Existence of Carcinogenic Threshold : Evidence from Mechanism-Based Carcinogenicity Studies

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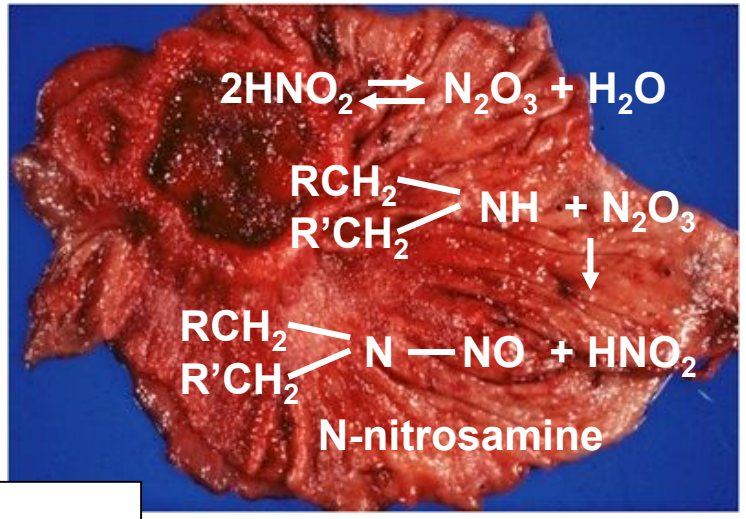
Japan Industrial Safety and Health Association

Genotoxic



Genotoxic

Environmental carcinogens

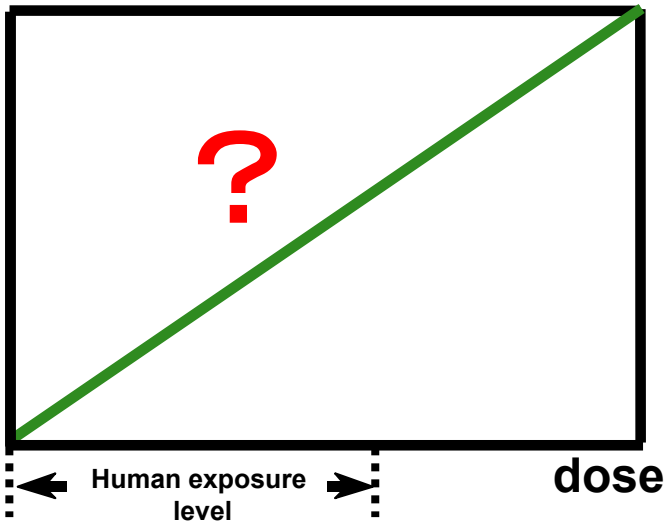
- Genotoxic or non-genotoxic
- Natural or synthetic
- Cooking process, contamination, or synthesis in the body
- Avoidable or unavoidable
- Human intake,1.5 g/day (B. Ames)

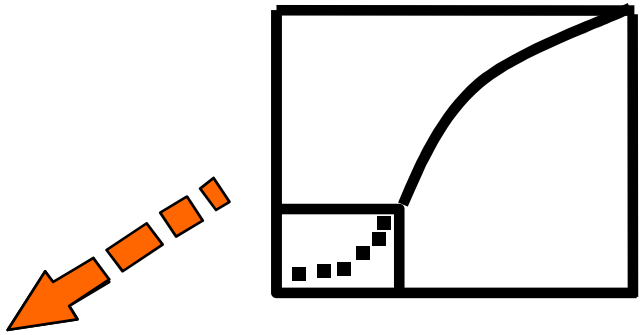
Genotoxic

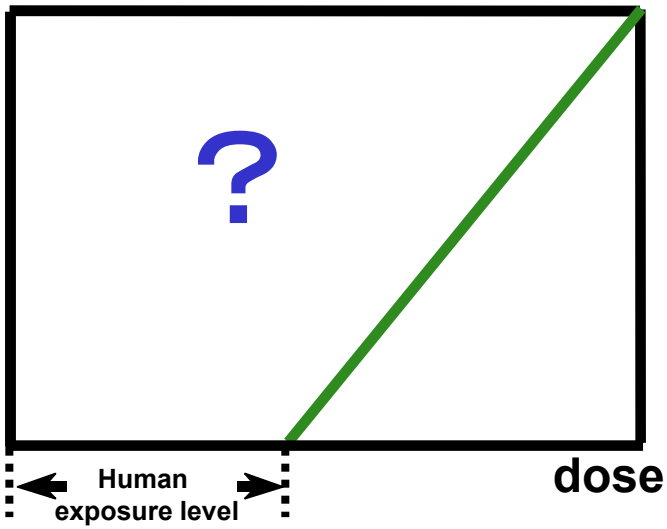
Non-genotoxic

Present concept of chemical carcinogenicity

Low-dose carcinogenicity curve of genotoxic (mutagenic) carcinogens: Extrapolation from high to low doses







- 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. There are, however, limited date available for these hypothesis.
- It has been argued that non-threshold theory is challenged based on the view that organism possess biological responses that can be ameliorate genotoxic activities.
- Therefore, it is important to resolve this question from the view point of cancer risk assessment and management.

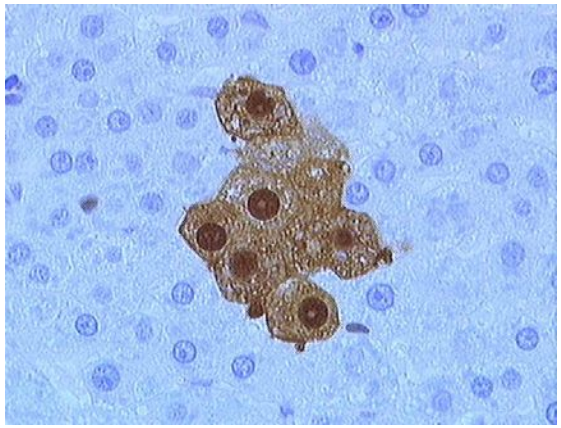
Merit of a medium-term bioassay for carcinogens

Normal tissue → Preneolastic lesion → Benign tumor → Cancer

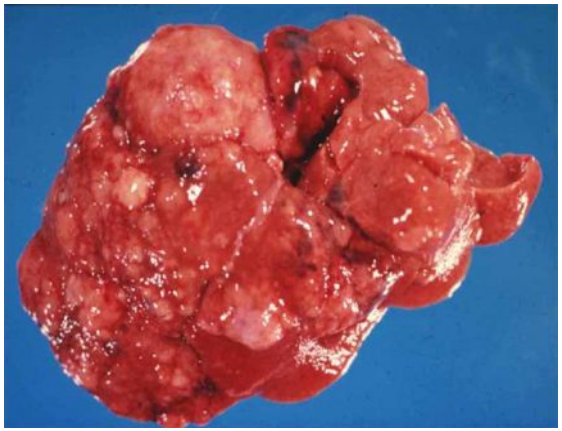
Liver medium-term bioassay

Carcinogenicity test

Liver

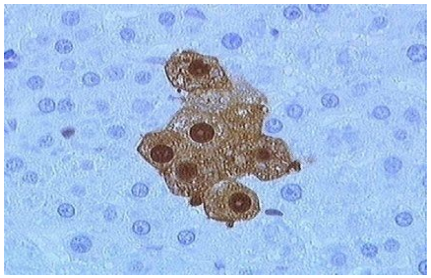


Number-Area / unit of glutathione *S*-transferase placental form (GST-P) positive foci

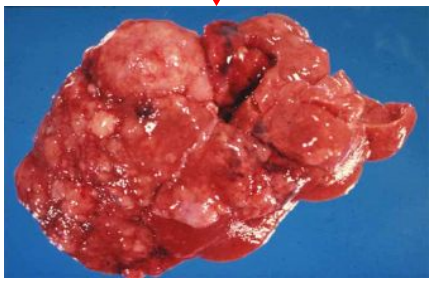


Incidence of tumors

Chemical carcinogenesis mechanism



GST-P positive foci



Cancer

Carcinogen

Inactivation

Non-DNA

Metabolic activation : ultimate carcinogen

Initiation

DNA adduct formation
Oxidative stress

DNA repair

DNA repair error

Cancer-irrelative mutations

Mutation: irreversible change

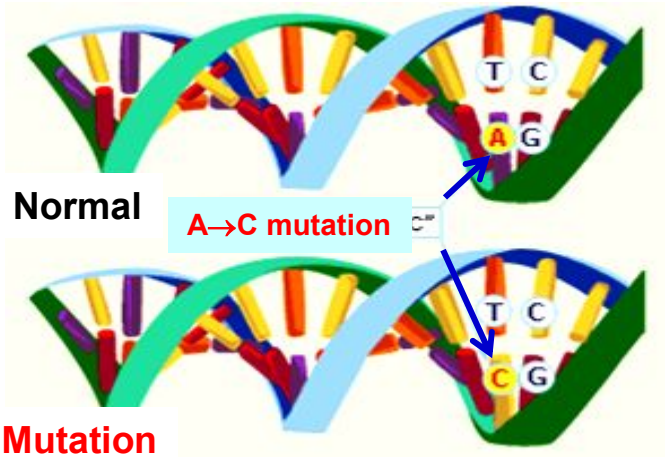
Promotion

Apoptosis

Preneoplasia : cell proliferation

Apoptosis

Cancer: malignancy



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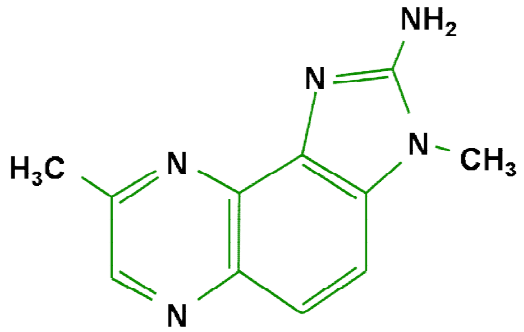
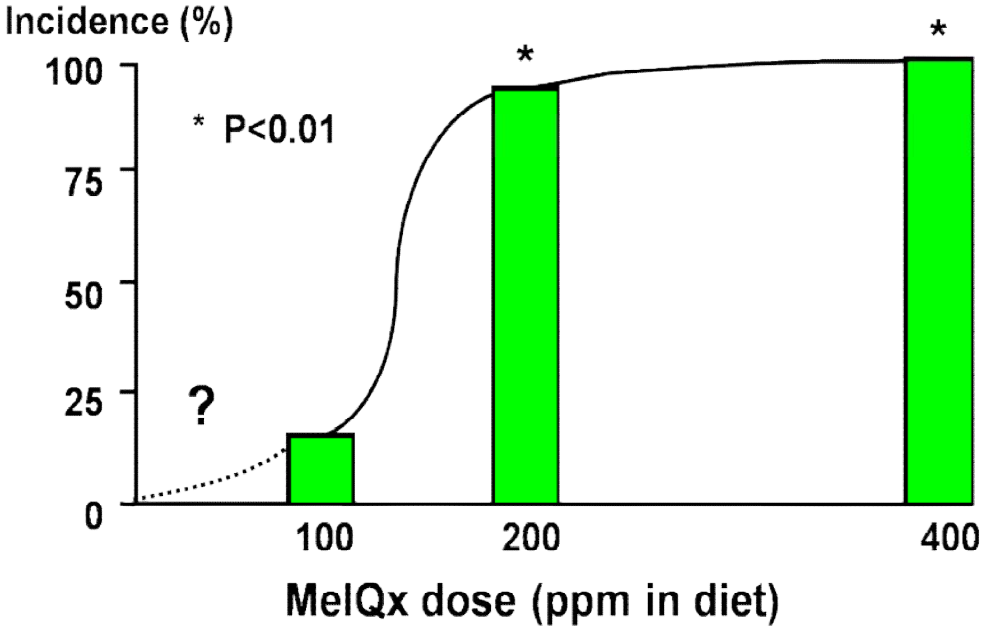
MeIQx



