

Evidence on the Carcinogenicity of N-Methyl-N-Formylhydrazine (MFH)

Carcinogen Identification Committee Meeting
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Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment, CalEPA



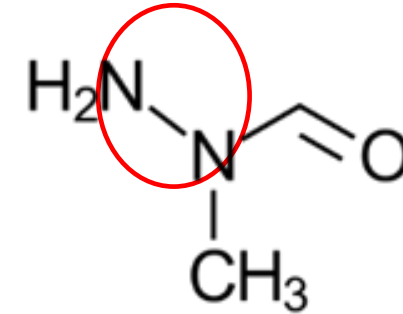
Overview

- **Occurrence and exposure**
- **Evidence of carcinogenicity**
 - **Animal data**
 - **Mechanistic considerations**
 - Pharmacokinetics and metabolism
 - Key characteristics (KCs) of carcinogens
 - Structure activity considerations
- **Summary of evidence**



N-Methyl-N-Formylhydrazine (MFH)

- **A hydrazine compound**
 - Water/solvent soluble; volatile
- **Occurrence**
 - Naturally occurring in *Gyromitra* mushrooms (“false morels”)
 - Co-occurs with other toxic chemicals, e.g., gyromitrin, monomethylhydrazine (MMH)
 - Synthesized and used
 - To produce a veterinary antibacterial medication
 - For research purposes
- **Exposure**
 - Consumption of *Gyromitra* mushrooms
 - Occupational settings



