OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Evidence on the Carcinogenicity of N-Methyl-N-Formylhydrazine

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Reproductive and Cancer Hazard Assessment Branch
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PREFACE

This document presents evidence relevant to the evaluation of the carcinogenicity of N-methyl-N-formylhydrazine.

Proposition 65[1] requires the publication of a list of chemicals known to the state to cause cancer or reproductive toxicity within the meaning of the Act (Health and Safety Code section 25249.8.). The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as the lead agency for implementing Proposition 65. The Carcinogen Identification Committee (CIC) advises and assists OEHHA and adds chemicals to the Proposition 65 list of chemicals that cause cancer, as required by Health and Safety Code section 25249.8.

The CIC serves as the state's qualified experts for determining whether a chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer within the meaning of the Act (Health and Safety Code section 25249.8.). The CIC also provides advice and consultation regarding which chemicals should receive their review. At their meeting in September 2010, the CIC recommended that N-methyl-N-formylhydrazine be placed in the 'medium' priority group for future listing consideration. OEHHA selected N-methyl-N-formylhydrazine for consideration for listing by the CIC, and in November 2024 OEHHA solicited from the public information relevant to the assessment of the evidence on its carcinogenicity. No information was received.

The CIC is scheduled to meet on November 18, 2025. OEHHA is providing this document to the CIC to assist the Committee in its deliberations on whether or not N-methyl-N-formylhydrazine should be listed under Proposition 65 for the cancer endpoint. The original papers and reports discussed in this document are provided to the CIC.

OEHHA is holding a public comment period on this hazard identification document. For information on how to comment go to https://oehha.ca.gov/comments. Comments on this document will be included in the hazard identification materials that are provided to the CIC members prior to the meeting.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 et seq.)

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LIST OF ABBREVIATIONS

Abbreviation	Full name
1,1-DMH	1,1-Dimethylhydrazine
1,2-DMH	1,2-Dimethylhydrazine
µg	Micrograms
µl	Microliters
µM	Micromolar
µmol	Micromoles
µmol/mL	Micromoles per milliliter
A	Absorption
AhR	Aryl hydrocarbon receptor
BW	Body weight
CAs	Chromosomal aberrations
CIC	Carcinogen Identification Committee
CompTox Chemical Dashboard	Computational Toxicology Chemicals Dashboard
CYP	Cytochrome P-450 monooxygenases
DNA	Deoxyribonucleic acid
D	Distribution
E	Elimination
EPR	Electron paramagnetic resonance
ER	Estrogen receptor
ESR	Electron spin resonance
FMO	Flavin adenine dinucleotide-containing monooxygenases
g	Grams
g/mol	Grams per mole
GSH	Glutathione
HAWC	Health Assessment Workspace Collaborative
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
KCs	Key characteristics
kg	Kilogram
Koa	Octanol-air partition coefficient
Kow	Octanol-water partition coefficient

Abbreviation	Full name
LD50	Median lethal dose
M	Metabolism
MFH	N-Methyl-N-formylhydrazine
MFHO	Methylformylhydrazone
mg	Milligram
mg/kg	Milligrams per kilogram
mg/kg-bw	Milligrams per kilogram body weight
mg/kg/day	Milligrams per kilogram per day
mg/l	Milligrams per liter
mg/ml	Milligrams per milliliter
min	Minute
mL	Milliliter
mM	Millimolar
MMH	Monomethylhydrazine
mmol	Millimole
MN	Micronuclei
mol/l	Moles per liter
N7MeGu	N7-methylguanine
NIOSH	National Institute of Occupational Safety and Health
N-N	Nitrogen-nitrogen
NS	Not significant
NT	Not tested
NTP	National Toxicology Program
ºC	Degrees Celsius
O^6MeGu	O^6-methylguanine
OEHHA	Office of Environmental Health Hazard Assessment
PPAR	Peroxisome proliferator-activated receptor
ppm	Parts per million
r	Rare
RoC	Report on Carcinogens
s.c.	Subcutaneous
SN	Substitution nucleophilic
SOD	Superoxide dismutase
UDMH	Unsymmetrical dimethylhydrazine

Abbreviation	Full name
UDS	Unscheduled DNA synthesis
US	United States
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
w/v	Weight per volume

SUMMARY

This document presents evidence relevant to the evaluation of the cancer hazard of N-methyl-N-formylhydrazine (MFH). MFH was placed in the “medium” priority group for future listing consideration by the Carcinogen Identification Committee (CIC) at their 2010 meeting.

MFH is naturally occurring in wild edible *Gyromitra* mushrooms. Mushrooms in the genus *Gyromitra* are commonly known as “spring false morels,” “false morels,” or “lorchels.” These mushrooms also contain other hydrazine compounds, such as monomethylhydrazine (MMH or methylhydrazine; a component in rocket propellants) and hydrazone compounds, such as gyromitrin (acetaldehyde N-methyl-N-formylhydrazone).

In addition to its natural occurrence in wild mushrooms, MFH can be synthesized by reacting MMH with ethyl formate. MFH is used in the production of marbofloxacin (a fluorinated quinolone antibacterial agent used in veterinary medicine) and for research purposes.