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

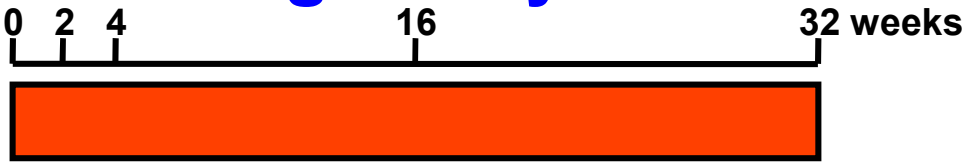
- **One of heterocyclic amines**
- **Exists in well-cooked fish and meat**
- **Mutagenicity: positive**
- **Hepatocarcinogen**
- **Human exposure level：0.2-2.6 μg/day**



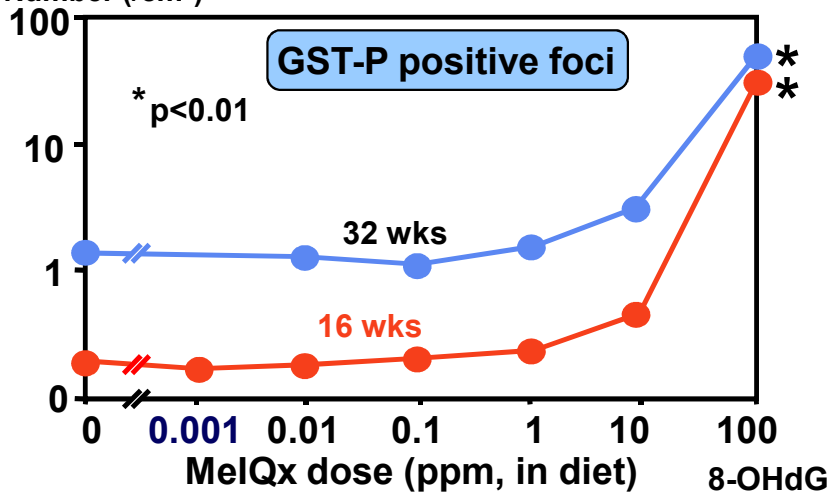
MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline

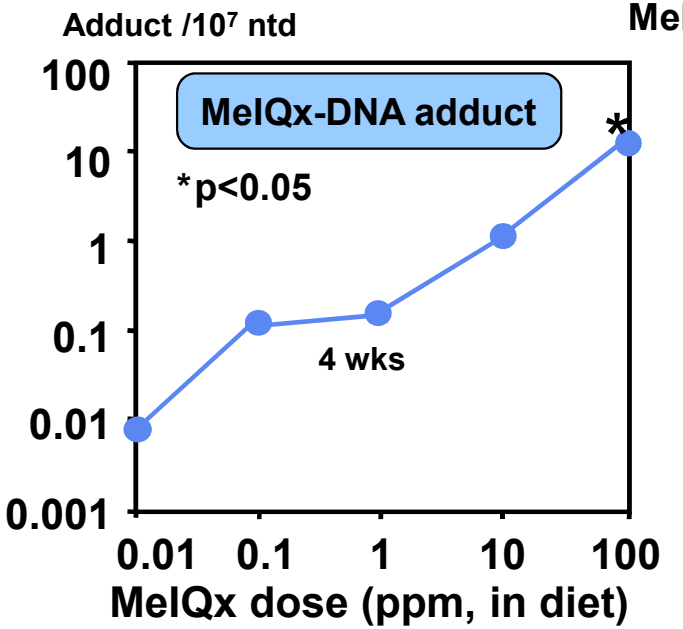
(Wakabayashi et al, 1995)

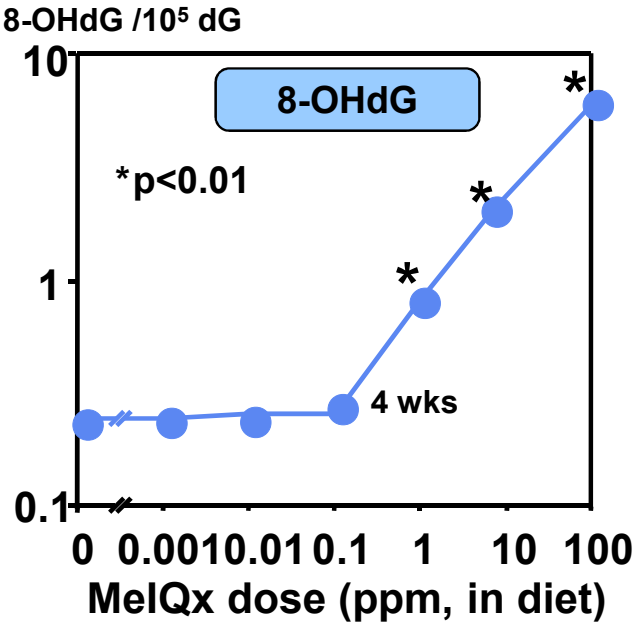
Rat hepatocarcinogenicity of MeIQx at low doses