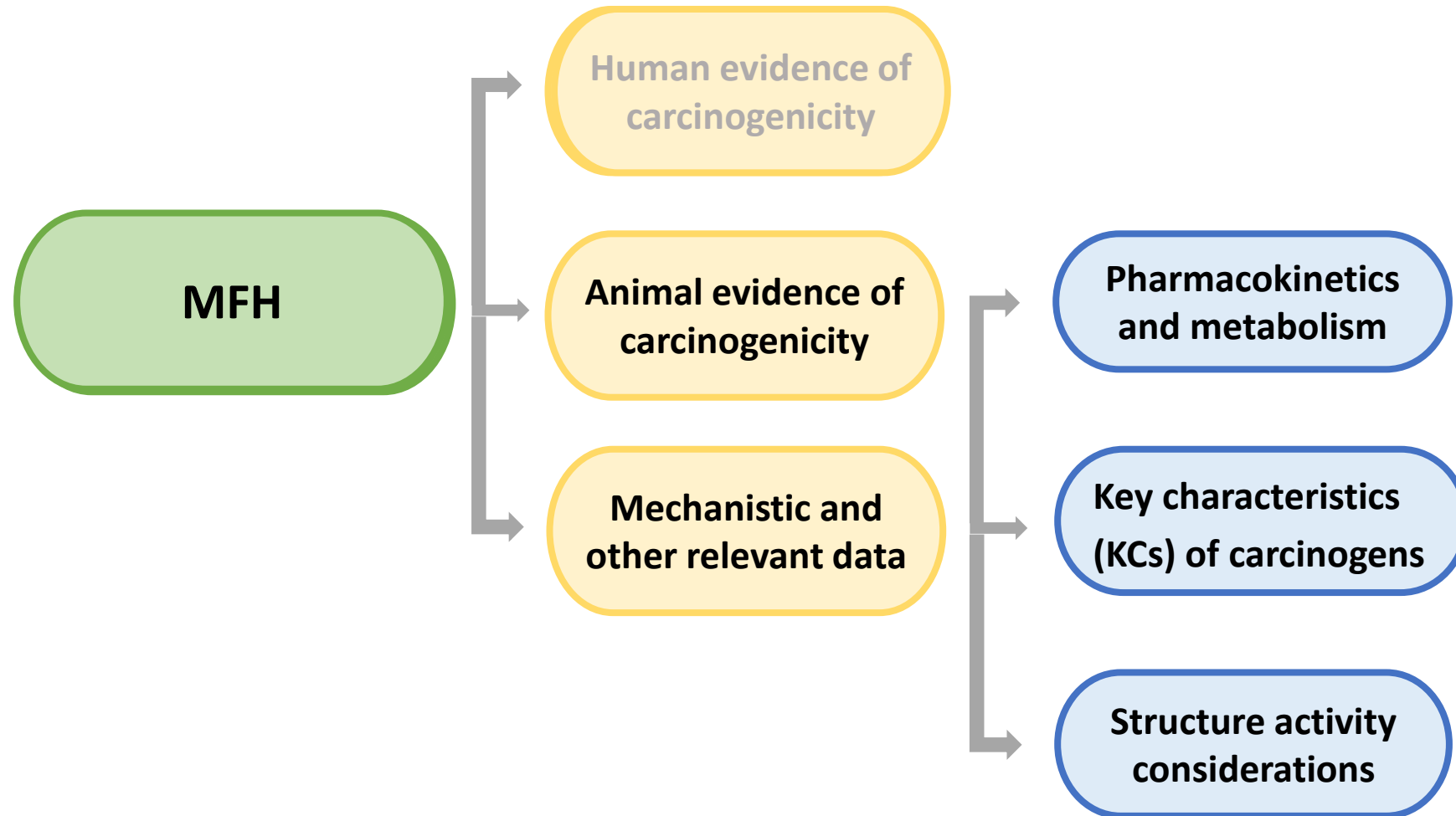
CAS RN: 758-17-8



Evidence of Carcinogenicity



Integration of Evidence Streams in MFH Cancer Hazard Identification



Carcinogenicity studies in animals



MFH Animal Studies - Overview

Strain & Species	Route	Treatment	Sex	Concentration or dose	Reference
Swiss mouse	Drinking water	Lifetime	M	0, 0.0078%, 0.0156% [w/v]	Toth and Nagel (1978)
			F		
			M	0, 0.0039% [w/v]	Toth et al. (1979)
			F		
			M	0, 0.001%, 0.002% [w/v]	Toth and Patil (1980b)
			F		
			M	0, 0.00025%, 0.0005% [w/v]	Toth and Patil (1982)
			F		
	Subcutaneous (s.c.)	Single injection	M	0, 100, 120 mg/kg-bw	Toth and Patil (1980a)
			F	0, 180 mg/kg-bw	
		40 weekly injections	M	0, 10 mg/kg-bw	Toth and Patil (1983)
			F	0, 20 mg/kg-bw	
Syrian golden hamster	Drinking water	Lifetime	M	0, 0.0078% [w/v]	Toth and Patil (1979)
			F		

Each study included 100/control group and 50/treated group, all treatments started at 6 weeks of age

M: male; F: female



MFH mouse drinking water studies (Section 4.1.1 of the HID):

Statistically significant tumor findings

Administered Concentration [w/v]	Male						Female			
	Lung	Liver	Gall-bladder	Bile duct	Blood vessel	Other	Lung	Liver	Gall-bladder	Blood vessel
0.0156%								√		
0.0078%	√	√	√ _r	√ _r			√	√	√ _r	
0.0039%	√	√	√ _r	√ _r		√ ^{1, r}	√	√	√ _r	√
0.0020%	√	√	√ _r		√		√		√ _r	√
0.0010%	√	√	√ _r		√		√			√
0.0005%	√					√ ^{2, r}	√			
0.00025%	√					√ ^{3, r}	√			

√: tumor incidence with a significant increase; blank cells: no significant increase; r: rare tumor

¹ Seminal vesicle adenoma

² Forestomach squamous cell carcinoma

³ Glandular stomach polypoid adenoma, and subcutis fibroadenoma



MFH mouse drinking water studies: Tumor sites and types

System	Tumor site (sex)	Tumor Type
Respiratory	Lung (M, F)	Adenoma, adenocarcinoma, or adenoma and adenocarcinoma combined
Digestive	Forestomach (M)	Squamous cell carcinoma (r)
	Glandular stomach (M)	Polypoid adenoma (r)
	Liver (M, F)	Hepatocellular adenoma, carcinoma, or adenoma and carcinoma combined
	Gallbladder (M, F)	Adenoma, or adenoma and adenocarcinoma combined (r)
	Bile duct (M)	Cholangioma, cholangiocarcinoma, or cholangioma and cholangiocarcinoma combined (r)
Vascular	Blood vessel (M, F)	Angioma, angiosarcoma, or angioma and angiosarcoma combined
Integumentary	Subcutis (M)	Fibrosarcoma
Reproductive	Seminal vesicle (M)	Adenoma (r)