Systematic Literature Review Approach

The Office of Environmental Health Hazard Assessment (OEHHA) conducted literature searches on the carcinogenicity of MFH (last comprehensive search in December 2024). The literature searches included primary searches in major biomedical databases, searches in other data sources such as reports by other health agencies, and additional focused searches. The literature searches were supplemented with a public data call-in period from November 29, 2024 to January 10, 2025. An overview of the systematic literature review approach is presented in Section 2, and more detailed information can be found in Appendix A.

Carcinogenicity Studies in Humans

No cancer epidemiological studies on the effects of human exposure to MFH were identified.

Carcinogenicity Studies in Animals

Carcinogenicity studies of MFH have been conducted in both sexes of Swiss mice in lifetime drinking water studies and subcutaneous (*s.c.*) injection studies with lifetime

observation and in both sexes of Syrian golden hamsters in lifetime drinking water studies. All studies were conducted by the same laboratory, and statistically significant tumor findings were observed in each of these studies.

Statistically significant tumor findings, including several tumor types seen in multiple studies and several that are considered rare, are as follows:

Respiratory system:

- Lung tumors (adenoma, or adenocarcinoma, or adenoma and adenocarcinoma combined)
    - Male Swiss mice
        - drinking water (0.0078%, 0.0039%, 0.002%, 0.001%, 0.0005%, 0.00025%)
        - *s.c.* injection (40 weekly 10 milligram per kilogram body weight [mg/kg-bw] injections)
    - Female Swiss mice
        - drinking water (0.0078%, 0.0039%, 0.002%, 0.001%, 0.0005%, 0.00025%)
        - *s.c.* injection (single injection: 180 mg/kg-bw, 40 weekly 20 mg/kg-bw injections)

Digestive system:

- Forestomach tumors (squamous cell carcinoma)
    - Male Swiss mice (rare) – drinking water (0.0005%)
- Glandular stomach tumors (polypoid adenoma)
    - Male Swiss mice (rare) – drinking water (0.00025%)
- Liver hepatocellular tumors (adenoma, or carcinoma, or adenoma and carcinoma combined)
    - Male Swiss mice - drinking water (0.0078%, 0.0039%, 0.002%, 0.001%)
    - Female Swiss mice - drinking water (0.0156%, 0.0078%, 0.0039%)
    - Male Syrian golden hamsters (rare) – drinking water (0.0078%)
    - Female Syrian golden hamsters (rare) – drinking water (0.0078%)
- Liver malignant histiocytoma (Kupffer cell sarcoma)
    - Male Syrian golden hamsters (rare) – drinking water (0.0078%)
    - Female Syrian golden hamsters (rare) – drinking water (0.0078%)

- Gallbladder tumors (adenoma, or adenoma and adenocarcinoma combined)
    - Male Swiss mice (rare) - drinking water (0.0078%, 0.0039%, 0.002%, 0.001%)
    - Female Swiss mice (rare) - drinking water (0.0078%, 0.0039%, 0.002%)
    - Male Syrian golden hamsters (rare) - drinking water (0.0078%)
- Bile duct tumors (cholangioma, or cholangiocarcinoma, or cholangioma and cholangiocarcinoma combined)
    - Male Swiss mice (rare) - drinking water (0.0078%, 0.0039%)
    - Male Syrian golden hamsters (rare) – drinking water (0.0078%)

Vascular System:

- Blood vessel tumors (angioma, or angiosarcoma, or angioma and angiosarcoma combined)
    - Male Swiss mice - drinking water (0.002%, 0.001%)
    - Female Swiss mice - drinking water (0.0039%, 0.002%, 0.001%)

Reproductive system:

- Seminal vesicle tumors (adenoma)
    - Male Swiss mice (rare) - drinking water (0.0039%)
- Preputial gland tumors (squamous cell papilloma, squamous cell carcinoma, or squamous cell papilloma and carcinoma combined)
    - Male Swiss mice (rare) - *s.c.* injection (single injection: 100 mg/kg-bw, 120 mg/kg-bw)

Integumentary system:

- Subcutis tumors (fibrosarcoma)
    - Male Swiss mice - drinking water (0.00025%)

Mechanistic Considerations and Other Relevant Data

Pharmacokinetics and metabolism

Data on the pharmacokinetics and metabolism of MFH are sparse; however, information may be inferred from reports on the onset and subsidence of poisoning symptoms in humans following the ingestion of *Gyromitra esculenta* mushrooms and from *in vivo* animal studies investigating the toxicity of MFH. Relevant information also comes from studies in animals *in vivo* and *in vitro* and cell-free systems investigating the toxicity and metabolism of MFH's parent compound, gyromitrin, and MFH's hydrolysis metabolite, MMH.

There is indirect evidence in humans for absorption of MFH by the oral route, following consumption of wild edible *Gyromitra* mushrooms and direct evidence for oral absorption in animals (e.g., mice, hamsters) from toxicity and carcinogenicity studies of MFH. There is also evidence in humans for absorption by the inhalation route, following occupational exposure to *Gyromitra* mushroom cooking steam or occupational handling of fresh *Gyromitra* mushrooms in an enclosed area. Animal studies on the distribution of gyromitrin and/or its metabolites in rabbits, rats, and mice suggest that MFH is likely to be widely distributed throughout the body. Indirect information on human elimination comes from the two- to five-day recovery period observed in most non-fatal *Gyromitra* mushroom poisoning cases, which suggests that residual gyromitrin, MFH and downstream metabolites are largely excreted within that time frame. Animal studies on the elimination of radiolabeled gyromitrin in rabbits and rats and radiolabeled MMH in rats and mice suggest that MFH or its metabolites may be eliminated in the urine, feces, and expired air.