Animals : 1,180 male F344 rats, 21-day-old







MeIQx

Big Blue Rat

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

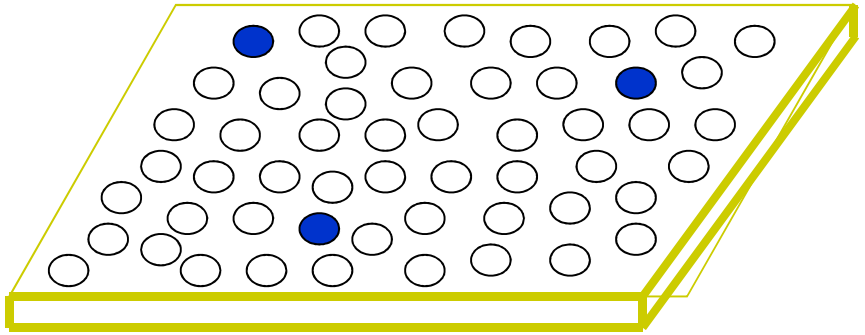
Liver

DNA isolation

in vitro packaging

Phage

Infection E.coli
Incubation containing X-gal



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

Mutation in the lacI gene

lacI lacO αlacZ

Transcriptional

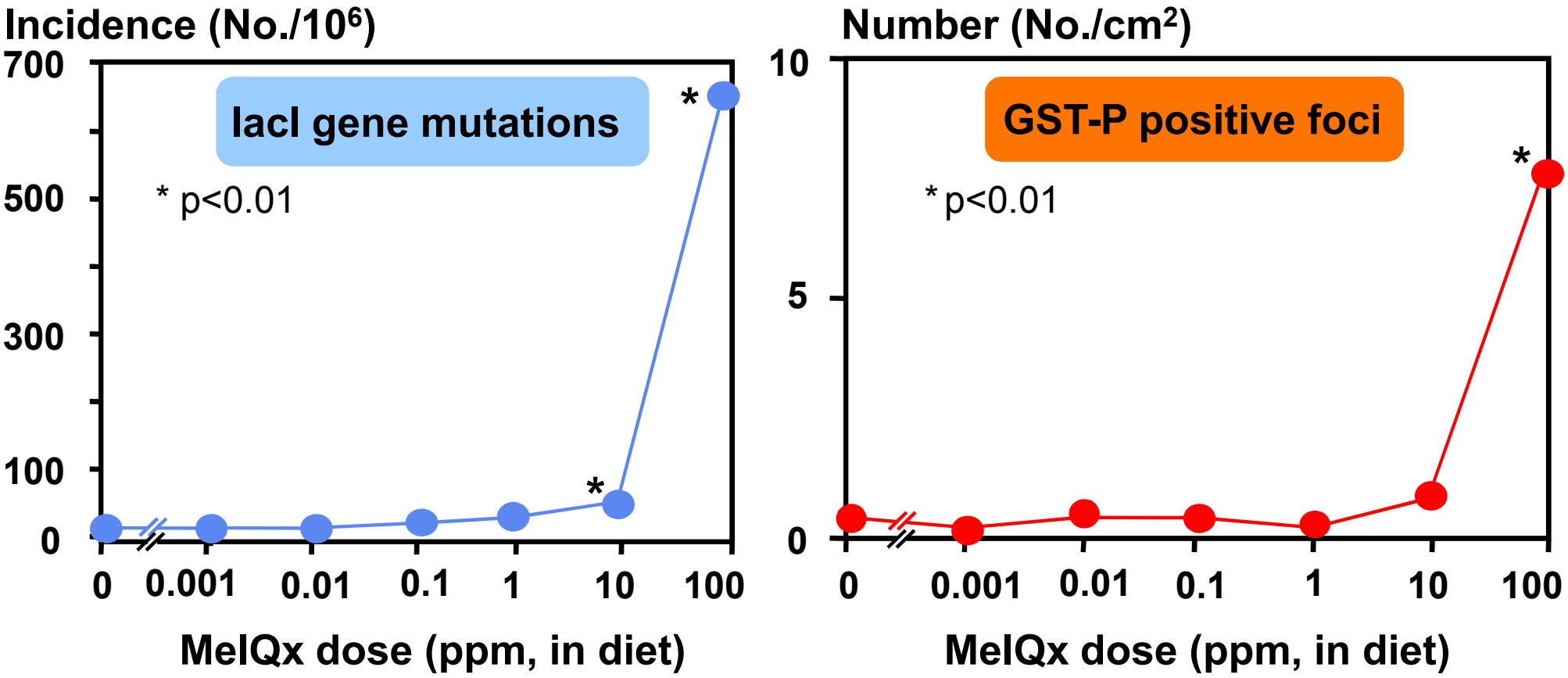
βgalactosidase

Repressor X-gal → Blue

In vivo mutagenicity test in Big Blue rats

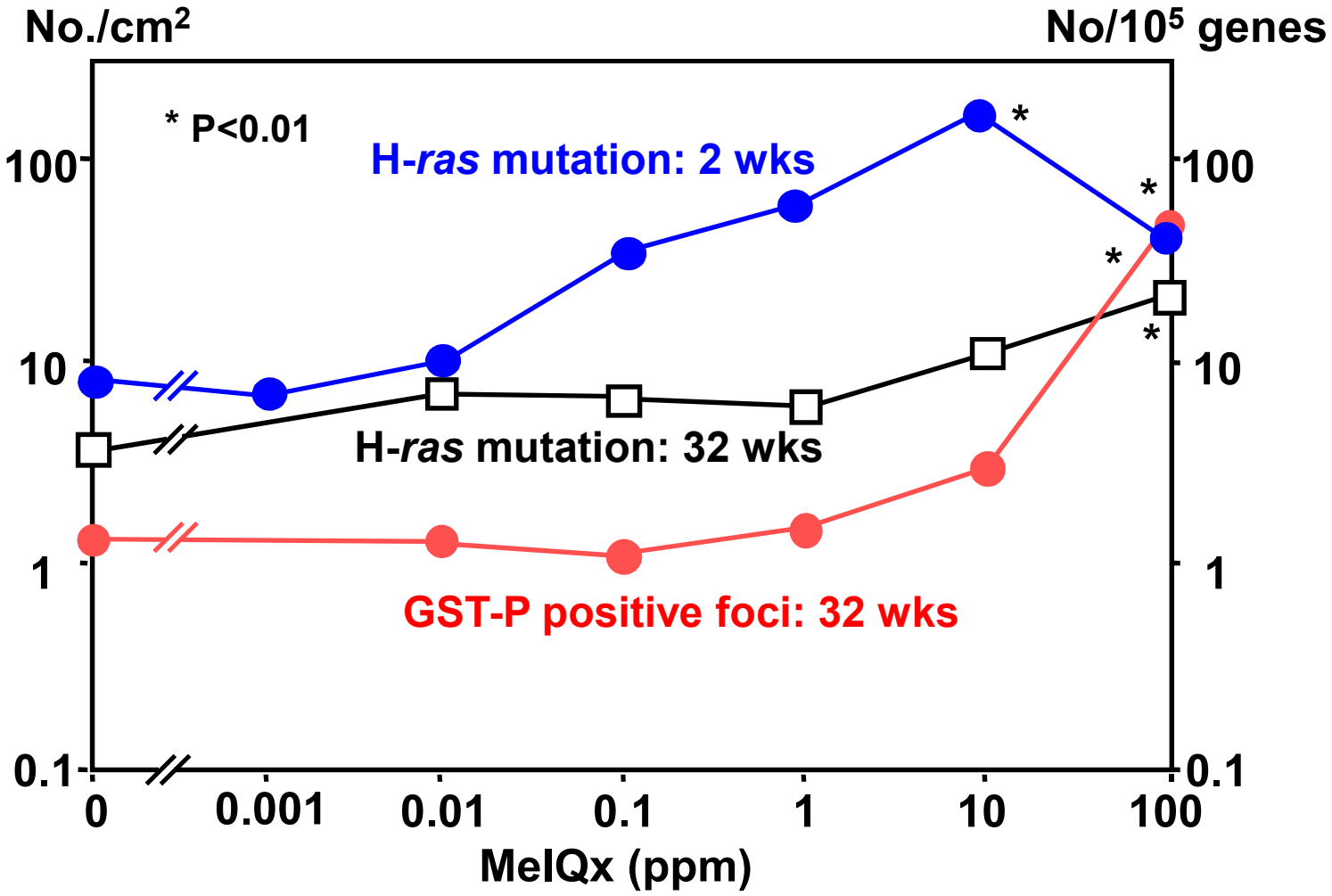
(Plaque Color Screening Assay)

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



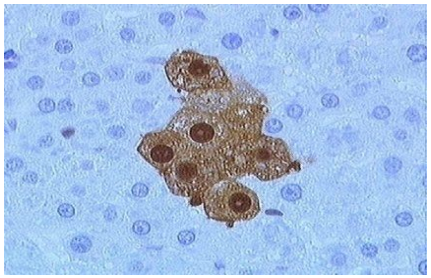
lacI gene: 30~40 copies on chromosome 4 in the F344 rat

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

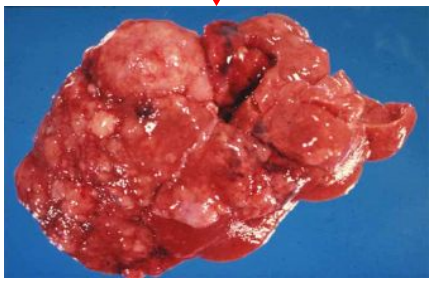


Detection of H-*ras* mutation:
Thermosequenase cycle end labeling (TCEL) method

Chemical carcinogenesis mechanism



GST-P positive foci



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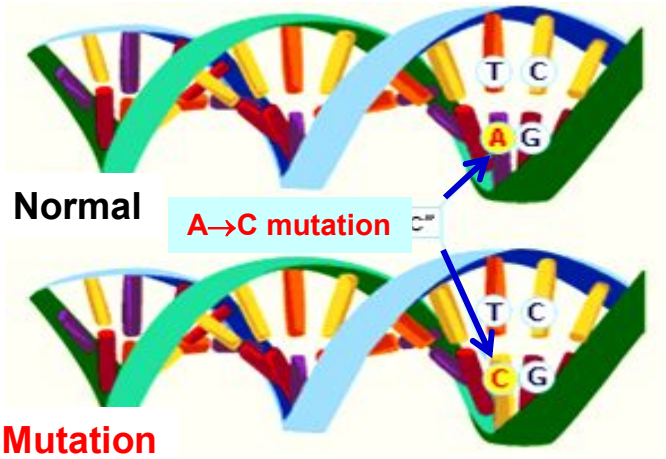

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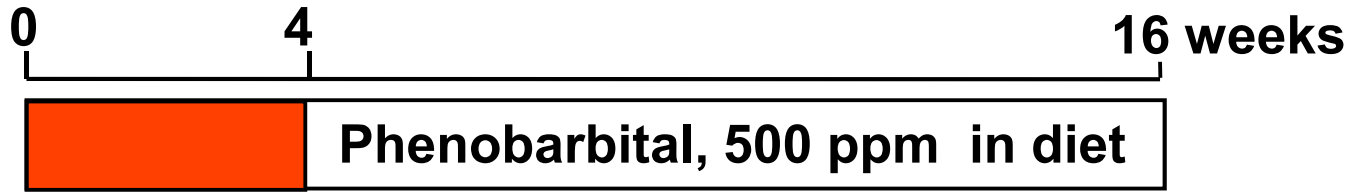
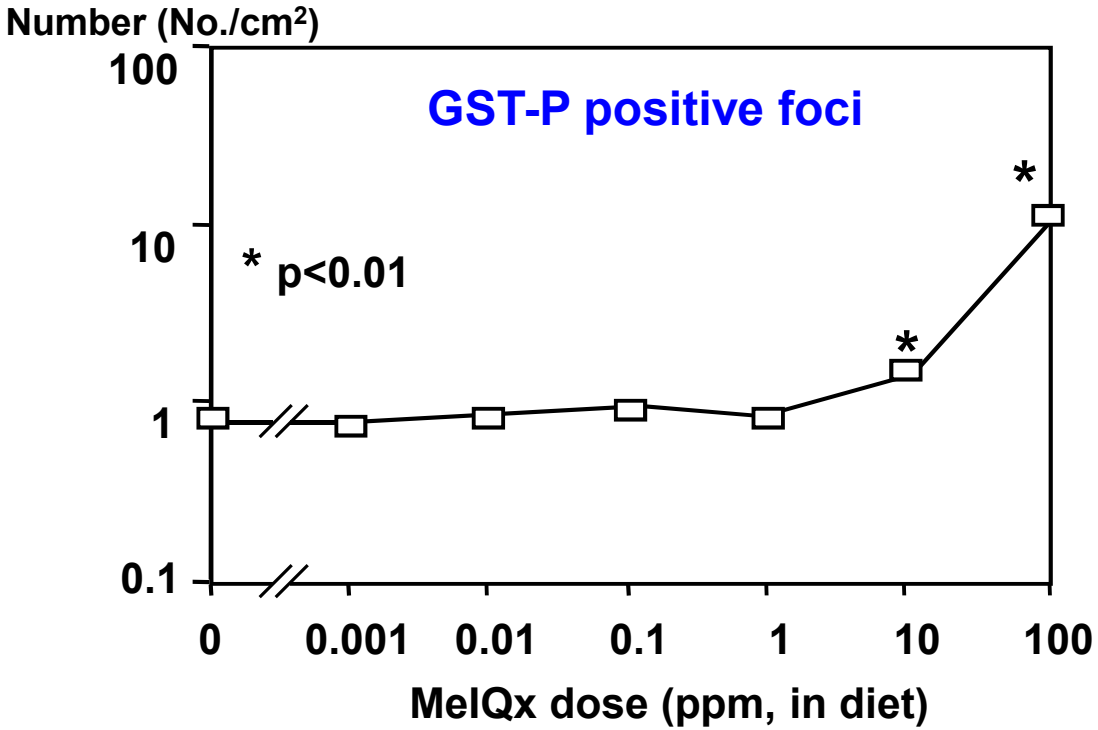
Initiation activity of MeIQx at low doses in the rat liver

