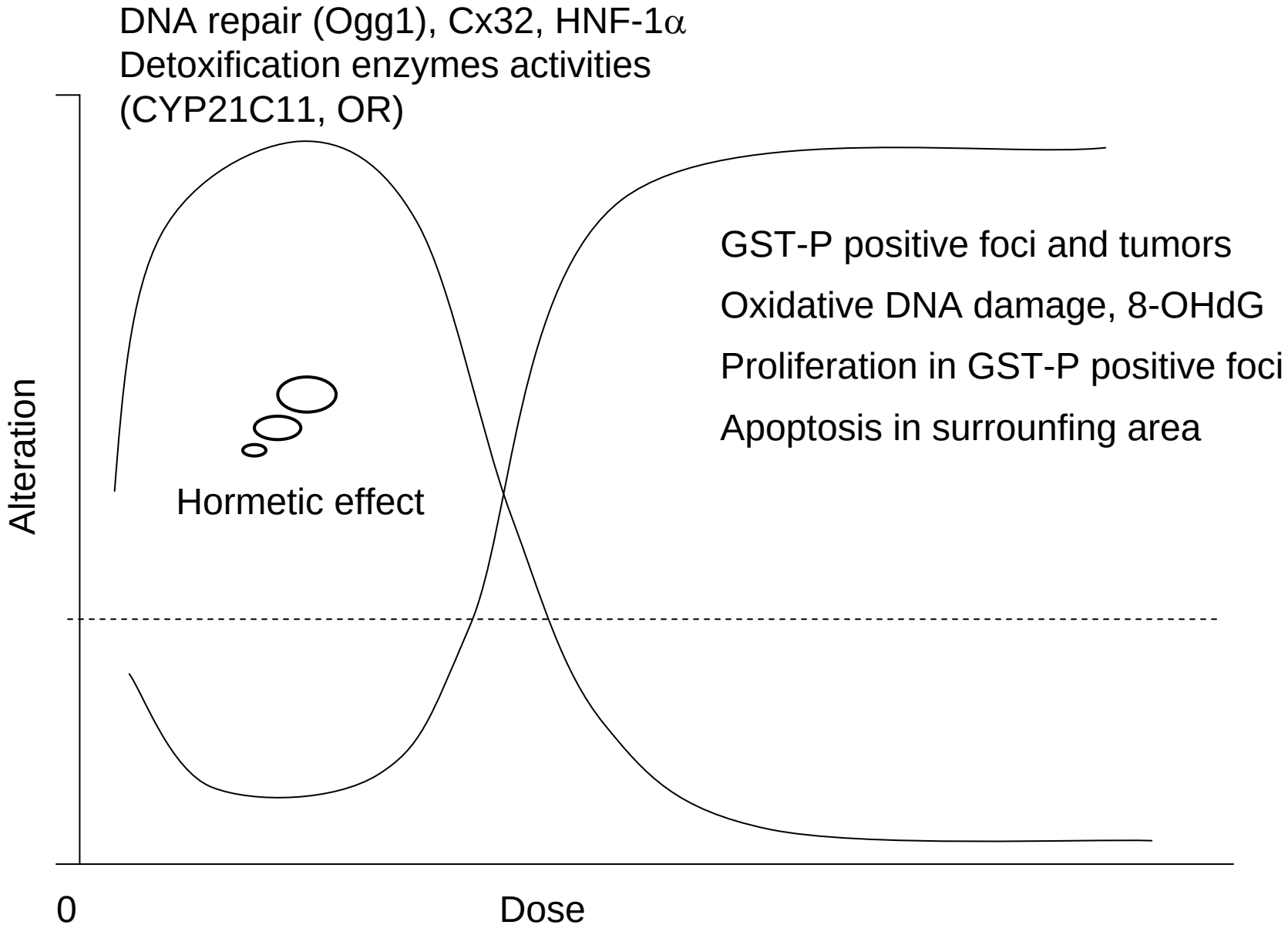






Etiology of human cancer (R. Doll 1981)

Potential mechanisms mediating hormesis in carcinogenesis



Chemical carcinogenesis mechanisms