Based on the integration of *in vivo* and *in vitro* studies of MFH, MMH, and gyromitrin, MFH is understood to undergo non-enzymatic hydrolysis to form MMH, although enzymatic hydrolysis of MFH via cytochrome P-450 monooxygenases (CYP) has also been proposed. MMH then undergoes enzymatic oxidation via CYP, flavin adenine dinucleotide-containing monooxygenases (FMO), or myeloperoxidase to generate methyl and formyl radicals via unconfirmed reactive intermediates and ultimately forms formaldehyde, acetaldehyde, methane, and carbon dioxide, as well as DNA adducts.

While little is known about possible detoxification pathways for MFH and its hydrolysis metabolite MMH, both acetylation and glutathione (GSH) reactions have been proposed. Several review papers have discussed the likelihood of acetylation as a detoxification pathway based on the apparent variation in severity and onset of adverse effects among *Gyromitra esculenta* poisoning cases, where it has been suggested that greater severity occurs in slow acetylators.

Key characteristics of carcinogens

The key characteristics (KCs) of carcinogens are characteristics of agents that cause cancer in humans and can encompass many types of mechanistic endpoints. OEHHA used the KCs of carcinogens to systematically identify, organize, and summarize mechanistic information from studies of MFH and its hydrolysis metabolite MMH. Evidence related to two of the KCs (KC1, KC2) was identified for MFH, and this evidence is briefly summarized here. See Section 5.2 for more detailed summaries of the data relevant to these KCs.

KC1. Is electrophilic or can be metabolically activated

MFH is hydrolyzed to MMH, which is further metabolized to form methyl radicals, formaldehyde, and acetaldehyde, which are each recognized as being electrophilic. Several studies of MMH report the *in vivo* and *in vitro* formation of DNA, RNA or protein adducts. In addition, methyl radical formation has been demonstrated in rat hepatocytes and neutrophils and liver microsomes incubated with MMH.

KC2. Is genotoxic

MFH has been tested for genotoxicity in a limited number of experimental systems and studies (rat and mouse hepatocytes *in vitro* and bacteria) for two genotoxicity endpoints (unscheduled DNA synthesis (UDS) and point mutations). The hydrolysis metabolite MMH has been tested in the same types of assays, as well as in additional *in vivo* and *in vitro* experimental systems. MFH induced mutations in the *Salmonella* reverse mutation assay in one strain, TA100, when tested at doses ≥ 0.04 mmol/plate, but not in other studies using lower doses or in other *Salmonella* strains, and did not induce UDS in mouse or rat hepatocytes *in vitro*. The hydrolysis metabolite MMH has been studied in a limited number of *in vivo* genotoxicity assays, and tested negative for the induction of UDS in the liver of rats and dominant lethal mutations in rats and mice. In *in vitro* studies, MMH induced UDS in mouse and rat hepatocytes, but not human fibroblasts, and forward mutations in Chinese hamster lung fibroblasts, but not mouse lymphoma cells. MMH induced mutations in the *Salmonella* reverse mutation assay in strains TA100 and TA102, and in some, but not all assays in strains TA1535 and TA1537. In *E. coli* assays MMH induced reverse mutations, SOS response, and DNA repair in all but one strain, and was negative in a gene recombination assay in *S. cerevisiae*. The MFH metabolites formaldehyde and acetaldehyde are genotoxic carcinogens.

Structure activity considerations

Five structurally related hydrazine or hydrazone compounds (gyromitrin, MMH, hydrazine, 1,1-dimethylhydrazine (1,1-DMH), and 1,2-dimethylhydrazine (1,2-DMH)) were identified for consideration and tumor site/type comparison with MFH, using findings from mouse and hamster cancer bioassays.

In mice, treatment-related tumor sites/types observed in common with MFH were lung (observed with all five comparator chemicals), liver hepatocellular (observed with MMH, hydrazine, and 1,1-DMH), and blood vessel (observed with MMH, 1,1-DMH, and 1,2-DMH). In hamsters, treatment-related tumor sites/types observed in common with MFH were liver hepatocellular (observed with hydrazine and 1,2-DMH) and liver malignant histiocytoma (Kupffer cell sarcoma) (observed with MMH).

1. INTRODUCTION

1.1 Chemical Identity of N-Methyl-N-formylhydrazine (MFH)

N-Methyl-N-formylhydrazine (MFH) is a hydrazine compound. Hydrazines, represented by the general chemical formula $R_2N\text{-}NR_2$, contain the nitrogen-nitrogen (N-N) covalent bond present in hydrazine ($H_2N\text{-}NH_2$). The chemical structure of MFH is shown in Figure 1. MFH is a colorless liquid at room temperature that is volatile and soluble in water and organic solvents (e.g., methanol, chloroform). Selected chemical properties of MFH are listed in Table 1.