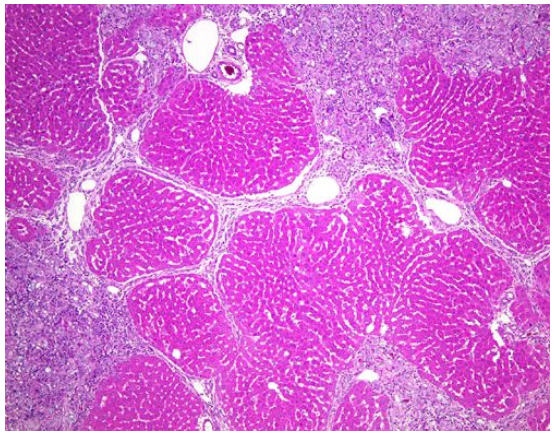


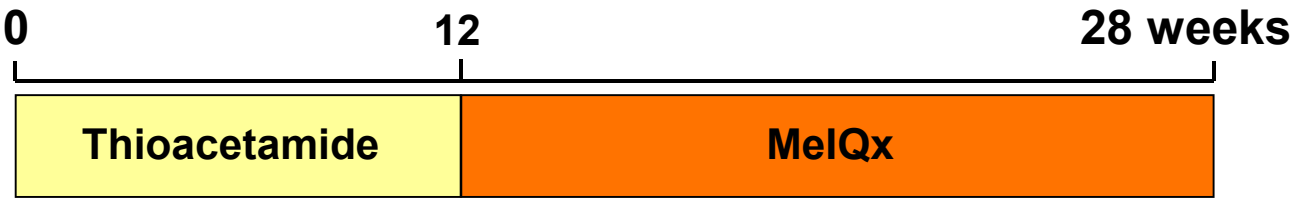
Animals: 850 male F344 rats, 21-day-old

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

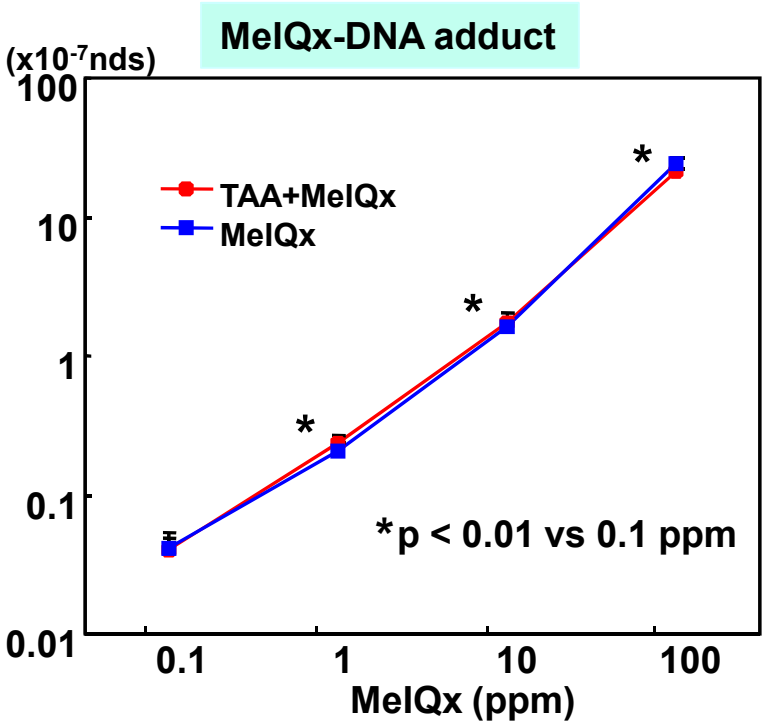


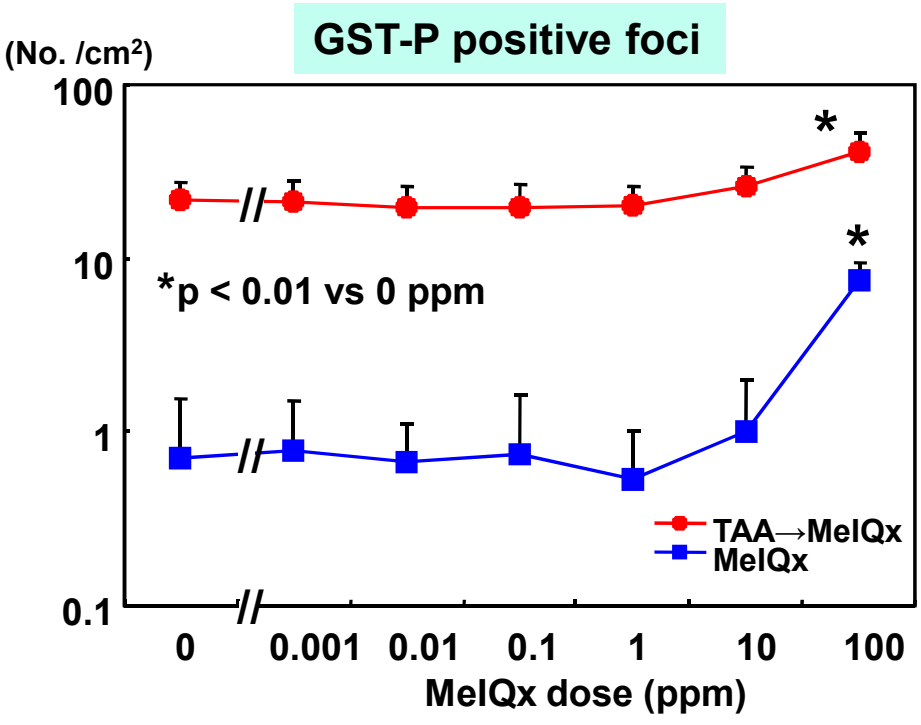
MeIQx DNA adduct level and number of GST-P positive foci in the damaged liver of rats



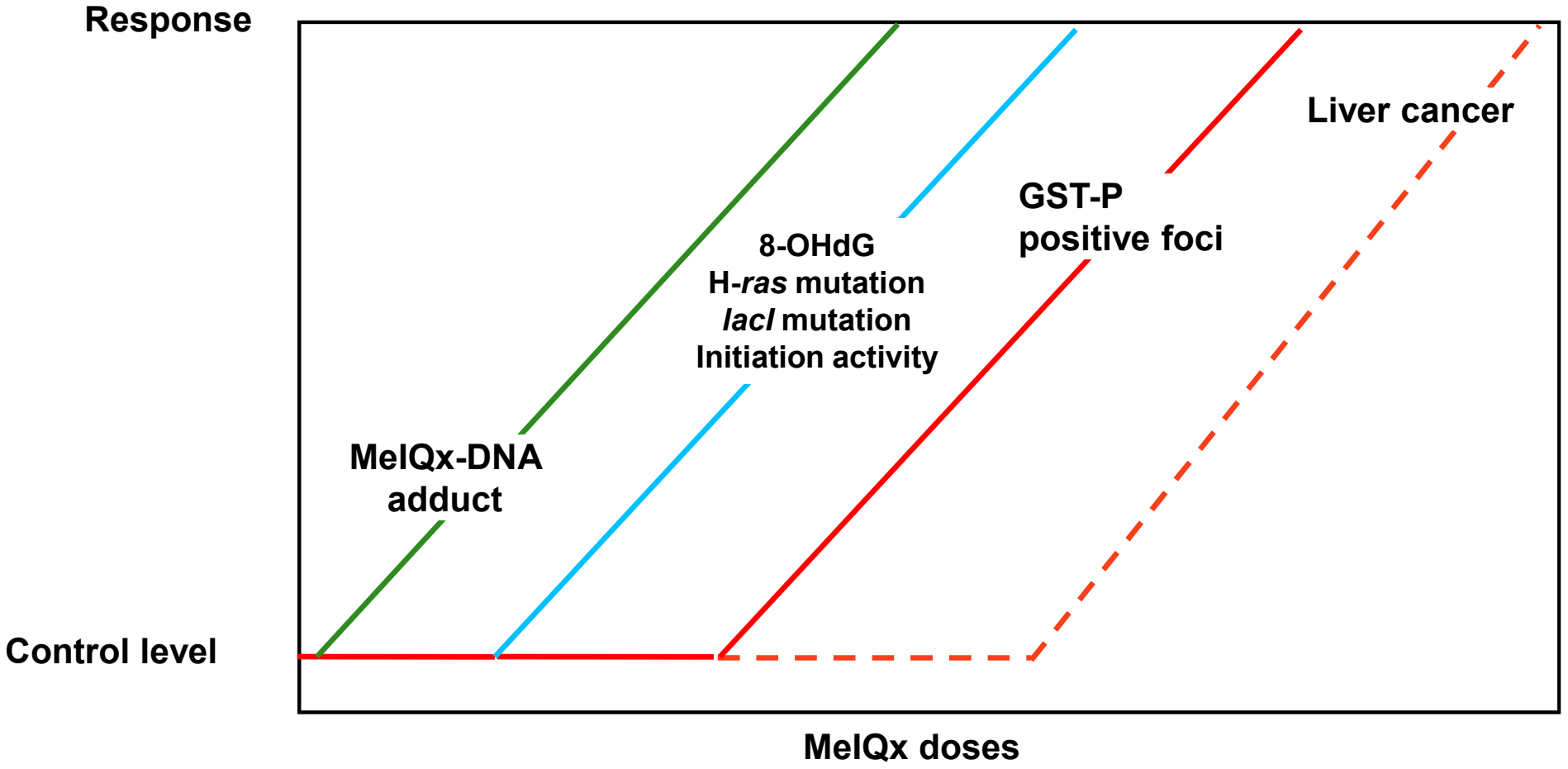


Animals: 280 male F344 rats, 21-day-old





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



Conclusion: Existence of a carcinogenic threshold, at least a practical threshold

Assessment of genotoxic carcinogens at low doses

Effects on various organs

Liver, Colon, Kidney

Effects on various biomarker

1. Carcinogen-DNA adduct
2. *In vivo* mutagenicity

   Mutation frequency of lacI or gpt gene
3. Oxidative DNA damage: 8-OHdG
4. Preneoplastic lesion

   Liver: GST-P positive foci

   Kidney: atypical tubular hyperplasia

   Colon：Aberrant crypt foci (ACF)
5. Tumor

Weights of evidence

Effects of IQ on development of GST-P positive foci and DNA adduct formation in livers of rats

Animal: 1,560 male F344 rats, 21-day-old

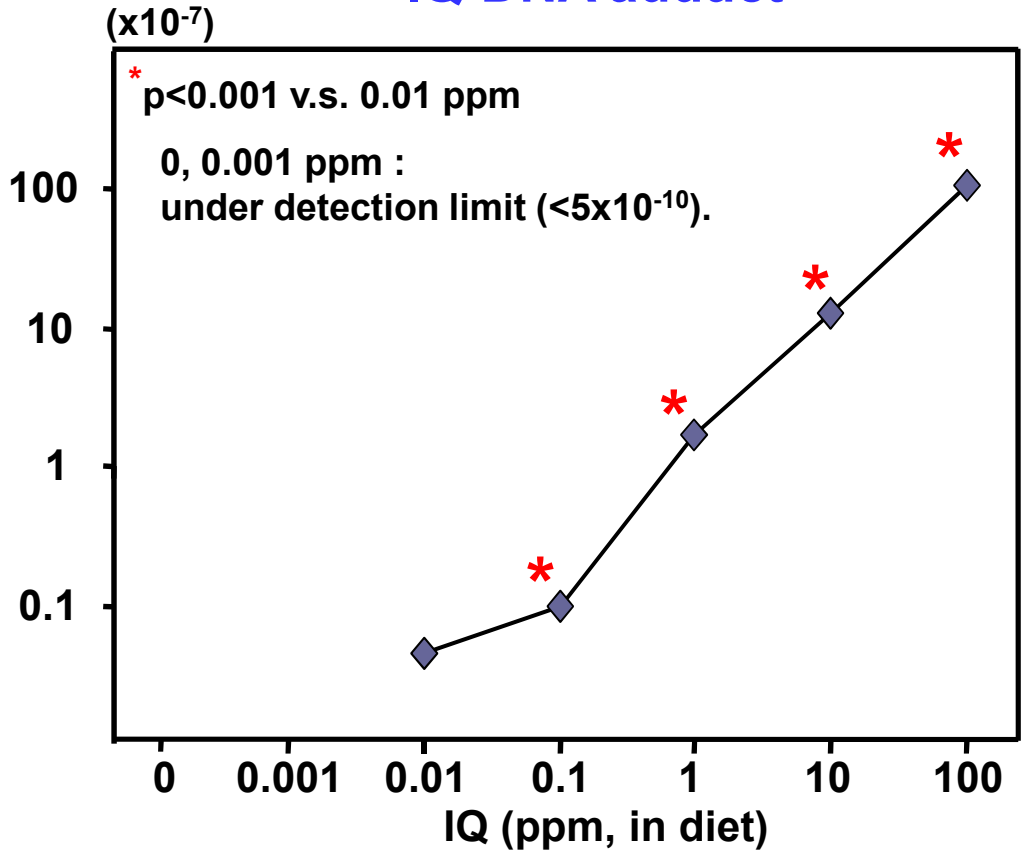
IQ : 0, 0.001, 0.01, 0.1, 1, 10, 100 ppm in diet

