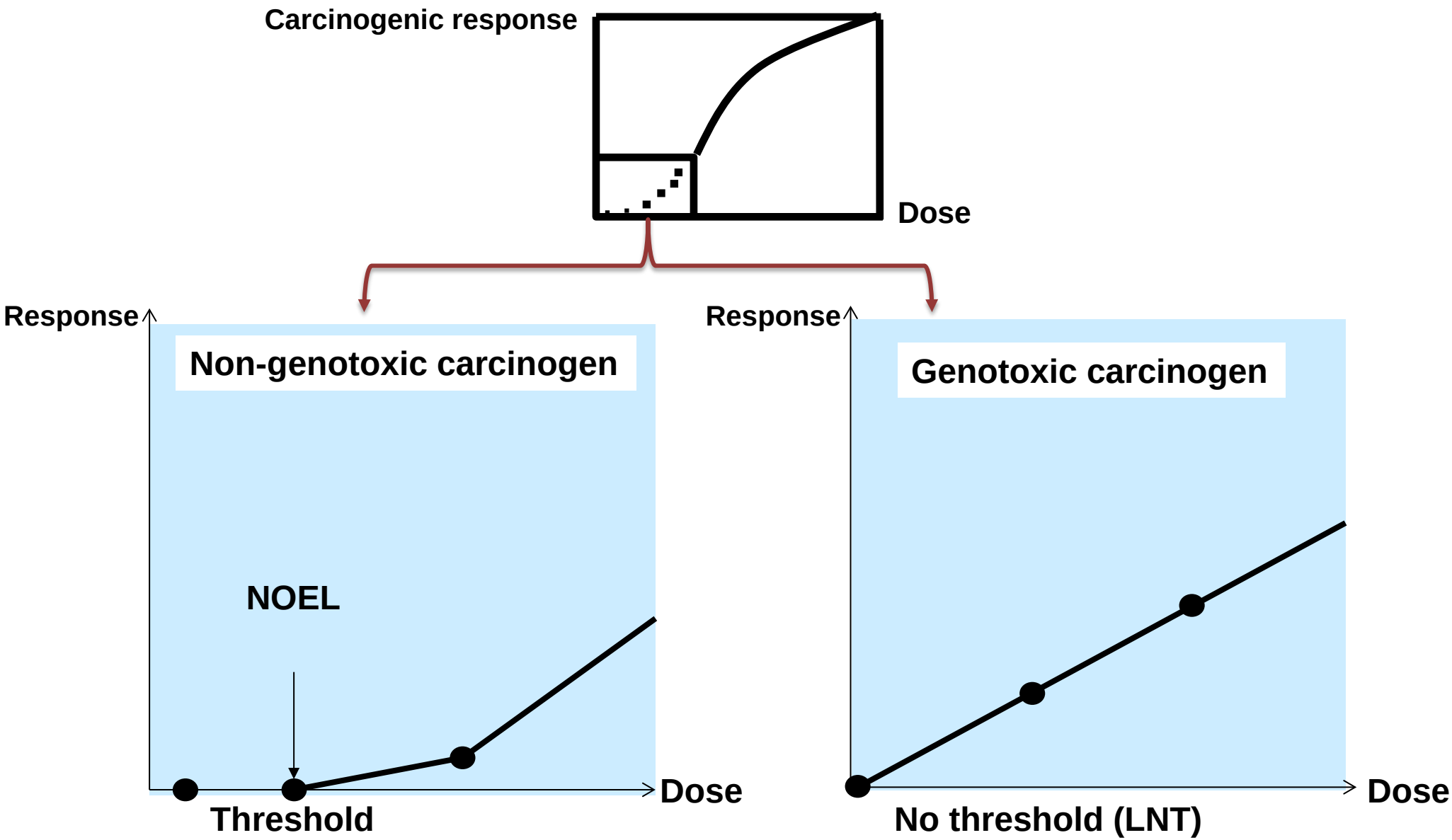
Qualitative and Quantitative Approaches in the Threshold of Genotoxic Carcinogens

Shoji Fukushima[1], Min Gi[2], Anna Kakehashi[2], Hideki Wanibuchi[2]

[1] Japan Bioassay Research Center, Japan Industrial Safety & Health Association, Hadano, Kanagawa 257-0015, Japan

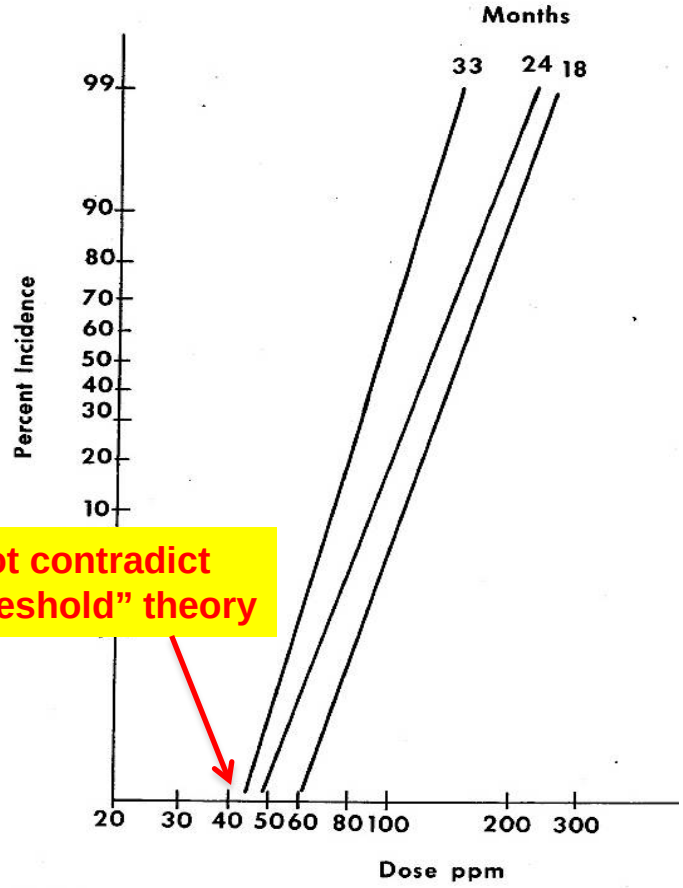
[2] Department of Pathology, Osaka City University Graduate School of Medicine, Abeno, Osaka 545-8585, Japan