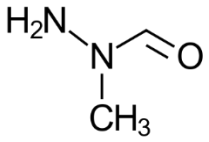


Figure 1. Chemical structure of N-methyl-N-formylhydrazine

Table 1. Selected chemical properties of N-methyl-N-formylhydrazine

Chemical name	N-Methyl-N-formylhydrazine (MFH)
Synonyms	1-Formyl-1-methyl-hydrazine; N-Methylformohydrazide
Chemical Abstracts Service Registry Number	758-17-8
Molecular formula	$C_2H_6N_2O$
Molecular weight (g/mol)	74.08
Boiling point (ºC)	143
Melting point (ºC)	10.7
Vapor pressure (mmHg)	5.55
Water solubility (mol/l)	7.84
Octanol-water coefficient (Log K_{ow})	-1.60
Octanol-air coefficient (Log K_{oa})	3.76

Values are from US EPA's CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard/chemical/synonyms/DTXSID2020840; accessed December 27, 2024).

1.2 Production, Sources, and Use

MFH is naturally occurring in wild edible *Gyromitra* mushrooms (List and Luft 1969; Schmidlin-Meszaros 1974; Toth 1983). Mushrooms in the genus *Gyromitra* are commonly known as “spring false morels,” “false morels,” or “lorchels.” These mushrooms also contain other hydrazine compounds, such as mono-methylhydrazine (MMH or methylhydrazine; a component in rocket propellants) and hydrazone compounds, such as gyromitrin (acetaldehyde N-methyl-N-formylhydrazone) (Dirks et al. 2023; Toth 2000). Gyromitrin may be nonenzymatically hydrolyzed to MFH, releasing acetaldehyde, and then further hydrolyzed to MMH (Figure 2). The nonenzymatic hydrolysis of gyromitrin to form MFH can occur at room temperature, and occurs more readily upon heating or under acidic conditions (*e.g.*, in the stomach) (Andary et al. 1985; Vohra et al. 2024).

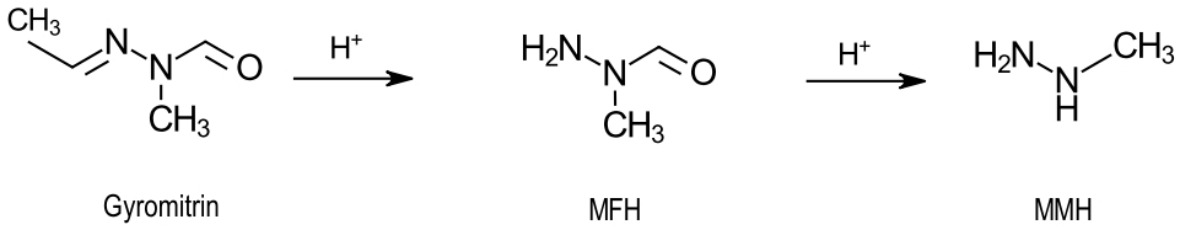


Figure 2. Nonenzymatic hydrolysis of gyromitrin to form MFH and MMH

In addition to its natural occurrence in wild mushrooms, MFH can be synthesized by reacting MMH with ethyl formate (Chemical Book 2023). MFH is used in the production of marbofloxacin (a fluorinated quinolone antibacterial agent used in veterinary medicine) and for research purposes (Santa Cruz Biotechnology 2025).

1.3 Occurrence and Exposure

Levels of MFH measured in mushrooms of the genus *Gyromitra* vary by the origin of the mushroom and the method of preservation (e.g., dried or freeze-dried). Reported concentrations range from 0.025 to 0.06% (or 250-600 mg/kg) in dried Swiss *G. esculenta* mushrooms (Schmidlin-Meszaros 1974; Toth et al. 1992), and from 10 (detection limit) to 200 mg/kg in dried Swiss false morels (Stijve 1978).

The occurrence in mushrooms of gyromitrin has been much better studied than the occurrence of MFH. Gyromitrin was identified (without quantification) in various *Gyromitra* species: *G. esculenta* (the most studied species with high levels of

gyromitrin), *G. antarctica*, *G. splendida*, *G. venenata*, *G. leucoxantha*, *G. gigas*, *G. fastigiate* and *G. montana* (Benjamin 2020; Dirks et al. 2023). Levels of gyromitrin in these mushrooms can vary by mushroom species (e.g., higher concentrations in *G. esculenta* vs. *G. gigas*), season, geological region, and altitude (Andary et al. 1985; Benjamin 2020). Reported concentrations of gyromitrin in fresh mushrooms range from 49.9 to 1676 mg/kg (Benjamin 2020; Dirks et al. 2023; Lima et al. 2012; List and Luft 1969; Pyysalo and Niskanen 1977; Stijve 1978), and in dried false morels range from 0.05 to 11.237% (or 500 to 112370 mg/kg) (Coulet and Guillot 1982; Lagrange et al. 2024; Schmidlin-Meszaros 1974).