M: male; F: female; r: rare tumor



Example: Tumor incidence in female Swiss mice administered MFH in drinking water (Toth and Patil 1980b) – Table 8 in the HID

Tumor site	Tumor type (age when first tumor was observed)	Concentration in drinking water			Trend test <i>p</i> -value
		0	0.001%	0.002%	
		Average dose (mg/day/animal)			
		0	0.12	0.21	
Lung	Adenoma	10/99	35/49***	44/50***	< 0.0001
	Adenocarcinoma	6/99	25/49***	25/50***	< 0.0001
	Combined (week 38)	15/99	39/49***	47/50***	< 0.0001
Gall-bladder	Adenoma (r)	0/95	0/44	2/48	NS
	Adenocarcinoma (r)	0/95	0/44	1/48	NS
	Combined (week 57) (r)	0/95	0/44	3/48*	< 0.05
Blood vessel	Angioma	4/95	5/44	9/48**	< 0.01
	Angiosarcoma	4/95	5/44	11/48**	< 0.001
	Combined (week 56)	8/95	10/44*	20/48***	< 0.0001

* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. Exact trend test conducted by OEHHA; NS- not significant



Mouse s.c. injection studies of MFH (Section 4.1.2 of the HID): Statistically significant tumor findings

Injection plan	Male			Female	
	Dose (mg/kg-bw)	Lung	Preputial gland	Dose (mg/kg-bw)	Lung
40 weekly injections	10	√	-	20	√
Single injection	120	-	√ ^r	180	√
	100	-	√ ^r		

Lung (M, F):

Adenoma, or adenocarcinoma, or adenoma and adenocarcinoma combined

Preputial gland (M):

Squamous cell papilloma, squamous cell carcinoma, or squamous cell papilloma and carcinoma combined (r)



Example: Tumor incidence in female Swiss mice administered MFH via a single s.c. injection (Toth and Patil 1980a)

– Table 12 in the HID

Tumor site	Tumor type (age when first tumor was observed)	Administered Dose (mg/kg-bw)	
		0	180
Lung	Adenoma	10/95	15/45**
	Adenocarcinoma	6/95	13/45***
	Adenoma and adenocarcinoma combined (59 weeks)	15/95	20/45***

** $p < 0.01$; *** $p < 0.001$



Syrian golden hamster drinking water studies (Section 4.2 of the HID): Statistically significant tumor findings

MFH Administered Concentration	Male			Female
	Liver	Gallbladder	Bile duct	Liver
0.0078% [w/v]	√ ^r	√ ^r	√ ^r	√ ^r

r: rare tumor

Liver (M, F)

- Hepatocellular adenoma, or carcinoma, or adenoma and carcinoma combined
- Malignant histiocytoma (Kupffer cell sarcoma)

Gallbladder (M)

- Adenoma, or adenoma and adenocarcinoma combined

Bile duct (M)

- Cholangiocarcinoma, or cholangioma and cholangiocarcinoma combined



Example: Tumor incidence in male Syrian golden hamsters administered MFH in drinking water (Toth and Patil 1979)

– Table 15 in the HID

Tumor site	Tumor type (age when first tumor was observed)	Concentration in drinking water (%)	
		0	0.0078
		Average dose (mg/day/animal)	
		0	1.35
Liver	Hepatocellular adenoma (r)	0/67	16/43***
	Hepatocellular carcinoma (r)	0/67	8/43***
	Combined (53 weeks) (r)	0/67	24/43***
	Malignant histiocytoma (73 weeks) (r)	0/18	10/26**
Gallbladder	Papillary adenoma (r)	0/36	5/37*
	Adenocarcinoma (r)	0/36	3/37
	Combined (65 weeks) (r)	0/36	8/37**
Bile duct	Cholangioma (r)	0/18	1/26
	Cholangiocarcinoma (r)	0/18	6/26*
	Combined (73 weeks) (r)	0/18	7/26*

* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$; r: rare tumor

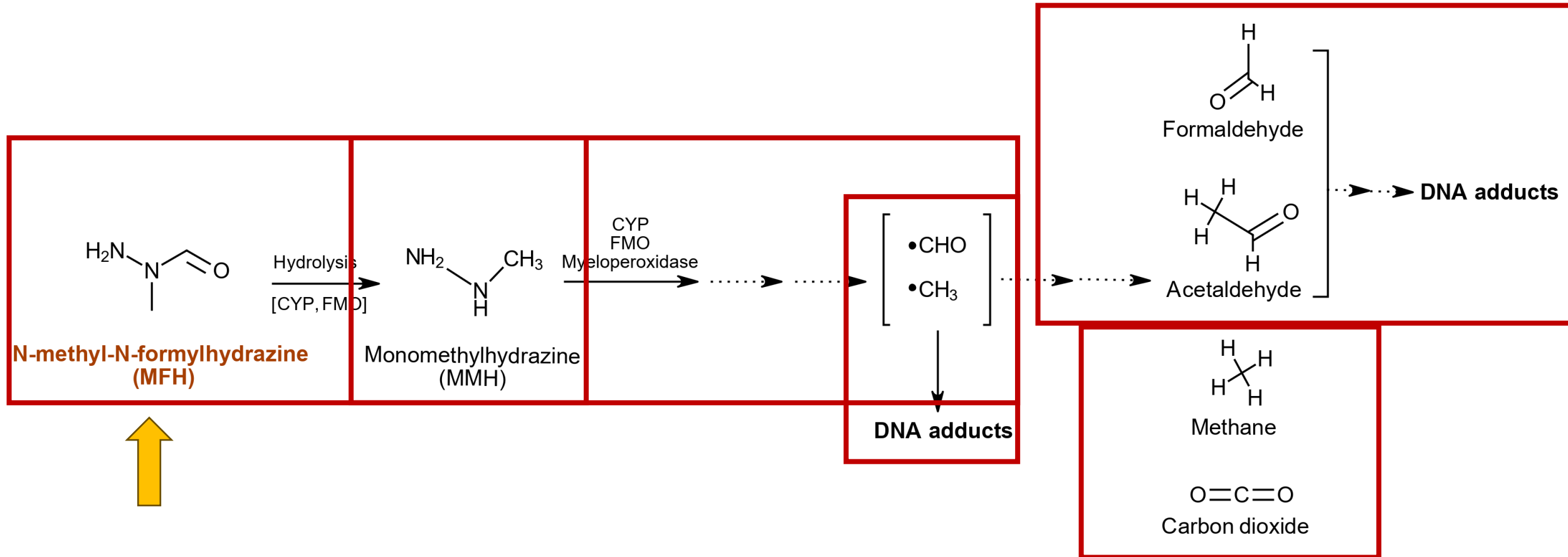


Pharmacokinetics and Metabolism

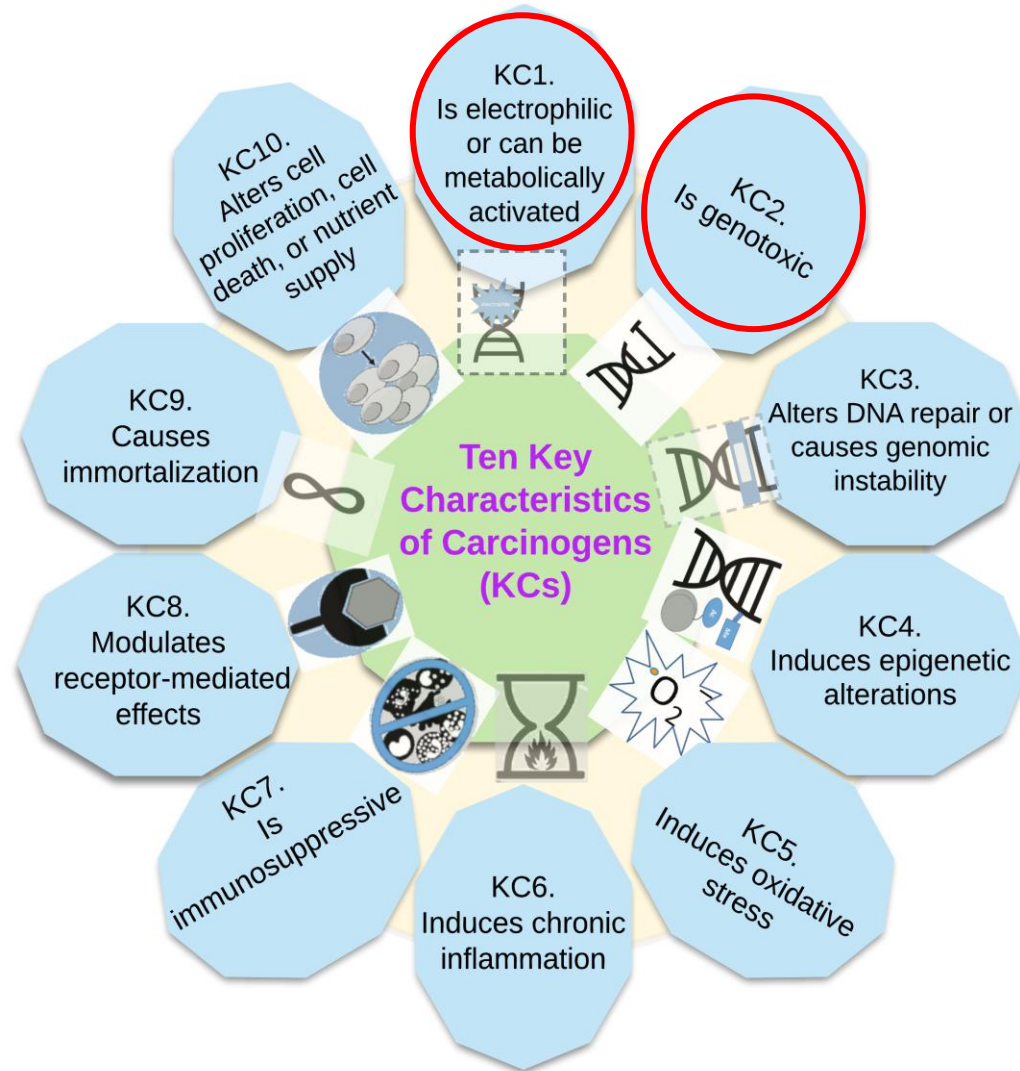
- Sparse ADME data from studies of MFH; information comes from:
 - MFH carcinogenicity and toxicity studies in animals
 - *Gyromitra* mushroom poisoning events in humans
 - MMH ADME studies in animals
- MFH is absorbed following oral and inhalation exposures & distributed throughout the body
- MFH is likely excreted in:
 - expired air as CO₂ and CH₄
 - urine
 - feces



Proposed metabolic pathways for MFH



Key Characteristics (KCs) of Carcinogens



Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications. See also Preamble to the IARC monographs (IARC 2019).

KC1: Is electrophilic or can be metabolically activated

Evidence comes from studies of MMH, the MFH hydrolysis metabolite

- **Formation of DNA and RNA adducts *in vivo***
 - O⁶MeGu was detected in liver and kidney DNA in mice
 - N7MeGu was detected in liver and large intestine DNA and in liver, large intestine, and kidney RNA in mice
 - N7MeGu was detected in liver DNA in rats
- **DNA and protein adducts *in vitro***
 - [¹⁴C]MMH binds covalently to nucleic acids in rat liver slices
 - MMH binds covalently to proteins in rat liver microsomes and S9
- **Electrophilic metabolites detected *in vitro***
 - Methyl radical formation in rat hepatocytes, neutrophils and liver microsomes
 - Acetaldehyde and formaldehyde formation in rat liver microsomes



KC2: Is genotoxic

Evidence comes from studies of MFH and MMH

Studies of MFH

- ↑ reverse mutations in *S. typhimurium* TA100 (dose-dependent with metabolic activation) in one study, but not in other studies or strains (TA98, TA1950, TA1535 and TA1537)
- No induction of unscheduled DNA synthesis (UDS) *in vitro* in rat or mouse hepatocytes

Studies of MMH

- *In vivo*
 - Formation of DNA adducts in mouse liver, kidney, and large intestine, and rat liver
 - No induction of UDS in rat liver
 - No induction of dominant lethal mutations in rats or mice