Present Concept of Chemical Carcinogenicity



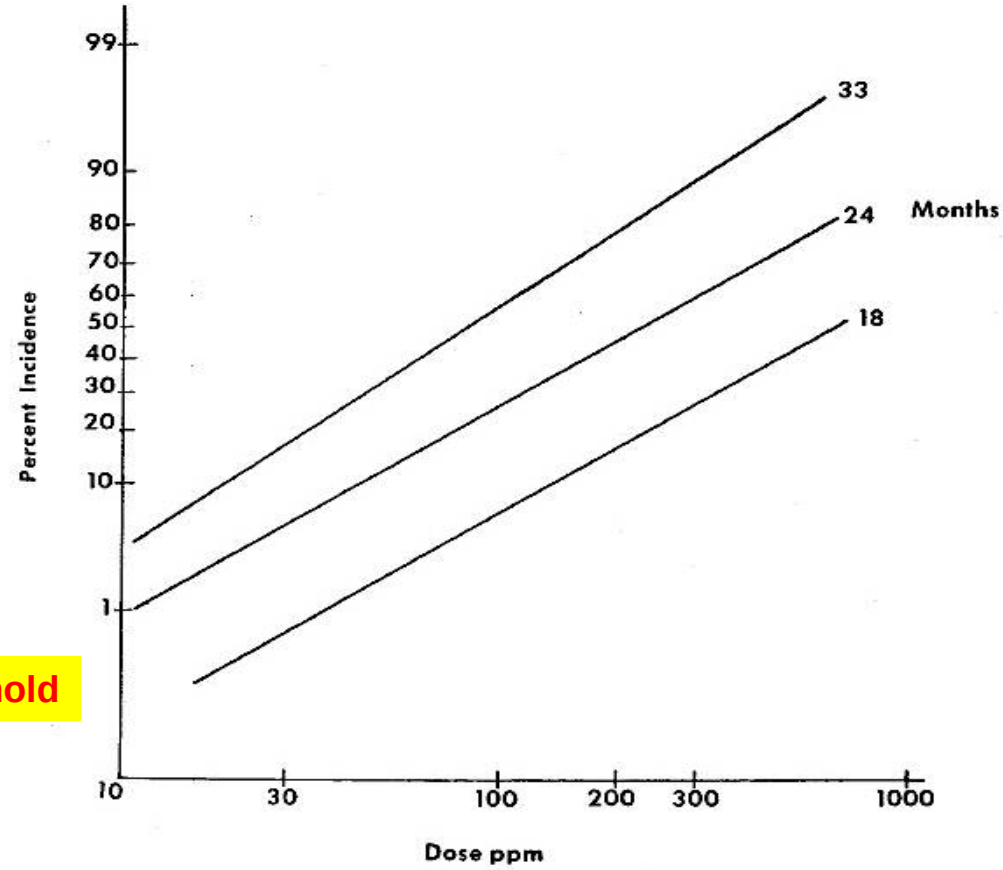
Low-dose Carcinogenicity Study of 2-Acetylaminofluorene (Megamouse Experiment)

- **Animal: 24,192 female BALB/c mouse, 3-4 weeks of age**
- **Doses of 2-acetylaminofluorene (2-AAF) in diet: 0, 30, 35, 45, 60, 75, 100, 150 ppm**
- **Time of sacrifice: 9 ~ 33 months**



Do not contradict "No Threshold" theory

No threshold



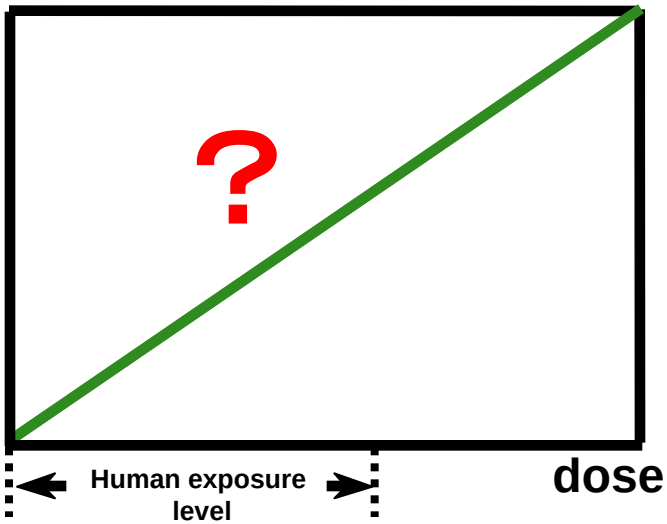
Dose model for bladder neoplasm

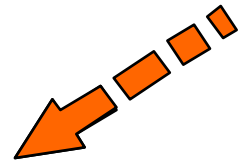
Dose model for liver neoplasm

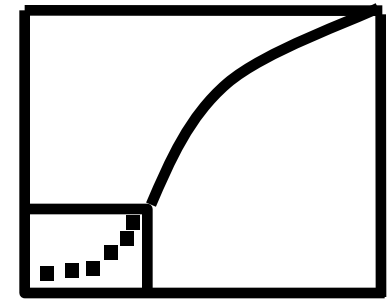
(Famer J.H., et al., J Environ Pathol Toxicol, 1979, 3: 55-68)

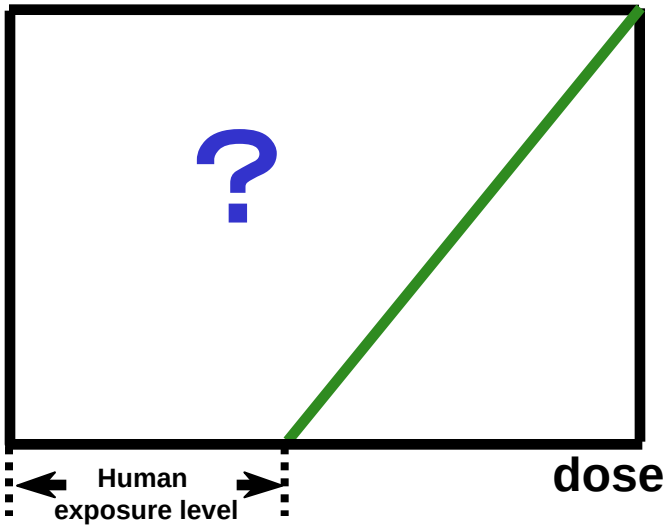
Reconsideration of Linear Non-threshold Theory

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



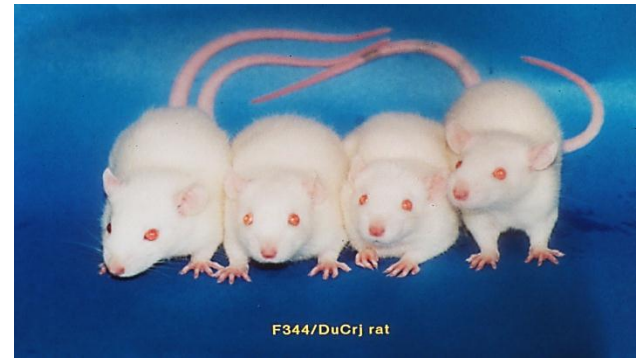






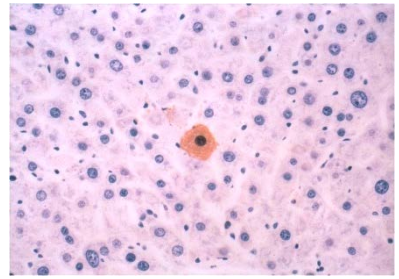
- It has been argued that non-threshold theory is challenged based on the view that organism possess biological responses that can ameliorate genotoxic activities.