Gyromitra mushrooms are consumed in the US and in other parts of the world; however, quantitative data on recent consumption levels in the US were not identified. In 1976, more than 100,000 people in the US annually consumed *Gyromitra esculenta* mushrooms (Toth 1995). More recent information on *Gyromitra* mushroom consumption in the US includes the following:

- The growing popularity of lorchel consumption in the US is illustrated by the Facebook group called “False Morels Demystified”, which was created in 2019 (Dirks et al. 2023).
- *Gyromitra* mushrooms are available to both professional and home cooks through online sources (e.g., dried false morels are available for purchase at sites such as Etsy), as well as from professional and/or amateur mushroom hunters.
- Continuing reports of gyromitrin-associated mushroom poisoning cases in the US (Benjamin 2020; Perisetti et al. 2018; Vohra et al. 2024). Note that raw or undercooked *Gyromitra* mushrooms are poisonous, with symptoms usually occurring six or more hours after consumption. Most cases reported gastrointestinal symptoms, neurotoxicity or hepatotoxicity. Among these reports, kidney failure and fatalities were not commonly observed (Benjamin 2020; Vohra et al. 2024).
    - For example, a total of 118 mushroom poisoning cases were reported to the Michigan Poison & Drug Information Center from 2002 to 2020 (Vohra et al. 2024). Most of the cases (91.5%) were related to *G. esculenta* and 8.5% were related to other *Gyromitra* species.

1.4 Review by Other Health Agencies

MFH has not been reviewed or classified as to its potential carcinogenicity by any of the authoritative bodies [i.e., the International Agency for Research on Cancer (IARC), the US Environmental Protection Agency (US EPA), the National Institute for Occupational

Safety and Health (NIOSH), the National Toxicology Program (NTP) Report on Carcinogens (RoC), the US Food and Drug Administration (US FDA)] or other international health agencies.

2. OVERVIEW OF SYSTEMATIC LITERATURE REVIEW APPROACH

2.1 Literature Search Process

Literature searches on the carcinogenicity of MFH were initiated in December 2024. The goal was to identify peer-reviewed journal articles, print and digital books, reports, and gray literature that potentially reported toxicological and epidemiologic information on the carcinogenicity of this chemical.

As described below, we used an approach similar to that recommended by the National Toxicology Program (NTP) Handbook for Preparing Report on Carcinogens (RoC) Monographs (NTP 2015).

The searches were conducted using the following three approaches:

- Primary searches in major biomedical databases, conducted by OEHHA librarian Nancy Firchow, MLS.
- Searches in other data sources, including authoritative reviews and reports, and databases or web resources, conducted by OEHHA scientists and the OEHHA librarian.
- Additional focused searches, conducted by OEHHA scientists.

Primary searches for MFH were executed using chemical synonyms in combination with search terms for human cancer studies, animal cancer studies, toxicokinetic studies, and mechanistic studies for genotoxicity and other key characteristics. There were no restrictions in the searches on exposure route or duration of exposure on cancer studies in humans, cancer studies in animals or mechanistic studies, or on publication language. For detailed information on the literature search process, please see Appendix A.

2.2 Literature Screening Process

HAWC (Health Assessment Workspace Collaborative, https://hawcproject.org) (Shapiro et al. 2018) was used as a tool to screen and tag the literature. First, citations retrieved from the literature searches were uploaded to EndNote libraries, and duplicates were removed. Next, these EndNote libraries were uploaded to HAWC for multi-level screening using specific inclusion and exclusion criteria (see Appendix A).

In Level 1 screening in HAWC, each citation was first screened by at least one OEHHA scientist, based solely on titles and abstracts, to eliminate studies or articles that do not contain information on MFH on any of the key topics covered in this cancer hazard identification document, such as cancer studies in humans and animals, toxicokinetics, metabolism, genotoxicity, or other cancer-associated mechanisms. The Level 1 screen was intended to identify all studies deemed to have a reasonable possibility of containing information that could be useful for the review process. Papers identified for inclusion during Level 1 screening were tagged in HAWC according to key topics.

In Level 2 screening, full-text papers for all citations that passed the Level 1 screening were obtained and screened by at least one OEHHA scientist, using similar inclusion/exclusion criteria as was used in the Level 1 screening.

Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC. Level 1 and 2 screenings were conducted and HAWC search results were updated if additional relevant studies in addition to those cited in the original set of publications ("secondary citations") were identified.

Total references

More than 200 references, including peer-reviewed journal articles and government reports, were identified through these search strategies. Among these, over 90 references were cited in this document.

3. CARCINOGENICITY STUDIES IN HUMANS

No cancer epidemiological studies on the effects of human exposure to MFH were identified in the literature search conducted by OEHHA (Appendix A).

4. CARCINOGENICITY STUDIES IN ANIMALS

OEHHA identified multiple carcinogenicity studies of MFH in animals, with 12 lifetime studies in Swiss mice and two lifetime studies in Syrian golden hamsters. These studies were all conducted at the University of Nebraska Medical Center's Eppley Institute for Research in Cancer, in the laboratory of Dr. Bela Toth. Table 2 provides an overview of the available carcinogenicity studies of MFH. In male and female mice, the studies include eight conducted via drinking water and four via subcutaneous (*s.c.*) injection. In hamsters there were two drinking water studies of MFH.

In addition to the carcinogenicity studies of MFH, two lifetime carcinogenicity studies of raw *Gyromitra esculenta* mushrooms were conducted in mice by the same research

group. Swiss mice (one study in males, the other in females) were fed the mushrooms *ad libitum* three days per week and semisynthetic diet for the other four days of the week for life (Toth et al. 1992). These *Gyromitra esculenta* studies are briefly discussed in Section 4.4.