KC2: Is genotoxic (MMH continued)

- *In vitro*

- ↑UDS in mouse and rat hepatocytes, but not in human fibroblasts
- ↑ forward mutations in Chinese hamster lung fibroblasts, but not in mouse lymphoma cells
- Covalent binding to nucleic acids in rat liver slices

- Bacteria and yeast

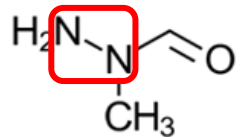
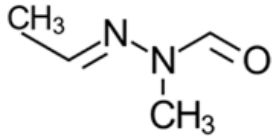
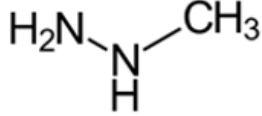
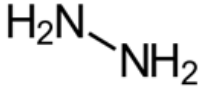
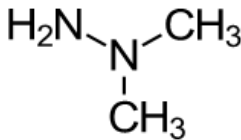
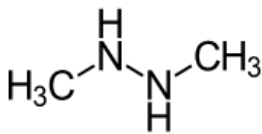
- ↑ reverse mutations in *S. typhimurium* TA100, TA102, TA1535, & TA1537 in some (but not all) studies
- ↑ reverse mutations, SOS response and DNA repair in *E. coli*
- No induction of gene recombination in one study in yeast

Additional considerations

- Genotoxic and carcinogenic metabolites of MFH and MMH – acetaldehyde and formaldehyde