Extrapolation of Genotoxic Carcinogenicity Study Results to Human

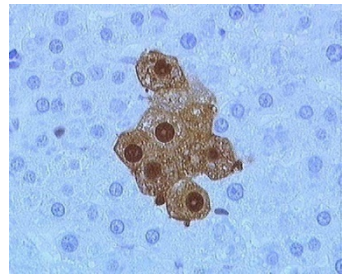


- ✓ Qualitative analysis only to classify into genotoxicity or non-genotoxicity is inadequate for carcinogenic risk assessment
- ✓ Qualitative and quantitative assessments are desirable in analysis for carcinogenicity, particularly at low doses
- ✓ Weight of evidence: *in vivo data are* more valuable than *in vitro* results in the quantitative analysis
- ✓ Point of departure (PoD) can be used for quantitative analysis of genotoxicity and carcinogenicity dose-response data
- ✓ PoD in markers of *in vivo* carcinogenic mechanism may contribute to resolution of putative non-threshold theory of genotoxic carcinogens

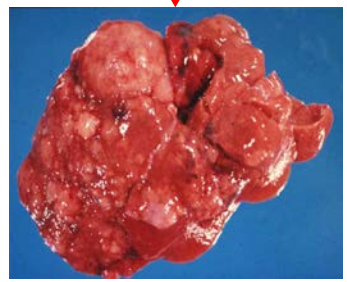
Chemical Carcinogenesis Mechanisms



GST-P positive cell
(possibly mutated cell)



GST-P positive foci



Cancer

Carcinogen

Inactivation

Metabolic activation :
ultimate carcinogen

Non-DNA

DNA adduct formation
Oxidative stress

DNA repair

DNA repair error

Initiation

Promotion

Cancer-irrelative
mutations

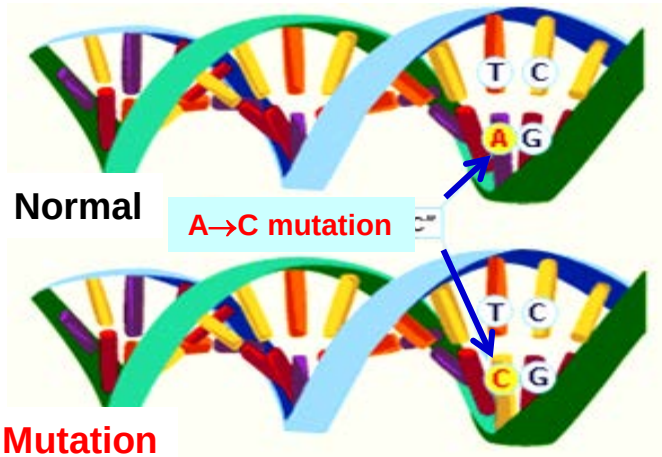
Mutation:
irreversible change

Apoptosis

Preneoplasia :
cell proliferation

Apoptosis

Cancer:
malignancy



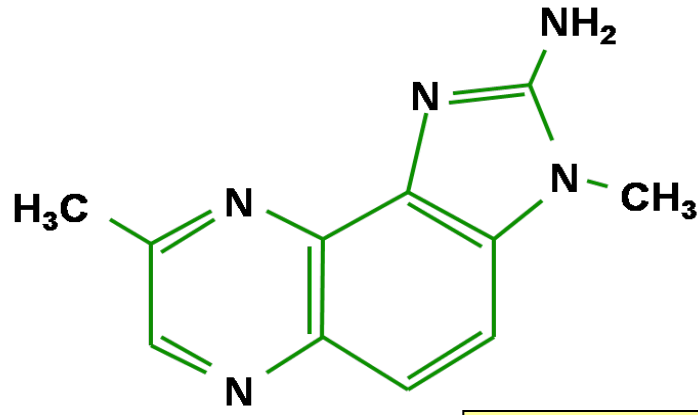
Normal

A→C mutation

Mutation

http://www.intelihealth.com

MeIQx

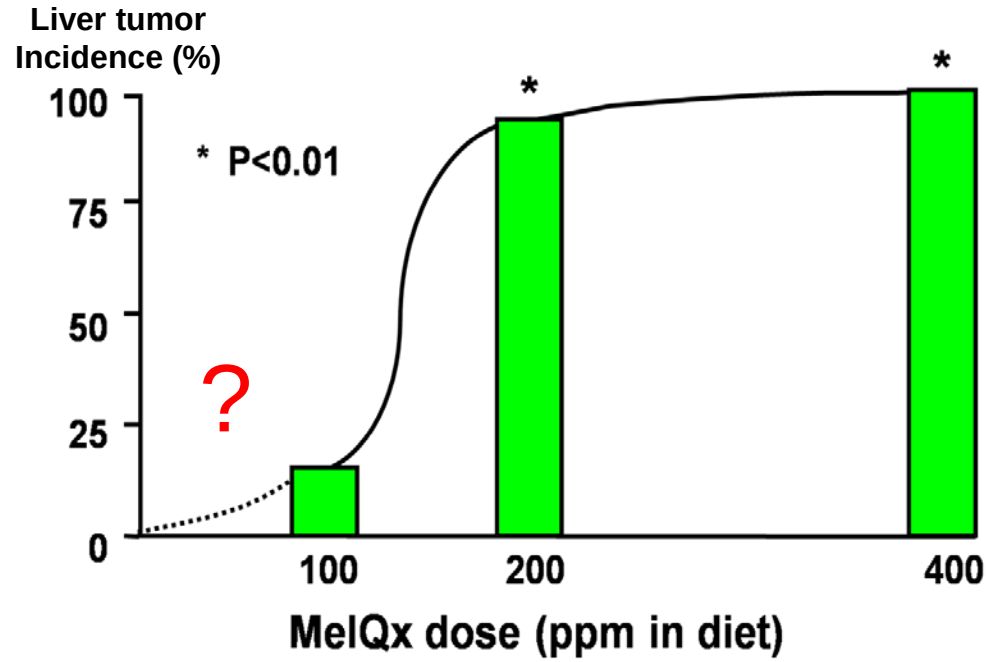


2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline

Hepatocarcinogenicity in rats

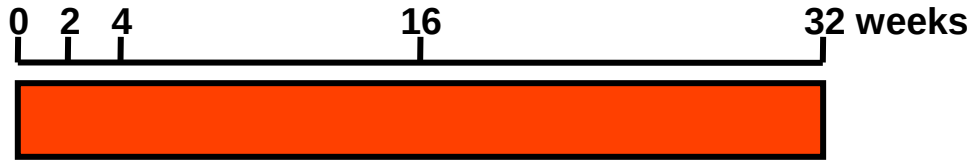
- One of heterocyclic amines
- Exists in well-cooked fish and meat
- Genotoxicity: Ames test, positive
  - Chromosome aberration test, positive
  - Structural aberration: positive
- Hepatocarcinogen
- Human exposure level: 0.2-2.6 μg/day
- IARC category: 2B

  (probably carcinogenic to humans)

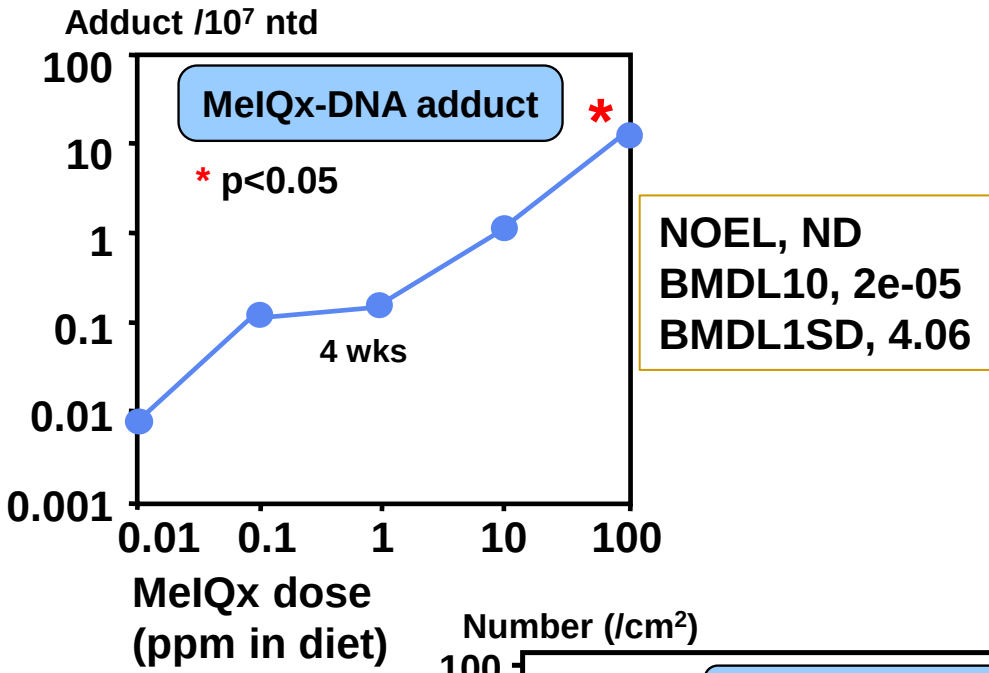


(Wakabayashi K., et al., Carcinogenesis, 1996, 17: 1029-1034)