The Toth laboratory had extensive experience conducting research in the area of chemical carcinogenesis, conducting and publishing findings from carcinogenicity studies over a period of more than 40 years. Chemicals studied included numerous hydrazines, urethane, 7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons, and many others. In addition to research designed to test for carcinogenic activity, the laboratory conducted studies to investigate age susceptibility to tumor development (e.g., urethane), dosing and dose rate effects, and the cancer preventative and therapeutic potential of various chemicals.

The MFH carcinogenicity studies were designed, conducted, and reported in a manner consistent with the practices at the time, and follow many of the recommendations discussed by contemporaneous working groups and committees convened by the IARC and NTP (IARC 1980, 1986; NTP 1984). The MFH studies included 50 animals in each treatment group and 100 animals in each control group. The purity of the administered MFH was ≥99% in each of the studies, and in the drinking water studies the dosing solutions were prepared every three days to ensure the integrity of the compound in aqueous solution. The pathology evaluation involved full necropsies and histopathologic examination of liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinals, lungs (four lobes), and other organs with gross pathologic changes, for all animals. Most of the studies provided a detailed description of the criteria used for the classification of each tumor type observed. Information on the incidence of spontaneous tumors observed in the Eppley Institute's randomly bred colony of Syrian golden hamsters, which was established in 1959, was available from Pour et al. (1976a, b, c).

Limitations in the Toth laboratory's MFH studies include absence of information on whether the pathologist was blinded to treatment status, high toxicity and poor survival in treated animals (mice) at early time points in some of the drinking water studies, and reduced survival in controls during the second year of life in the hamster studies. No individual animal time-to-tumor data were provided; however, the authors reported the number of animals alive at 10-week increments and time of first death for each tumor type. In some of the MFH mouse studies the same set of untreated mice was used as the control group and reported in multiple publications. Specifically, data from the same male and female control mice were used in four publications (Toth and Nagel 1978; Toth and Patil 1980a, b; Toth et al. 1979), and data from a different set of studies in male and female control mice were used in two publications (Toth and Patil 1982; Toth and Patil 1983). OEHHA was not able to identify a report or publication that summarized

historical control data on the incidence of spontaneous tumors for the Eppley Institute's randomly bred Swiss mouse colony, which was established in 1951.

Table 2. Carcinogenicity studies of MFH in Swiss mice and Syrian golden hamsters

Species, strain	Group size	Sex	Route, age at first exposure, exposure duration	Administered concentration [w/v] or dose[1]	Reference
Mouse, Swiss	100 (control), 50 (treated)	Male	drinking water, starting at 6 weeks of age, lifetime	0, 0.0078%, 0.0156%	Toth and Nagel (1978)
		Female			
		Male	drinking water, starting at 6 weeks of age, lifetime	0, 0.0039%	Toth et al. (1979)
		Female			
		Male	drinking water, starting at 6 weeks of age, lifetime	0, 0.001%, 0.002%	Toth and Patil (1980b)
		Female			
		Male	drinking water, starting at 6 weeks of age, lifetime	0, 0.00025%, 0.0005%	Toth and Patil (1982)
		Female			
		Male	*s.c.*, 6 weeks of age, single injection	0, 100, 120 mg/kg-bw (males); 0, 180 mg/kg-bw (females)	Toth and Patil (1980a)
		Female			
		Male	*s.c.*, starting at 6 weeks of age, 40 weekly injections	0, 10 mg/kg-bw (males); 0, 20 mg/kg-bw (females)	Toth and Patil (1983)
		Female			
Hamster, Syrian golden	100 (control), 50 (treated)	Male	drinking water, starting at 6 weeks of age, lifetime	0, 0.0078%	Toth and Patil (1979)
		Female			

[1] The administered concentrations in the drinking water studies are expressed as percent in weight per volume (w/v), i.e., grams of MFH per 100 milliliters of water. The administered doses in the *s.c.* injection studies are expressed as milligram per kilogram body weight (mg/kg-bw).

4.1 Carcinogenicity Studies of MFH in Mice

4.1.1. Lifetime drinking water studies in male and female Swiss mice

Toth and Nagel (1978)

Male and female Swiss albino mice were administered 0, 0.0078%, or 0.0156% [w/v] MFH in drinking water starting at 6 weeks of age for their lifetime. In each study 100 animals were included as controls and 50 animals were included in each treatment group. The purity of MFH used in the studies was > 99%. The average daily intake of MFH was reported to be 0, 0.99, and 1.35 mg per animal for males and 0, 0.80, and 1.34 mg per animal for females in the control, low-, and high-dose groups, respectively. The authors did not report information on body weights or body weight changes for any groups during the studies.

The authors noted that the high dose of MFH (0.0156% in drinking water) administered in each study had a severe effect on survival with the majority of animals in the high dose groups dying between 20 and 30 weeks of age. The early mortality of some animals may decrease the likelihood of developing treatment-related tumors before death. This could lead to misleading conclusions when reporting tumor incidence based on the total number of animals in the group at the start of the exposure. The authors did not report time-to-tumor information for individual animals. Thus, for the male mouse study (Table 3) and the female mouse study (Table 4), OEHHA presents tumor incidence data for each tumor site tabulated as the number of tumor-bearing animals divided by the number of animals alive at 20 weeks of age.