GST-P positive foci

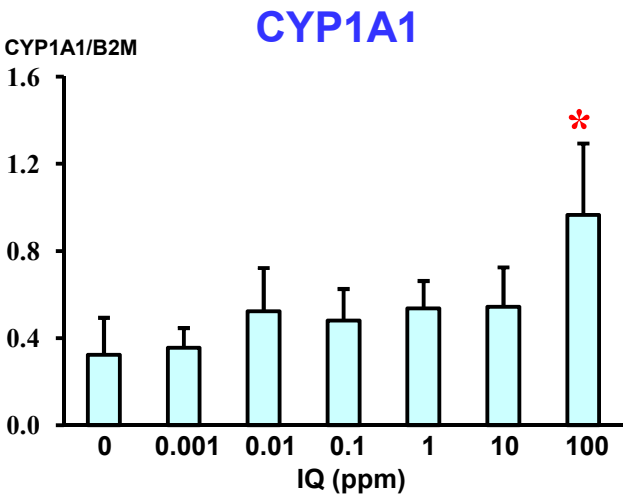
IQ (ppm)	No. of rat	GST-P positive foci (No./cm^2)
0	240	0.15 ± 0.31
0.001	240	0.16 ± 0.31
0.01	240	0.26 ± 1.30
0.1	240	0.15 ± 0.35
1	240	0.14 ± 0.33
10	240	0.74 ± 0.88 *
100	120	88.03 ± 50.41 *

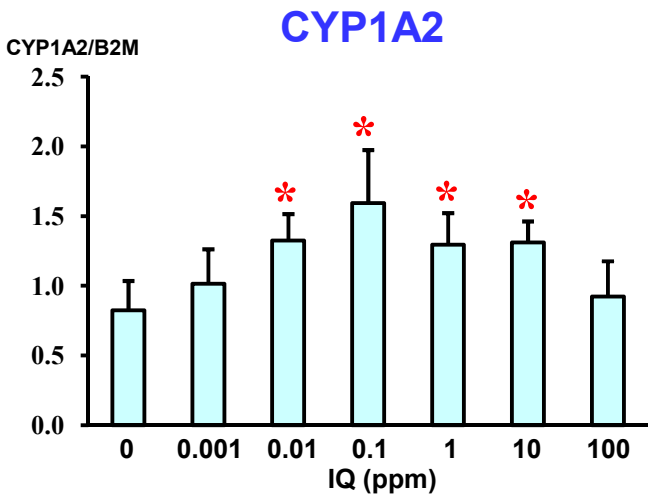
* p<0.01 v.s. 0 ppm

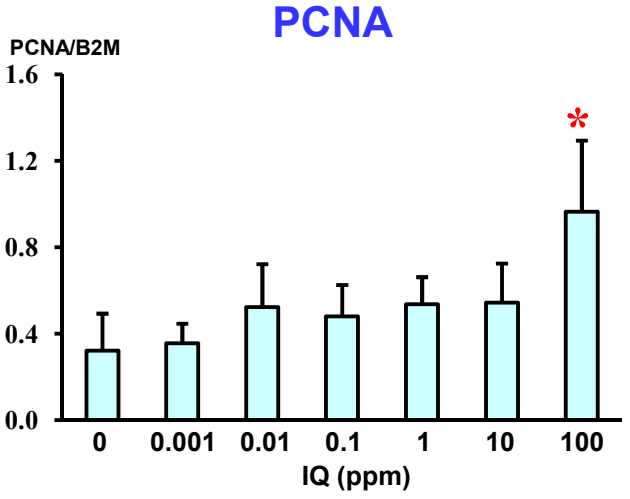
IQ-DNA adduct

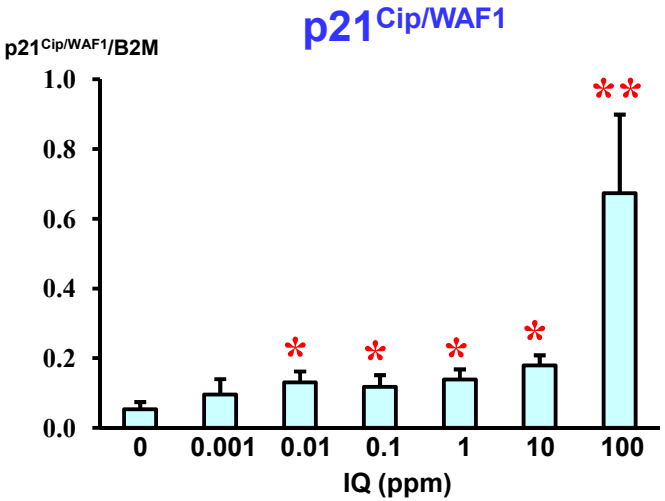


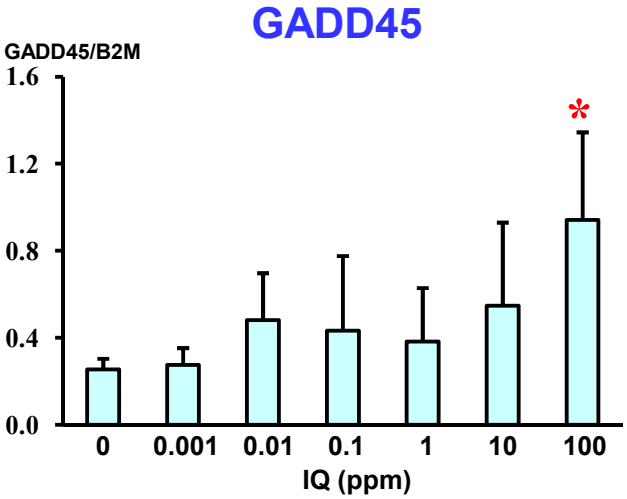
mRNA expression in liver of IQ-treated rats at week 16

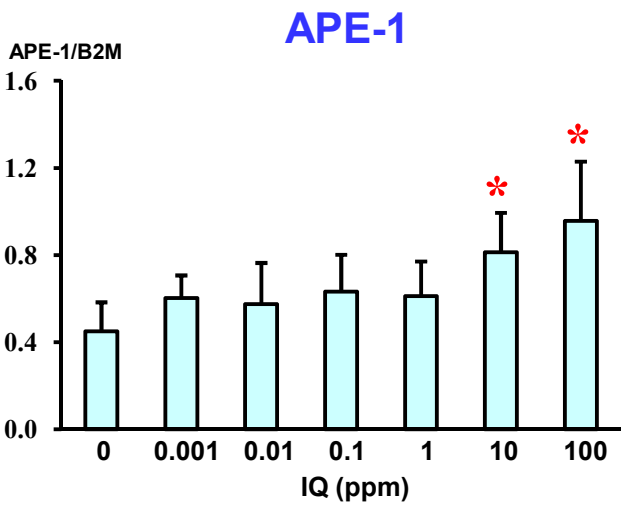






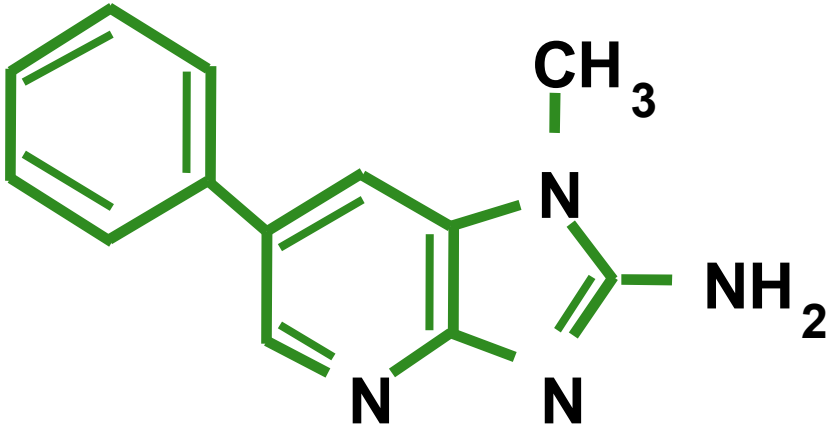






*p<0.05 v.s. 0 ppm

PhIP



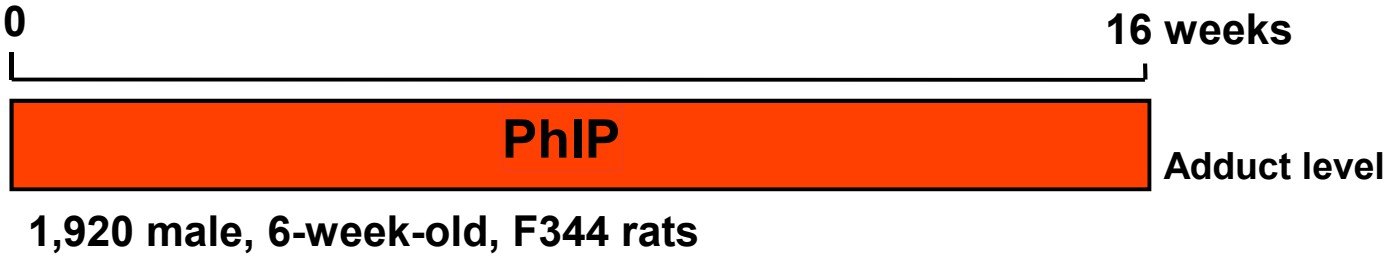
One of food-derived heterocyclic amines

Mutagenicity: positive

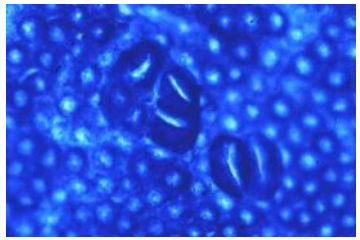
Carcinogenicity: colon

Daily intake: 0.005-0.3 μg/day

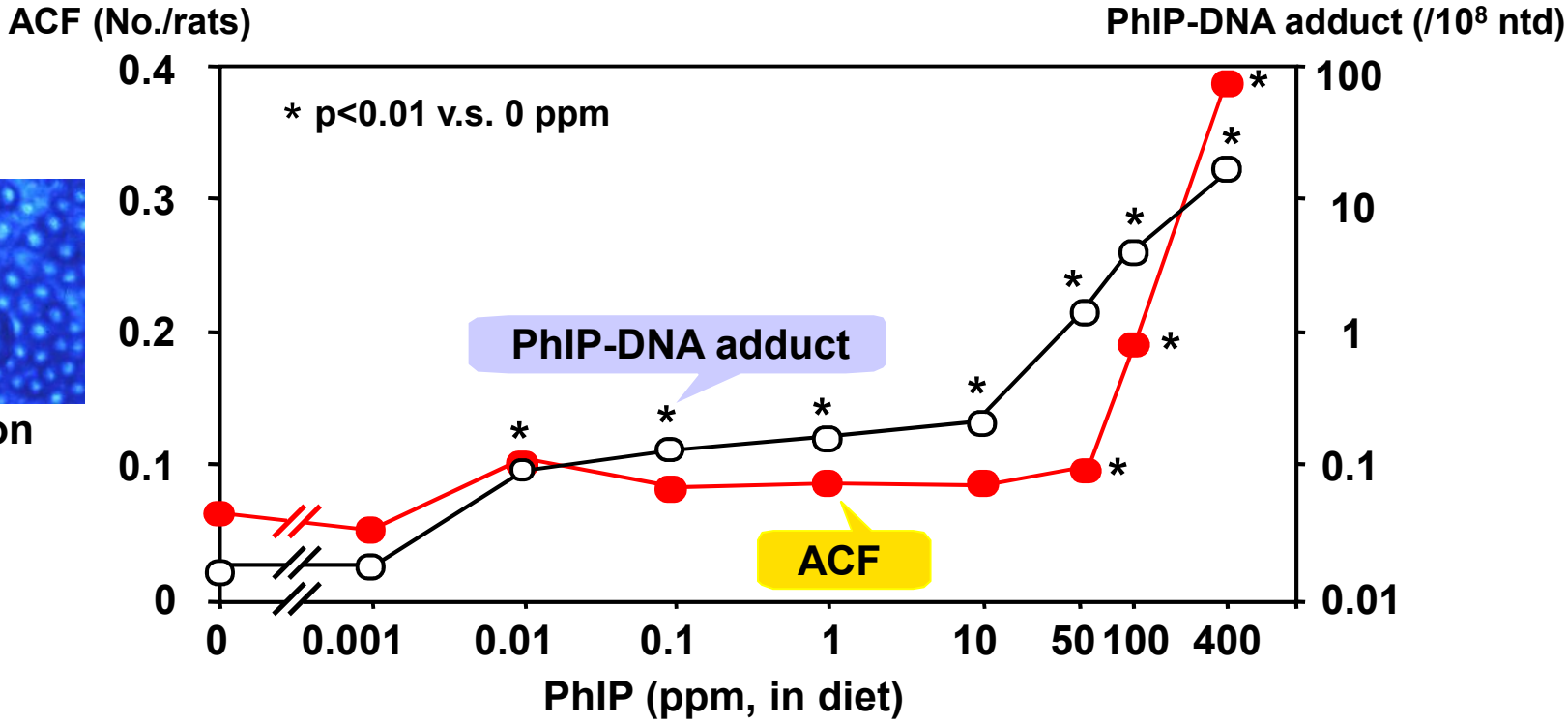
Rat colon carcinogenicity of PhIP at low doses: Aberrant crypt foci (ACF) and PhIP-DNA adducts