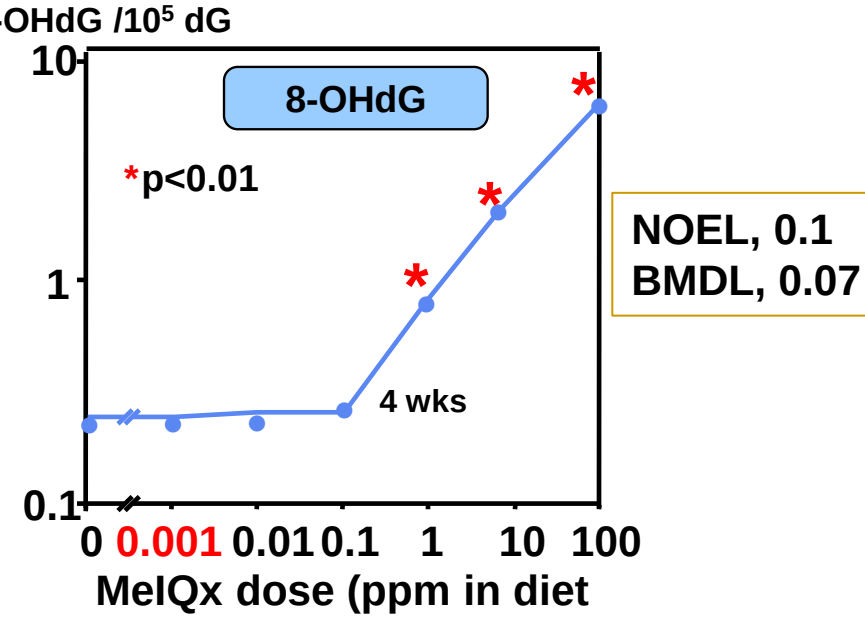
Rat Hepatocarcinogenicity of MeIQx at Low Doses



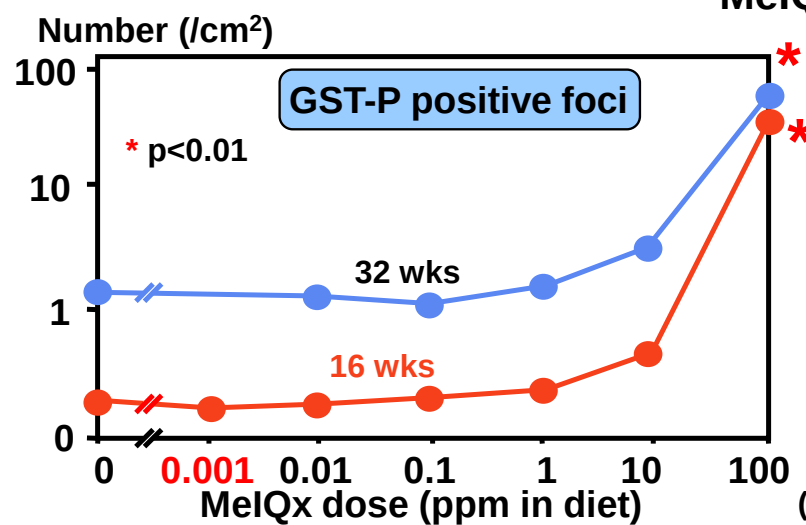
Animals: 1,180 male F344 rats, 21-day-old