Males

In males, survival was significantly lower in both treatment groups compared to the controls. At 20 weeks of age, the survival rates in the low- (0.0078%) and high-dose (0.0156%) groups were 94% and 52%, while the control group had a survival rate of 98%. By 30 weeks of age, the survival rates in the low- and high-dose groups were 58% and 16%, respectively, compared to 92% in controls, and by 50 weeks of age these rates were 24% and 4%, respectively, compared to 80% in controls. No animals in either MFH treatment group survived to 80 weeks of age.

As shown in Table 3, statistically significant increases in tumors were observed in the lung, liver, gallbladder, and bile duct in the 0.0078% (low-dose) group.

In the lung, the incidence of adenoma in the 0.0078% group was statistically significantly increased compared to the control group by pairwise comparison. Lung adenomas observed in mice by the Toth laboratory were described as tumors of the alveolar type B (type II) cells (Toth and Shimizu 1974), and most likely correspond to

alveolar/bronchiolar adenomas, which in mice consist of cells with cytologic features of alveolar type II cells (Dixon et al. 1999).

In the liver, statistically significant increases in hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma and carcinoma combined were observed in the 0.0078% group by pairwise comparison with controls, with dose-related trends for hepatocellular carcinoma, and hepatocellular adenoma and carcinoma combined. Hepatoma, used by Toth and Nagel (1978), is another term for hepatocellular adenoma (Frith et al. 1994). In this document, we will use the term hepatocellular adenoma. Hepatocellular adenoma can progress to the malignant hepatocellular carcinoma (McConnell et al. 1986).

In the gallbladder, five adenomas were observed in the 0.0078% group and the increase was statistically significant by pairwise comparison with controls. Spontaneous occurrence of gallbladder tumors in mice is rare (Yoshitomi and Boorman 1994), and data collected from four published studies from the Toth laboratory indicate no occurrence of gallbladder adenoma or adenocarcinoma among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

In bile ducts, statistically significant increases in cholangiocarcinoma, and cholangioma and cholangiocarcinoma combined were observed in the 0.0078% group. Spontaneous occurrence of cholangioma or cholangiocarcinoma is very rare (Harada et al. 1999), and data collected from four published studies from the Toth laboratory indicate no occurrence of bile duct tumors among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

Table 3. Tumor incidence in male Swiss mice administered MFH in drinking water (Toth and Nagel 1978)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water			Trend test *p*-value
		0	0.0078%	0.0156%	
		Average dose (mg/day/animal)			
		0	0.99	1.35	
Lung [2]	**Adenoma (week 20) [3]**	17/98	20/47**	4/26	NS
Liver [2]	**Hepatoma (hepatocellular adenoma)**	2/98	5/47*	2/26	NS
	Hepatocellular carcinoma	0/98	6/47***	1/26	< 0.05
	Hepatoma (hepatocellular adenoma) and carcinoma combined (week 24)	2/98	11/47***	3/26	< 0.01
Gallbladder	**Adenoma (week 24) (r)**	0/98	5/47**	0/26	NS
Bile duct	**Cholangioma (r)**	0/98	2/47	0/26	NS
	Cholangiocarcinoma (r)	0/98	3/47*	0/26	NS
	Cholangioma and cholangiocarcinoma combined (week 33) (r)	0/98	5/47**	0/26	NS

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 20 weeks of age (denominator). In the case of bile duct tumors, the selection of 20 weeks instead of 30 weeks was based on poor survival in the treated groups at 30 weeks of age. The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Nagel 1978). For bile duct tumor incidence, a sensitivity analysis was performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B1 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

[1] Age when first tumor was observed is "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Nagel 1978).

[2] The authors (Toth and Nagel 1978) reported the combined incidence of lung and liver tumors for the control group. Tumor incidences in controls for lung adenoma, hepatocellular adenoma, and hepatocellular carcinoma are as reported in Toth and Erickson (1978).

[3] Age when first lung adenoma was observed is as reported by Toth and Nagel (1978) for lung adenomas and adenocarcinomas, combined.

NS, not significant; r, rare tumor

Females

In females, survival in the treated groups was significantly lower in both treatment groups compared to the controls. In the high-dose (0.0156%) group, survival decreased to 74% at 20 weeks of age and dropped to 22% by 30 weeks of age. In the low-dose (0.0078%) group, survival was 96% at 20 weeks, 86% at 30 weeks, and 66% at 40 weeks of age. Survival in the control group was 100% at 20 weeks, 99% at 30 weeks,

and 96% at 40 weeks of age. No animals in either MFH treatment group survived to 80 weeks of age.

As shown in Table 4, statistically significant increases in tumors were observed in the lung, liver, and gallbladder in the 0.0078% (low-dose) group, and in the liver in the 0.0156% (high-dose) group. In addition, rare bile duct tumors were observed in treated, but not control females.

In the lung, the incidence of adenoma in the 0.0078% group was statistically significantly increased compared to the controls by pairwise comparison, with a dose-related trend.

In the liver, statistically significant increases in hepatocellular adenoma (in the 0.0078% and 0.0156% groups), hepatocellular carcinoma (in the 0.0078% group), and hepatocellular adenoma and carcinoma combined (in the 0.0078% and 0.0156% groups) were observed by pairwise comparison with controls, with dose-related trends for hepatocellular adenoma and hepatocellular adenoma and carcinoma combined.