1,920 male, 6-week-old, F344 rats

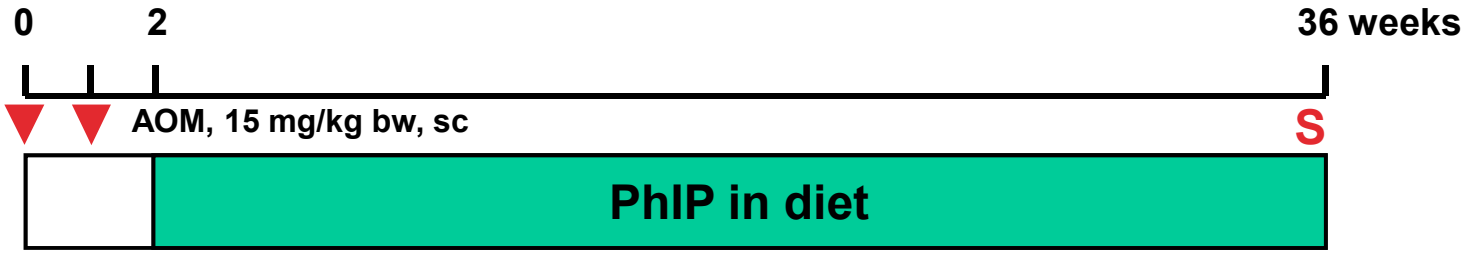


ACF in colon



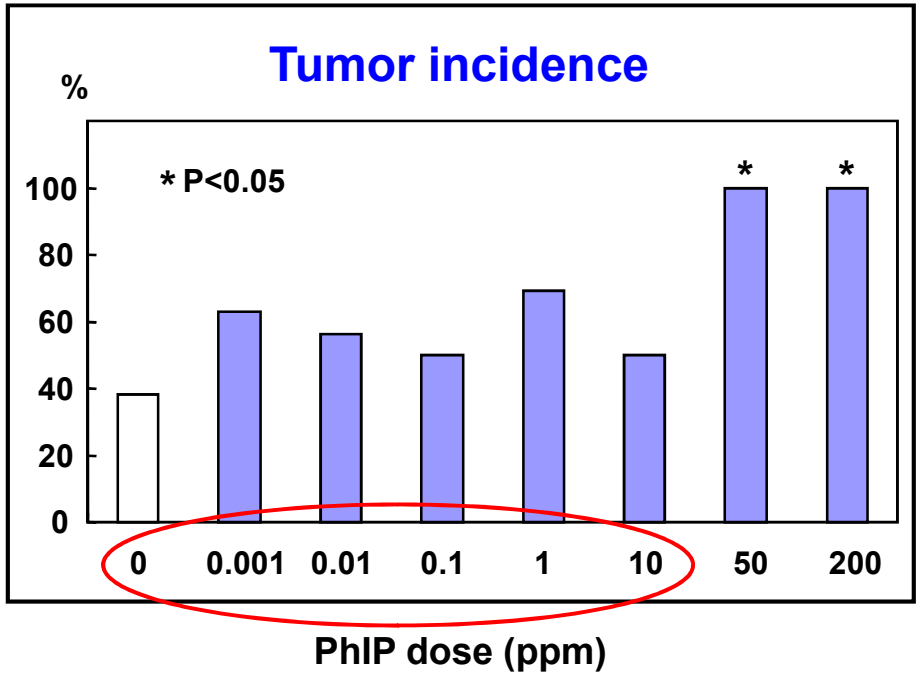
PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

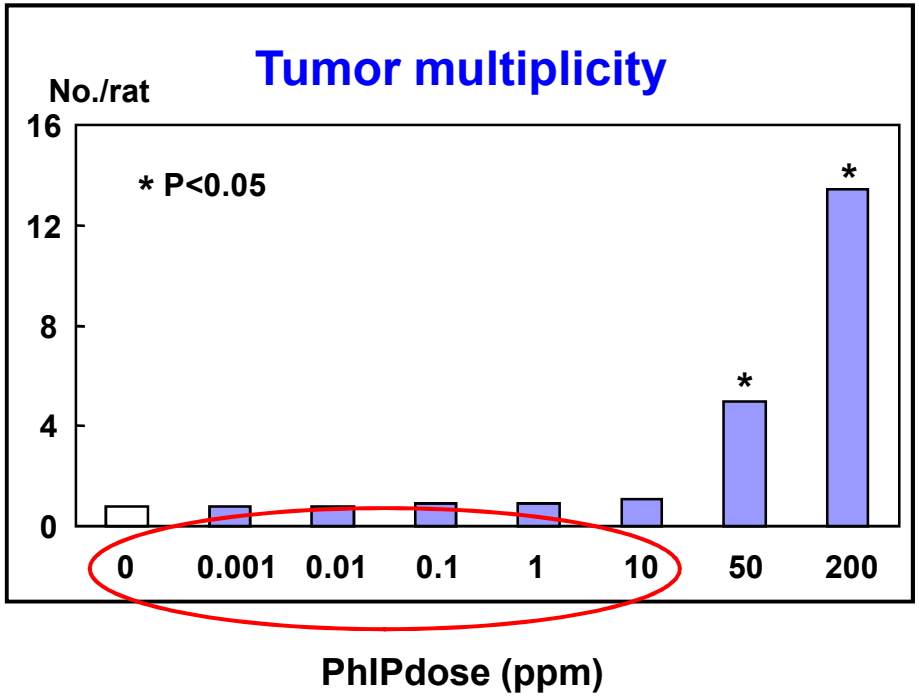
PhIP carcinogenicity in azoxymethane-initiated rat colon carcinogenesis