NOEL, ND
BMDL10, 2e-05
BMDL1SD, 4.06



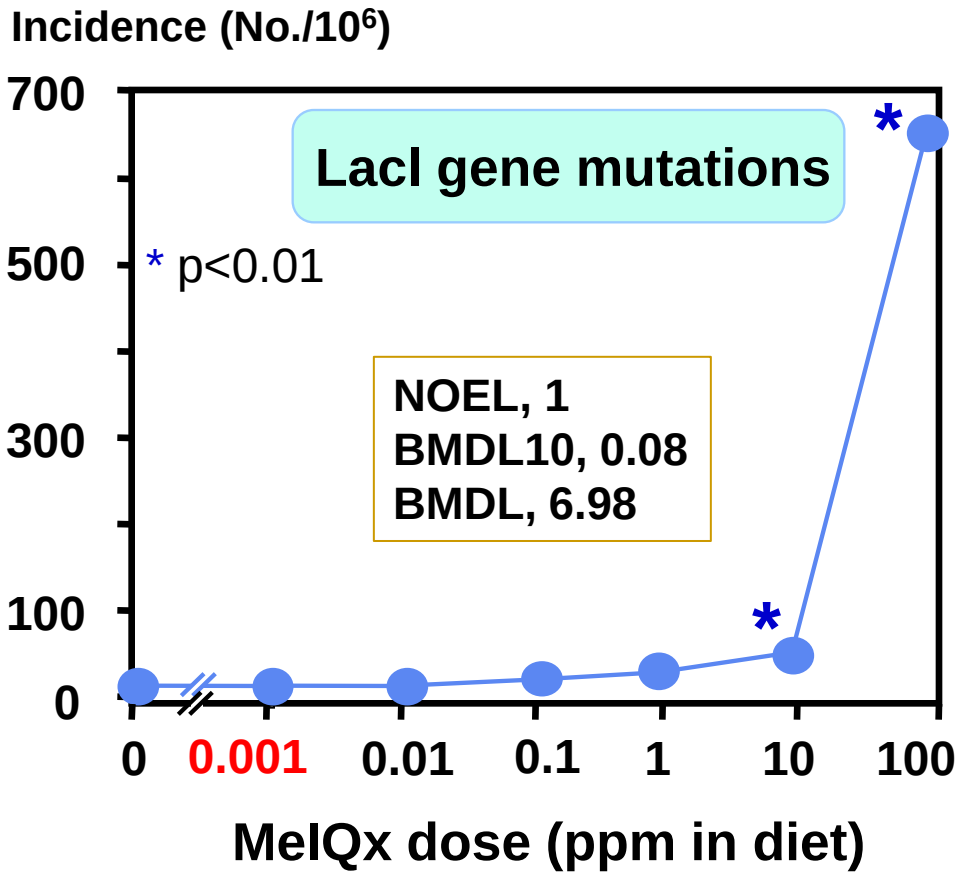
NOEL, 0.1
BMDL, 0.07

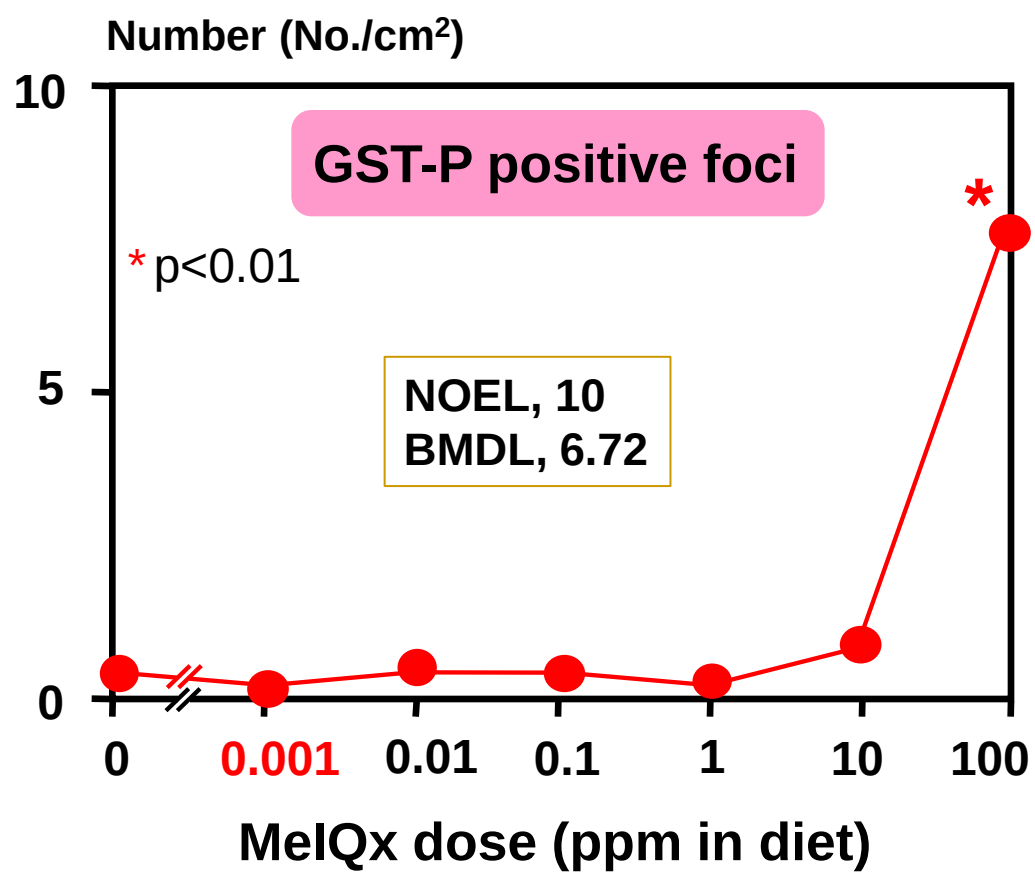


NOEL, 10 (16, 32 wks)
BMDL10, 0.14 (16 wks)
0.23 (32 wks)
BMDL, 21.95 (16 wks)
15.12 (32 wks)

(Fukushima S., et al., JJCR, 2002, 93: 1076-1082)

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

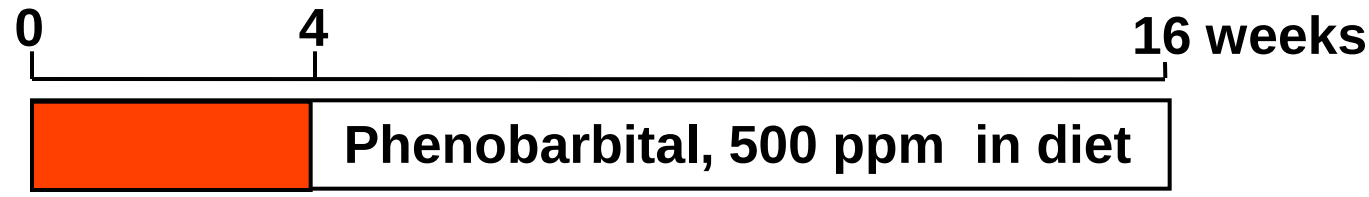




***LacI* gene: 30~40 copies on chromosome 4 in the F344 rat**

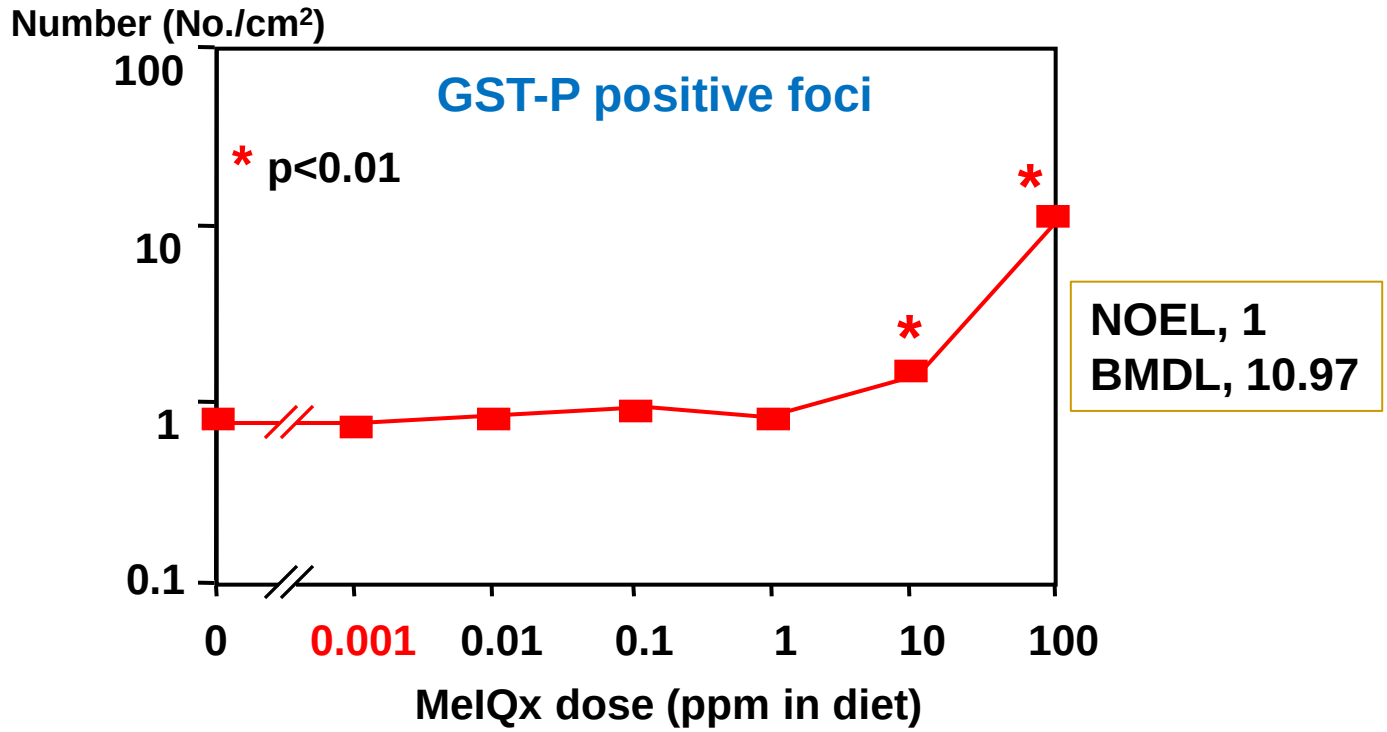
(Hoshi M., et al., Toxicol Sci, 2004, 81: 273-279)