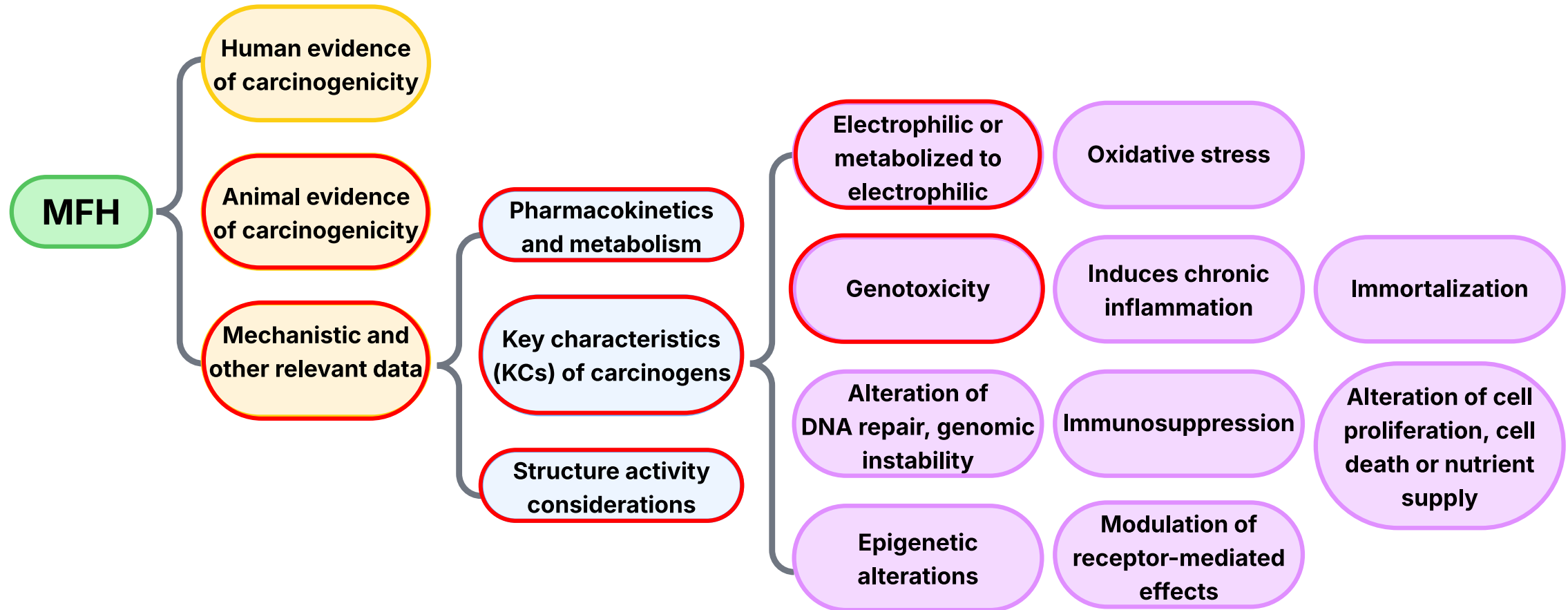


Structure Activity Considerations

Chemical [Cancer classification]	Structure	Tumor sites	
		Mouse	Hamster
N-Methyl-N-formylhydrazine (MFH)		<u>Lung</u> , <u>liver hepatocellular</u> , gallbladder, bile duct, <u>blood vessel</u> , seminal vesicles, forestomach, glandular stomach, subcutis, preputial gland	<u>Liver hepatocellular</u> , <u>liver malignant histiocytoma (Kupffer cell sarcoma)</u> , gallbladder, bile duct
Gyromitrin [P65, <u>IARC Group 3</u>]		<u>Lung</u> , forestomach, preputial gland, clitoral gland	Not tested
Monomethylhydrazine (MMH) [P65]		<u>Lung</u> , <u>liver hepatocellular</u> , <u>blood vessel</u> , nasal, duodenum (adenoma)	<u>Liver malignant histiocytoma (Kupffer cell sarcoma)</u> , large intestine, nasal
Hydrazine [P65, <u>IARC Group 2A</u> , <u>NTP RA</u>]		<u>Lung</u> , <u>liver hepatocellular</u> , myeloid leukemia and lymphoma	<u>Liver hepatocellular</u> , nasal cavity, colon, thyroid
1,1-Dimethylhydrazine (1,1-DMH) [P65, <u>IARC Group 2B</u> , <u>NTP RA</u>]		<u>Lung</u> , <u>liver hepatocellular</u> , <u>blood vessel</u> , kidney	Large intestine, peripheral nerve sheath
1,2-Dimethylhydrazine (1,2-DMH) [P65, <u>IARC Group 2A</u>]		<u>Lung</u> , <u>blood vessel</u> , colon, small intestine	<u>Liver hepatocellular</u> , blood vessel, large intestine



Integration of Evidence Streams in MFH Cancer Hazard Identification



Summary – MFH tumor findings in animals

Swiss mice		Syrian golden hamsters
Drinking Water	s.c. Injection	Drinking Water
Respiratory system • Lung (M, F)	Respiratory system • Lung (M, F)	-
Digestive system • Forestomach (M) • Glandular stomach (M) • Liver hepatocellular (M, F) • Gallbladder (M, F) • Bile duct (M)	-	Digestive system • Liver hepatocellular (M, F) • Liver malignant histiocytoma (Kupffer cell sarcoma) (M, F) • Gallbladder (M) • Bile duct (M)
Vascular system • Blood vessel (M, F)	-	-
Reproductive system • Seminal vesicle (M)	Reproductive system • Preputial gland (M)	-
Integumentary system • Subcutis (M)	-	-



Summary – Mechanistic data

Pharmacokinetics and metabolism

- MFH → MMH →→ methyl radicals, formaldehyde, acetaldehyde, and DNA adducts

Key characteristics of carcinogens

- **KC1. Is electrophilic or can be metabolically activated**
 - MFH → MMH →→ electrophilic species (methyl radicals, formaldehyde, acetaldehyde)
 - MMH forms DNA and RNA adducts *in vivo*, and binds to nucleic acids and proteins *in vitro*
- **KC2. Is genotoxic**
 - MFH increases reverse mutations in one *S. typhimurium* strain (TA100)
 - MMH
 - Forms DNA adducts *in vivo* and binds to nucleic acids *in vitro*
 - Increases forward mutations and UDS *in vitro*
 - Increases reverse mutations, SOS response and DNA repair in bacteria
 - Genotoxic metabolites: formaldehyde and acetaldehyde

Structure activity considerations

Common treatment-related tumor findings between five comparator chemicals and MFH:

- Mice: lung, liver hepatocellular, and blood vessel
- Hamsters: liver hepatocellular and liver malignant histiocytoma (Kupffer cell sarcoma)



Thank you!

Any questions?