Animals: 192 male, 6-week-old, F344 rats

Tumor: Adenoma + Carcinoma

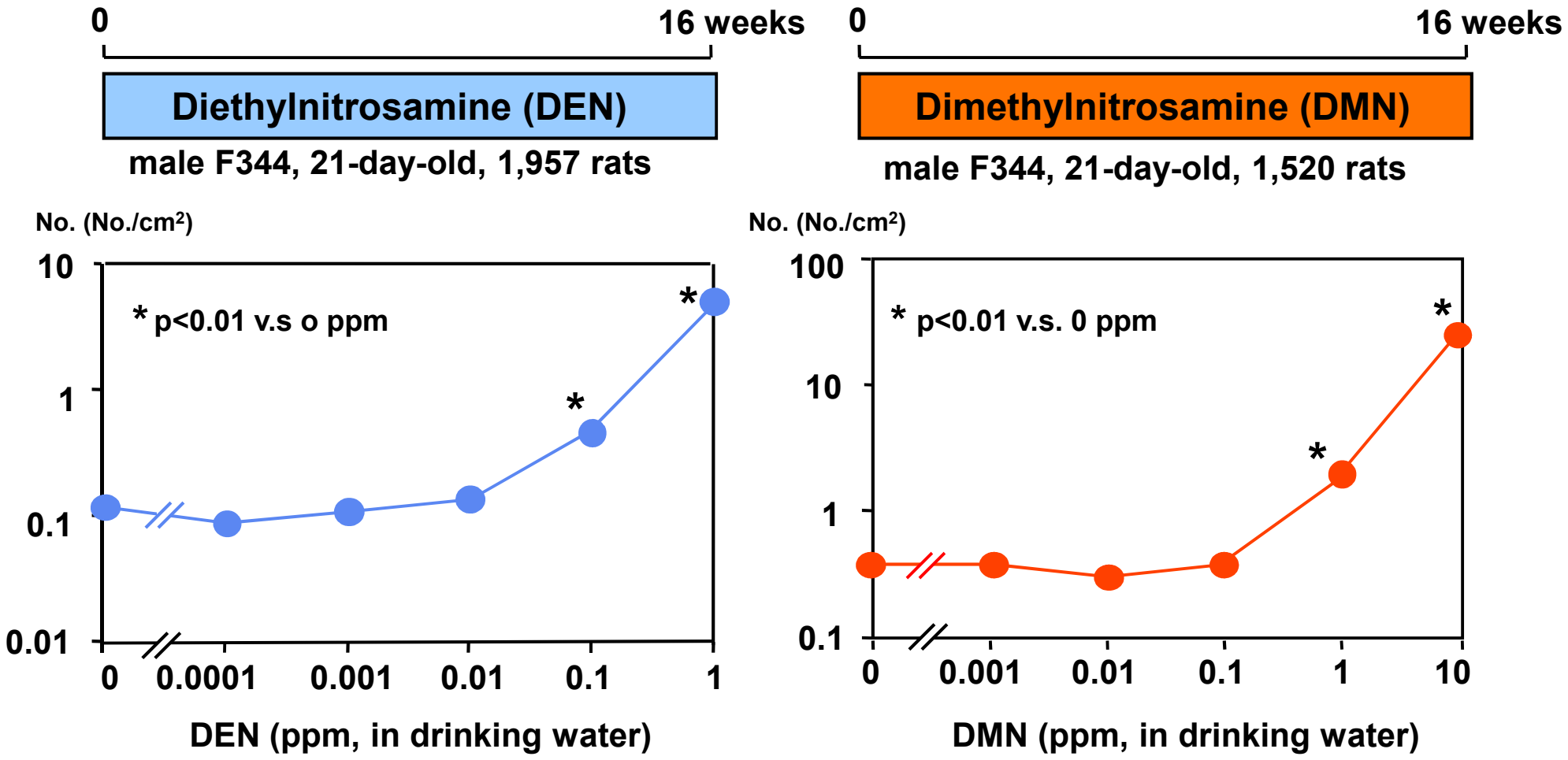




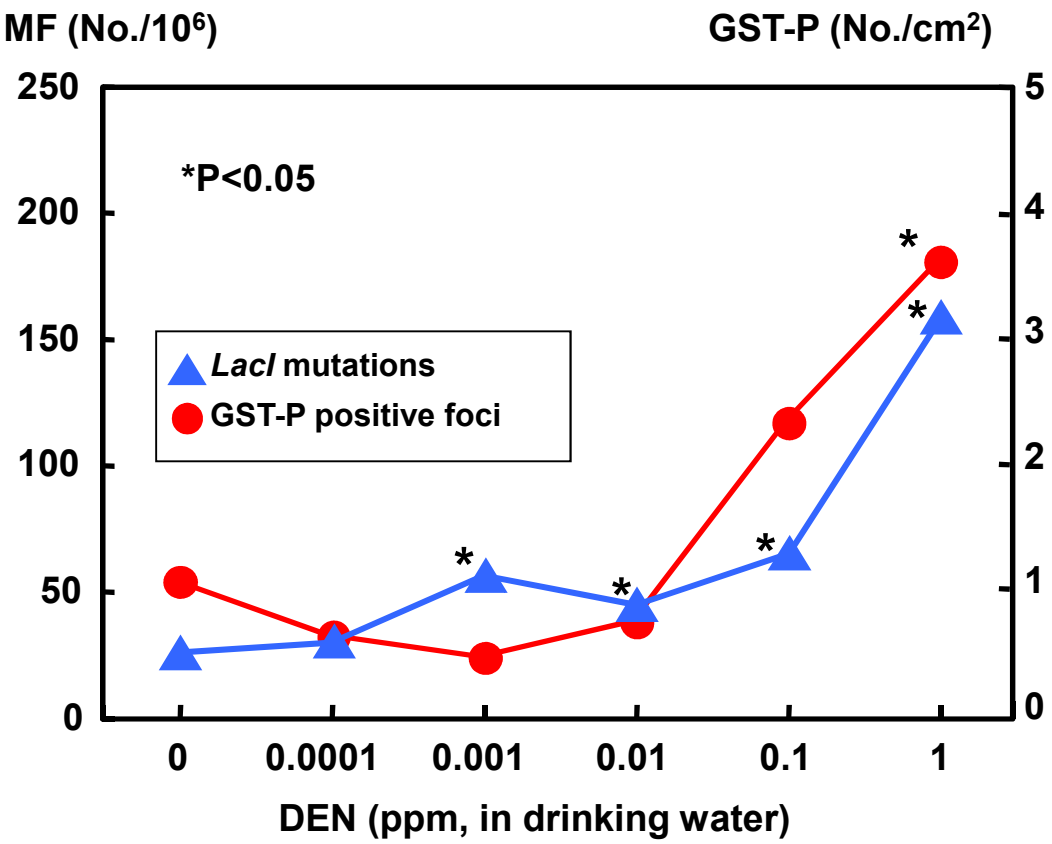
N-Nitroso Compounds

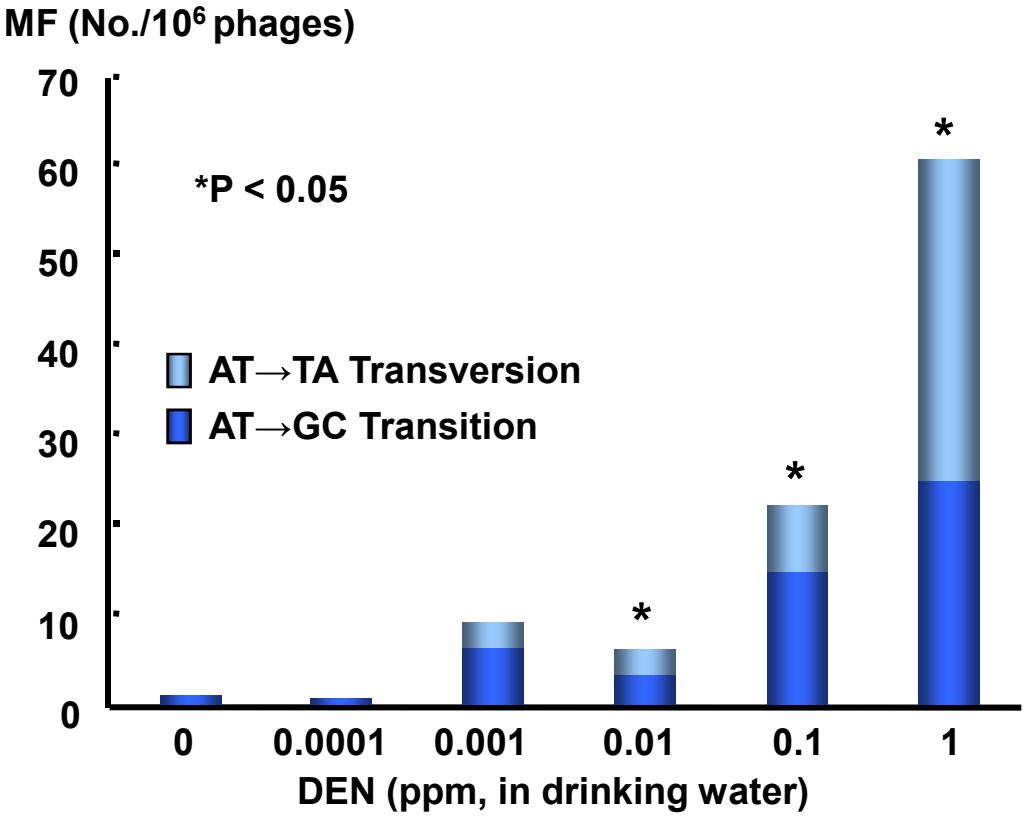
- Air, water, and food,
  notably in nitrite-treated meat and fish products
- *in vivo* formation from nitrites or nitrates
  and secondary amines
- DiethylNitrosamine
- DimethylNitrosamine
- Mutagen
- Hepatocarcinogen
- Daily intake : μg/day range level

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

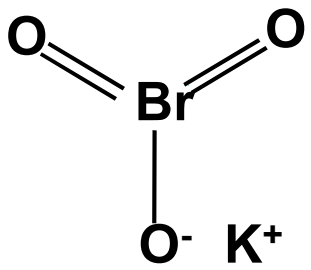


LacI mutation frequency and development of GST-P positive foci in the liver of Big Blue rats treated with DEN for 16 weeks





Potasium bromate



Potasium bromate
($KBrO_3$)

Food additive
Contaminant in tap water

Genotoxicity

- **Ames test: +**
- **Chromosome aberration test: +**
- **Micronucleus assay: +**

Renal carcinogenicity in rats

- **≥250 ppm: + (Kurokawa Y, 1983)**

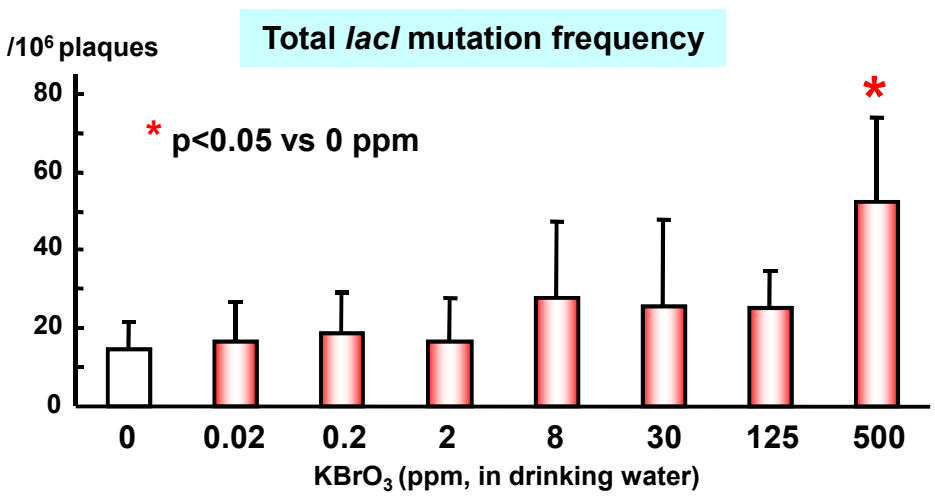
Mutation frequencies and oxidative DNA damage in kidney of Big Blue rats treated with potassium bromate

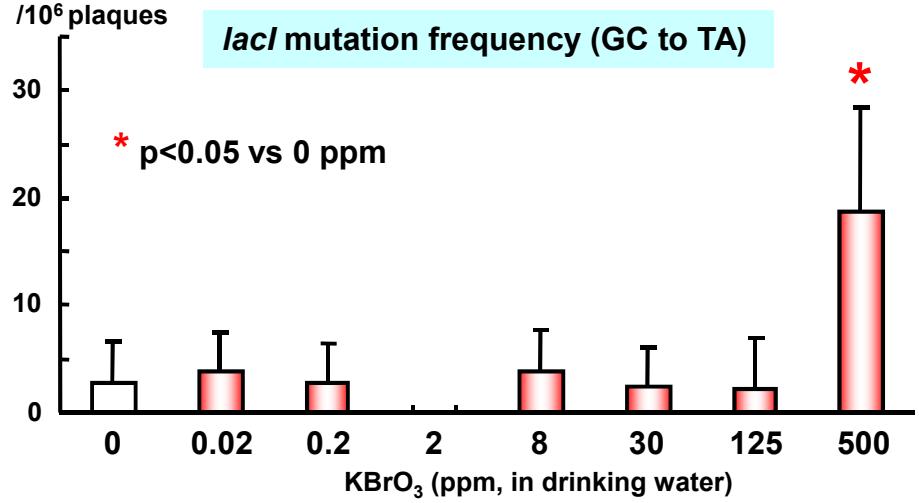
0 — 16 weeks

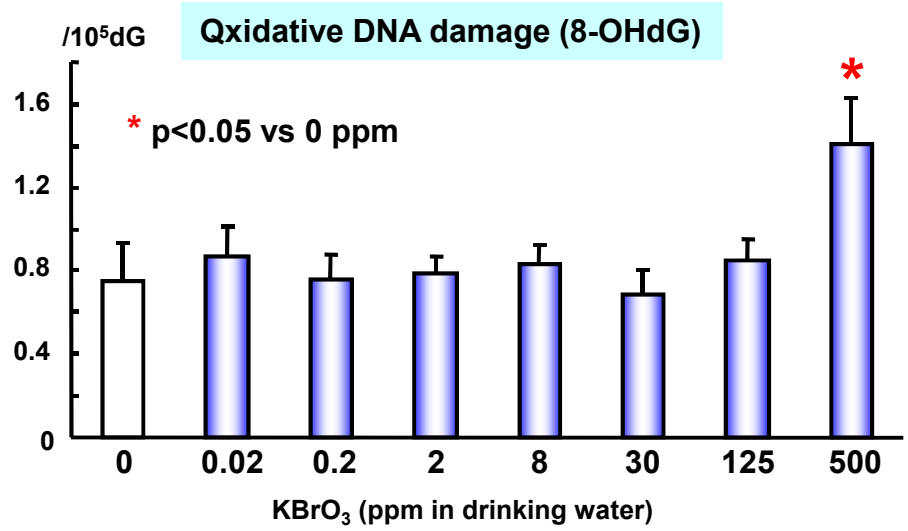
5 rats/group

$KBrO_3$, 0, 0.02, 0.2, 2, 8, 30, 125, 500 ppm

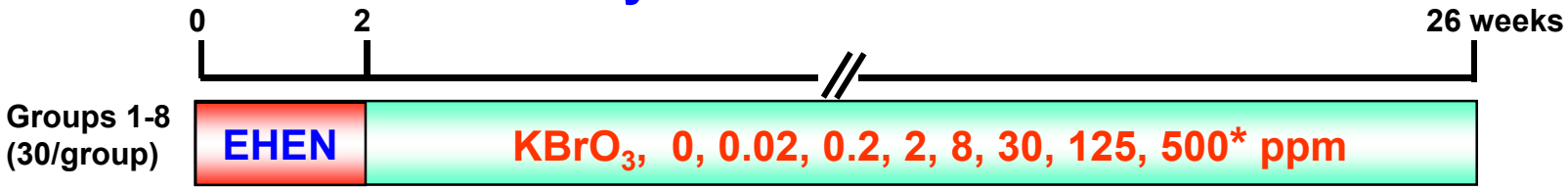
$KBrO_3$: potasium bromate







Promotion effects of $KBrO_3$ in kidney carcinogenesis induced by EHEN in Wistar rats