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



(Fukushima S., et al., Cancer Lett, 2003, 191: 35-40)

Rat Heatocarcinogenicity of MeIQx in Long-term Carcinogenicity Test

Liver tumors (54 wks)

MeIQx (ppm)	No. of rats	Incidence (%)	
		Hepatocellular adenoma	Hepatocellular carcinoma
0	15	0	0
100	30	5 (17)	0
200	29	13 (45) *	13 (45) *
400	19	1 (6)	15 (94) *

*p<0.01 v.s. 0 ppm

(Kushida H., et al., Cancer letters, 1994, 83: 31-35)

NOEL, Adenoma: < 100
Carcinoma: 100
BMDL10, Tumors: 11.4
BMDL10, Adenoma: 60.25
Carcinoma: 72.68

Liver tumors (104 wks)

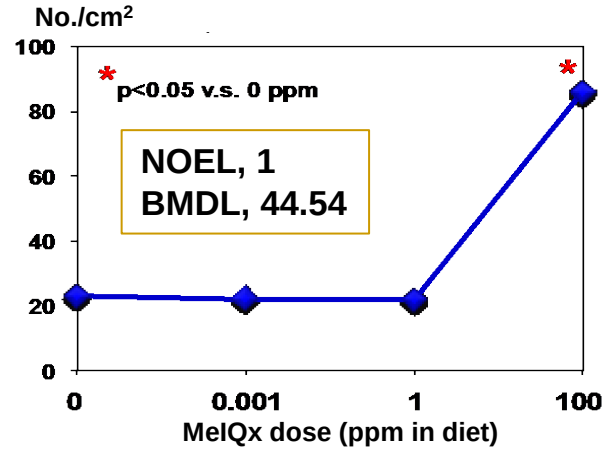
MeIQx (ppm)	No. of rats	Incidence (%)	
		Hepatocellular adenoma	Hepatocellular carcinoma
0	51	0	0
0.001	51	0	0
1	51	0	0
100	51	14 (27) *	6 (12) *

*p<0.01 v.s. 0 ppm

GST-P positive foci (104 wks)

Adenoma:
NOEL, 1
BMDL, 22.54

Carcinoma:
NOEL, 1
BMDL, 47.08



(Murai, T., Fukushima, S., et al., Toxicol Pathol, 2008, 36: 472-477)

Markers of MeIQx Rat Hepatocarcinogenesis and the Comparison with Point of Departure (PoD)

	DNA adduct	Mutation	GST-P^{+} Foci	Adenoma	Carcinoma
NOEL	ND	1	10	< 100	100
BMDL10	2e-05	0.08	0.14	11.4 (tumors)	
BMDL	4.06	6.98	15.12	60.25	72.69

ND, not detected

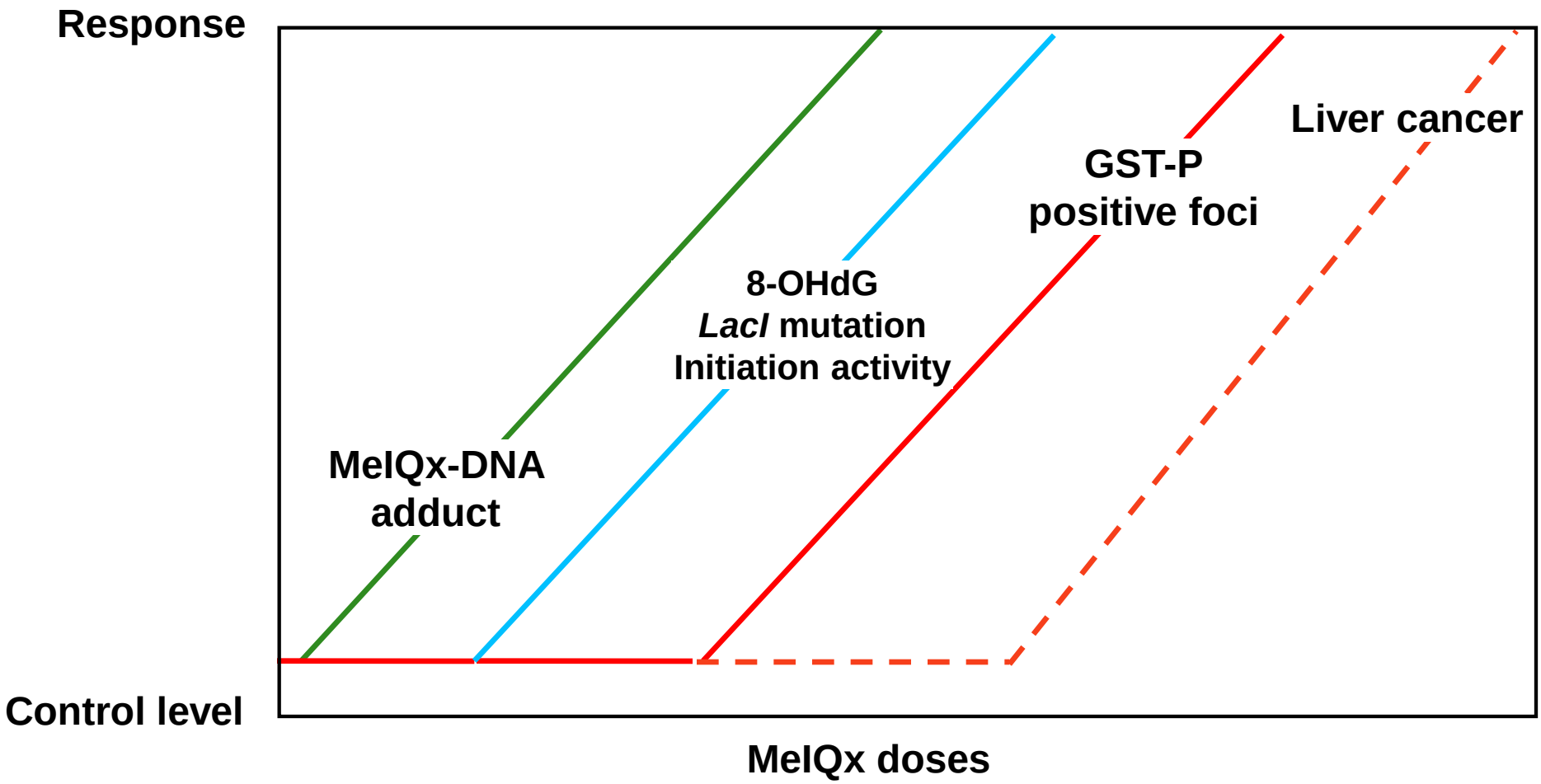
BMDL ranking: DNA adduct < Mutation < Preneoplasia < Tumor

Preneoplastic Lesions or Tumors in MeIQx Rat Hepatocarcinogenesis and the Comparison with PoD

	GST-P$^+$ Foci	Adenoma		Carcinoma	
	104 wks	54 wks	104 wks	54 wks	104 wks
NOEL	1	< 100	1	100	1
BMDL	44.52	60.25	22.54	72.69	47.08

BMDL ranking: Adenoma < Carcinoma (54 weeks, 104 wks)
Adenoma < Preneoplasia & Carcinoma (104 wks)

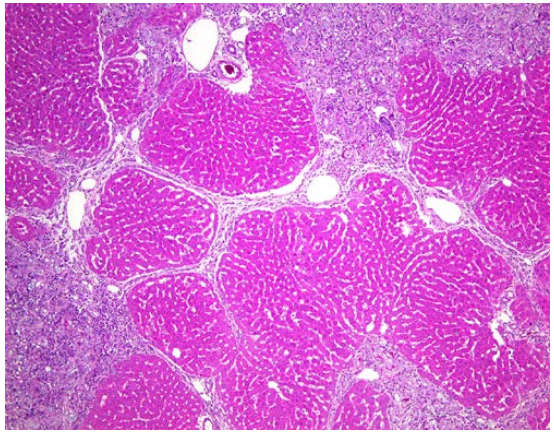
Risk of Liver Cancer: Reaction Curves for the Carcinogenicity Markers Dependent on the Dose of MeIQx



DNA adduct < Mutation < Preneoplasia < Tumor

Existence of a carcinogenic threshold

MeIQx DNA Adduct Levels and Number of GST-P Positive Foci in the Liver of Rats under Damaged Liver Condition

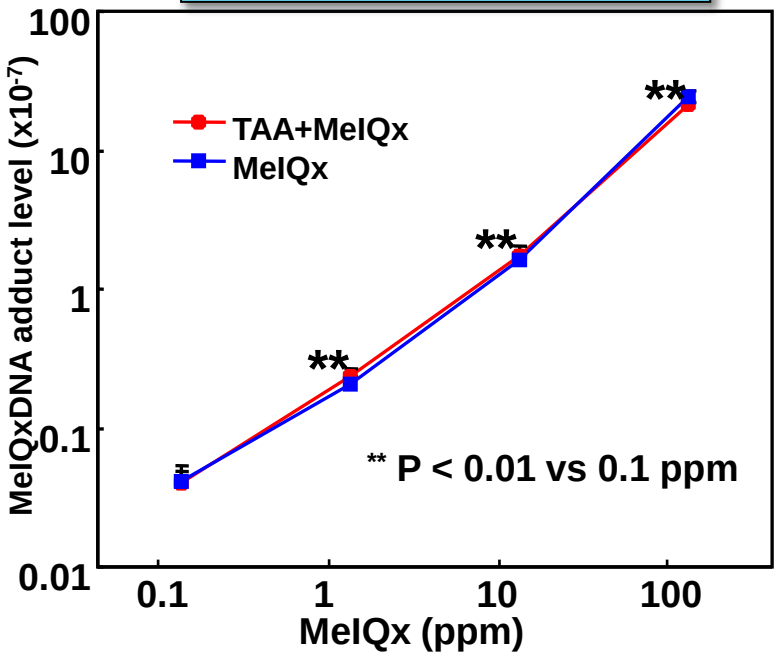


Male 280 F344 rats, 21-day-old

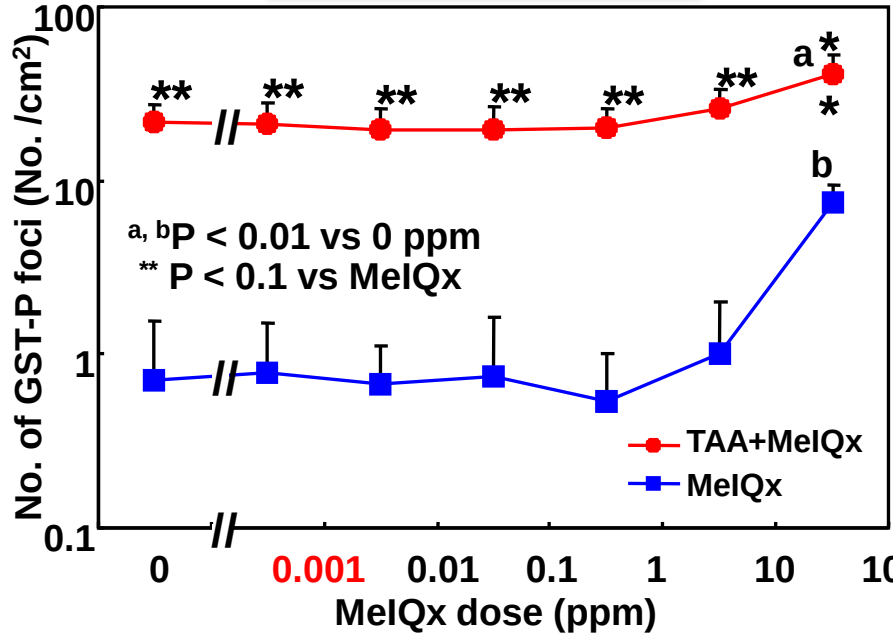
MeIQx-DNA adduct

TAA+MeIQx
NOEL, ND
BMDL, 1.46

MeIQx
NOEL, ND
BMDL, 3.02



GST-P positive foci



TAA+MeIQx
NOEL, 1
BMDL, 9.68

MeIQx
NOEL, 16
BMDL, 32.45

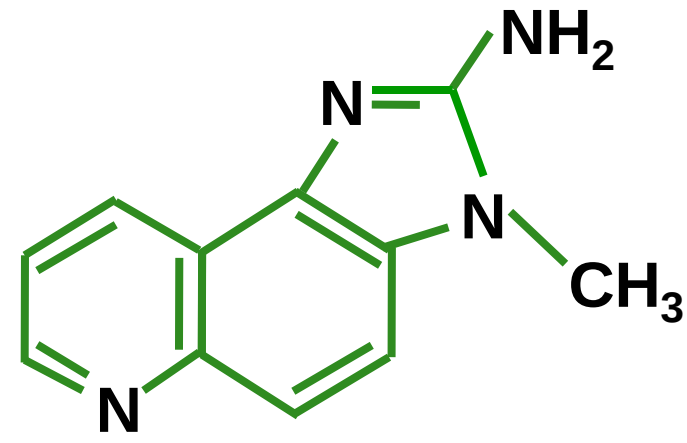
(Kang J.S., et al., Cancer Sci, 2006, 97: 453-458)

PoDs of DNA Adduct and GST-P Positive Foci in MeIQx Rat Hepatocarcinogenesis under Damaged Liver Condition

	DNA adduct	GST-P^{+} Foci
TAA-MeIQx	1.46	9.68
MeIQx	3.02	32.45

BMDL values: TAA→MeIQx < MeIQx
BMDL ranking: DNA adduct < Preneoplasia

IQ



2- amino-3-methylimidazo[4,5-*f*]quinoline

- Food-derived heterocyclic amine
- Mutagenicity: positive
- Genotoxicity (Chromosome aberration test):

  Structural aberration: positive
- Carcinogenicity in male rats: liver, colon, etc.