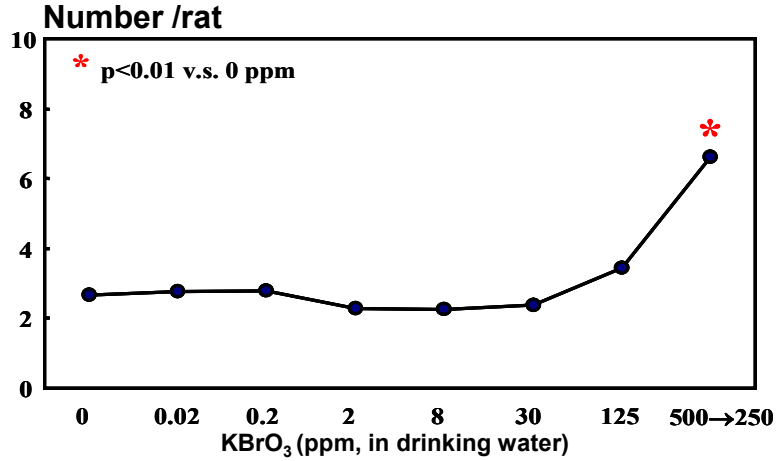


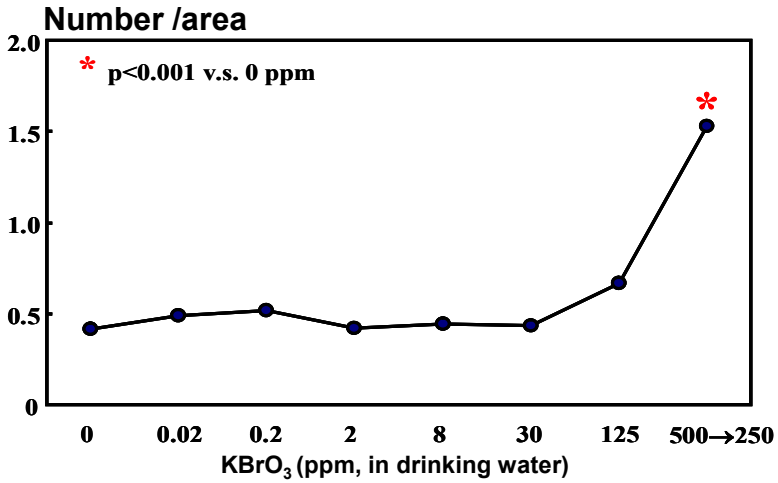
Animal: 240, 6-week-old, male Wistar rats

* 500 → 250 ppm from week 12

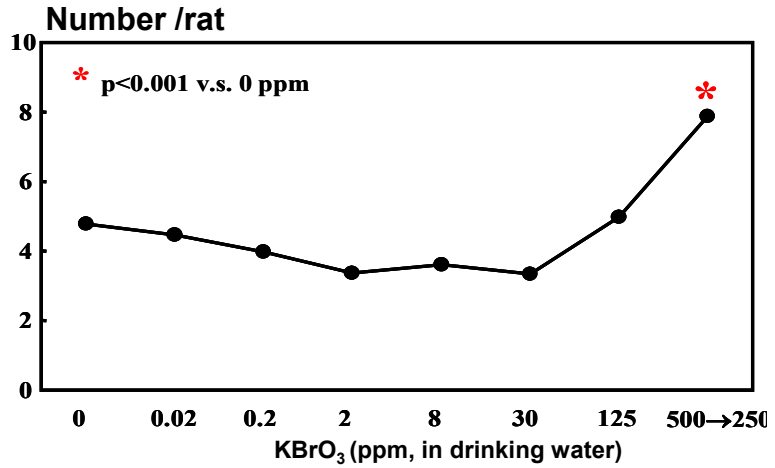
Kidney: 8 slices/kidney (16 slices/rat)

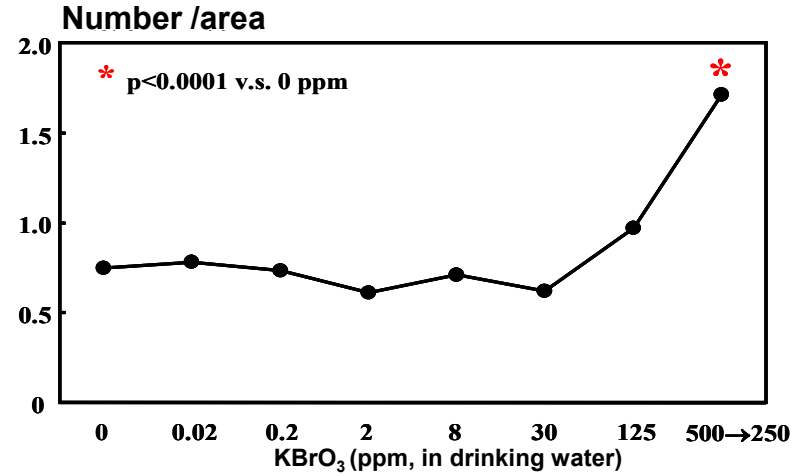
Renal tumor





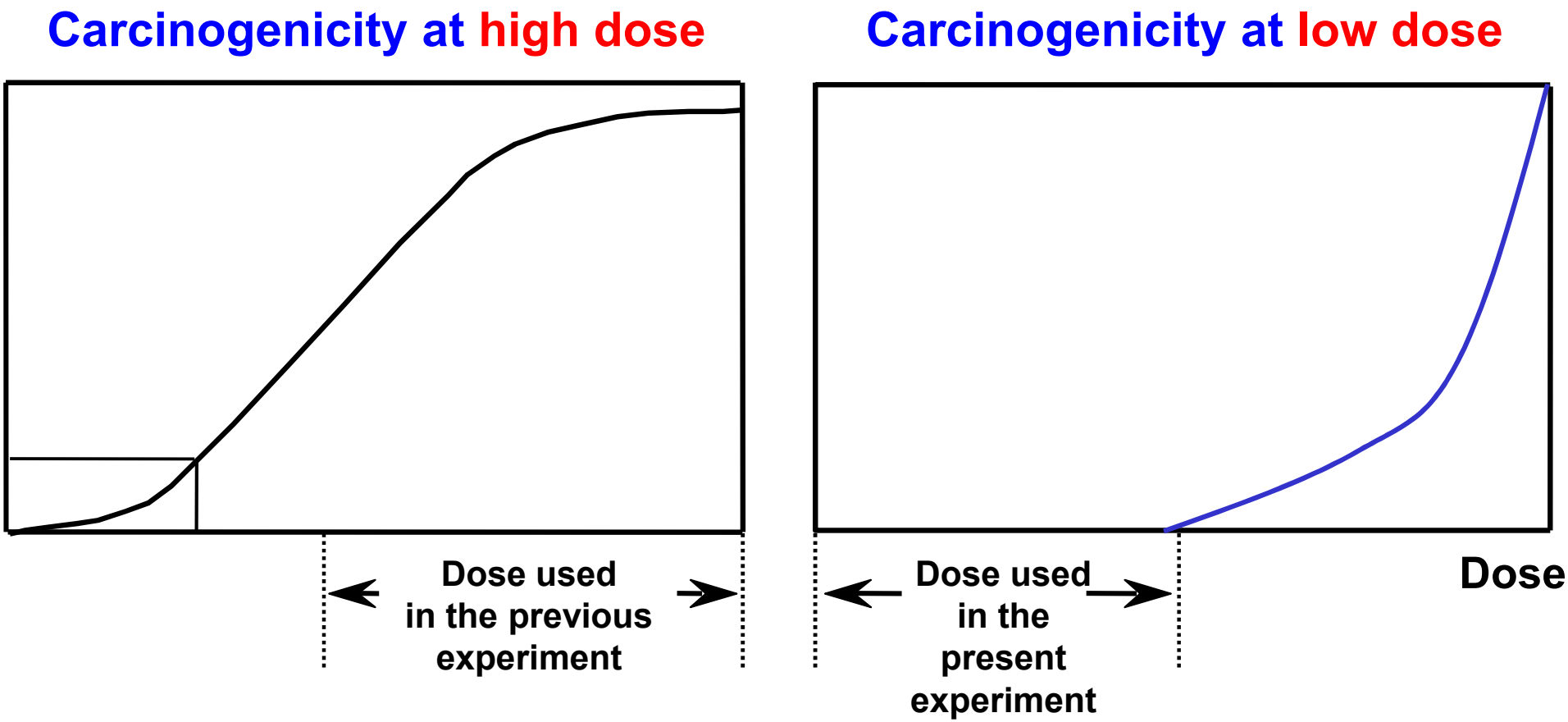
Atypical tubular hyperplasia





Conclusions

Response curves for the effects of genotoxic carcinogens dependent on the dose



Existence of threshold (practical or perfect)

Thresholds in carcinogenicity

Recently, the concepts of “practical” and “perfect” thresholds for genotoxic carcinogens have been proposed. In these cases, activities of carcinogens are usually associated with a no-observed effect level (NOEL).

Genotoxic carcinogens and thresholds

1. Primary mutagenic carcinogen

   → Practical threshold

   : Heterocyclic amines, *N*-nitroso compounds

2. Secondary mutagenic carcinogen

   → Perfect threshold

   : Potassium bromate

3. Primary or secondary mutagenic carcinogen, but carcinogenicity based on cytotoxic mechanism

   → Perfect threshold

   : 1,4-Dioxane

4. Genotoxic, but non-mutagenic carcinogen

   → Perfect threshold

   : Dimethylarsinic acid

Risk assessment for genotoxic carcinogens in near future

Since the threshold exists for genotoxic carcinogens, we should accept it for human risk assessment and management of environmental carcinogens, in particular for substances contained in food at low doses.

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