  (300 ppm in diet, 2-year carcinogenicity study)
- IARC category: 2A

Induction of DNA Adduct and GST-P Positive Foci in the Livers of Rats Administered IQ for 16 weeks

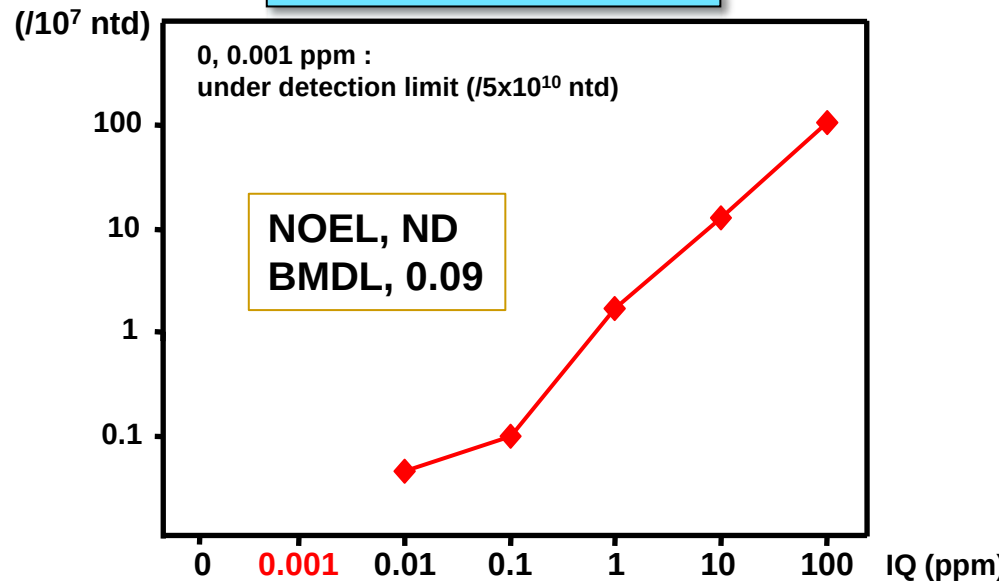
GST-P positive foci

IQ (ppm)	No. of rats	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

NOEL, 1
BMDL, 61.96

IQ-DNA adduct



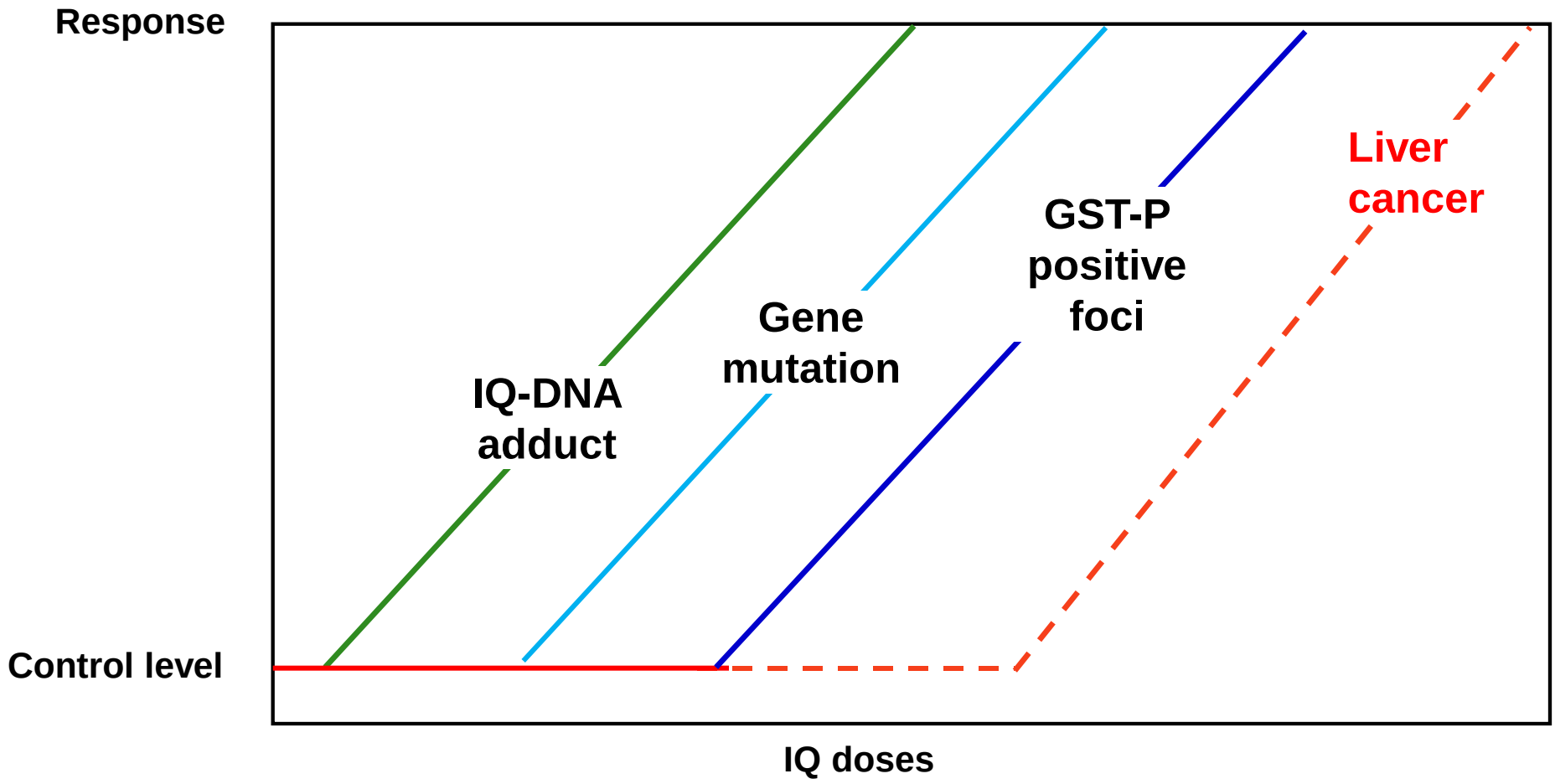
(Wei M., et al., Cancer Sci., 2010, 102: 88-94)

Relationship between Markers in IQ Carcinogenesis of Rat Livers and PoD

	DNA adduct	Mutation	GST-P$^+$ Foci
NOEL	ND	10	1
BMDL	0.09	1.22	61.96

BMDL ranking: DNA adduct < Mutation < GST-P$^+$ Foci

Reaction Curves for the Carcinogenicity Markers Dependent on the Dose of IQ



DNA adduct < Mutation < Preneoplasia < Tumor

Existence of a carcinogenic threshold

Conclusions

- ✓ **In qualitative analysis, Mode of Action in genotoxic carcinogens is an important tool for the analysis of low dose carcinogenicity**
- ✓ **In quantitative analysis, PoD is a useful tool for the determination of exposure level in each marker of carcinogenesis. BMD may be an appropriate method.**
- ✓ **In MeIQx or IQ carcinogenicity, values of PoD were different and increased in order of DNA adduct, mutation, GST-P positive foci and tumor**
- ✓ **These data will contribute to understand whether genotoxic carcinogenic threshold exists or not**

Collaborators

Hirose, Masao (Div. of Pathology, National Institute of Health Sciences)

Konishi, Yoichi (Dept. of Oncological Pathology, Cancer center, Nara Medical University)

Nakae, Dai (Dept. of Pathology, Sasaki Institute, Sasaki Foundation)

Otani, Shuzo (Dept. of Biochemistry, Osaka City University Med. Sch.)

Shirai, Tomoyuki (Dept. of Pathology, Nagoya City University Med. Sch.)

Takahashi, Michihito (Div. of Pathology, National Institute of Health Sciences)

Tatematsu, Masae (Div. of Oncological Pathology, Aichi Cancer Center Research Institute)

**Tsuda, Hiroyuki (Experimental Pathology and Chemotherapy Div.,
National Cancer Center Research Institute)**

**Wakabayashi, Keiji (Cancer Prevention Research,
National Cancer Center Research Institute)**