In the gallbladder, a statistically significant increase in adenoma was observed in the 0.0078% group. Spontaneous occurrence of gallbladder tumors is rare in mice (Yoshitomi and Boorman 1994), and data collected from four published studies from the Toth laboratory confirm this, with one gallbladder tumor (an adenoma) reported among 399 untreated female Swiss mice (spontaneous rate 0.25%) (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

Bile duct tumors were observed in treated, but not control animals. In the 0.0078% group, one animal developed cholangioma and another developed cholangiocarcinoma. In the 0.0156% group one animal developed a cholangioma. Spontaneous occurrence of cholangioma or cholangiocarcinoma is very rare (Harada et al. 1999), and data collected from four published studies from the Toth laboratory indicate no occurrence of bile duct tumors among 399 untreated female Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

Table 4. Tumor incidence in female Swiss mice administered MFH in drinking water (Toth and Nagel 1978)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water			Trend test *p*-value
		0	0.0078%	0.0156%	
		Average dose (mg/day/animal)			
		0	0.80	1.34	
Lung [2]	**Adenoma (week 22) [3]**	10/100	30/48***	8/37	< 0.01
Liver	**Hepatoma (hepatocellular adenoma)**	0/100	12/48***	3/37*	< 0.01
	Hepatocellular carcinoma	0/100	10/48***	0/37	NS
	Hepatoma (hepatocellular adenoma) and carcinoma combined (week 35)	0/100	22/48***	3/37*	< 0.01
Gallbladder	**Adenoma (week 59) (r)**	0/100	4/48**	1/37	NS
Bile duct	**Cholangioma and cholangiocarcinoma combined (week 31) (r)**	0/100	2/48	1/37	NS

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 20 weeks of age (denominator). In the case of liver, gallbladder, and bile duct tumors, the selection of 20 weeks instead of 30, 50, or 30 weeks, respectively, was based on poor survival in the treated groups at 30 weeks of age. The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Nagel 1978). For liver, gallbladder, and bile duct tumor incidences, sensitivity analyses were performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B2 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p \leq 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Nagel 1978).

[2] The authors (Toth and Nagel 1978) reported the combined incidence of lung adenoma and adenocarcinoma for the control group. Tumor incidence in controls for lung adenoma is as reported in Toth and Erickson (1978).

[3] Age when first lung adenoma was observed is as reported by Toth and Nagel (1978) for lung adenomas and adenocarcinomas, combined.

NS, not significant; r, rare tumor

Toth et al. (1979)

Male and female Swiss albino mice were administered 0 or 0.0039% [w/v] MFH in drinking water starting at 6 weeks of age for their lifetime. In each study there were 100 animals in the control group and 50 animals in the treated group. The purity of MFH used in these studies was reported as 99%. Dose selection was based on the substantial decreases in survival observed in the studies of Toth and Nagel (1978). The average daily intake of MFH in the 0.0039% dose groups was reported to be 0.4 mg per animal for both males and females. The authors did not report information on body weights or body weight changes for any groups during the studies.

Males

In males, survival in the 0.0039% MFH treated group was significantly lower than in controls, with a large number of animals dying between 40 and 50 weeks of age. In the treated animals, survival was 82% at 40 weeks and dropped to 60% by 50 weeks of age. In controls, survival was 88% at 40 weeks and 80% at 50 weeks of age. No animals in the MFH treated group survived to 90 weeks of age. Given the reduced survival in the treated males after 40 weeks of age, OEHHA presents the tumor incidence data from this study as the number of tumor-bearing animals divided by the number of animals alive at 40 weeks of age.

As shown in Table 5, statistically significant increases in tumors were observed in the lung, liver, gallbladder, bile duct, and seminal vesicles in male mice treated with 0.0039% MFH in drinking water.

In the lung, the incidences of adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were statistically significantly increased by 0.0039% MFH treatment compared to controls by pairwise comparison. These mouse lung tumors described by the Toth laboratory as adenomas and adenocarcinomas most likely correspond to alveolar/bronchiolar adenomas and carcinomas (Dixon et al. 1999). Alveolar/bronchiolar adenoma can progress to the malignant alveolar/bronchiolar carcinoma (McConnell et al. 1986).

In the liver, statistically significant increases in hepatocellular adenoma, carcinoma, and hepatocellular adenoma and carcinoma combined were observed with 0.0039% MFH treatment.

In the gallbladder, five rare adenomas were observed, and the increase was statistically significant.

In the bile duct, the increased incidence of rare cholangioma was statistically significant.

In the seminal vesicles, a statistically significant increase in adenoma was observed. Spontaneous occurrence of seminal vesicle adenoma is rare in mice, with an incidence

rate of 0.07% (one out of 1355 animals) in untreated male B6C3F1 mice (Haseman et al. 1999; Radovsky et al. 1999). Data collected from four published studies from the Toth laboratory indicate no occurrence of seminal vesicle tumors among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

A nonsignificant increase in blood vessel tumors was observed in treated males. Among the 0.0039% MFH dosed animals, four developed angioma (three in the liver, one in testis) and one of the four also had an angiosarcoma (liver). In the controls (as reported in Toth (1979)), three animals developed angioma (two in the liver, one in the anal gland). Two other control animals developed angiosarcoma (one in the liver, another in the pararenal fat).

Table 5. Tumor incidence in male Swiss mice administered MFH in drinking water (Toth et al. 1979)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water	
		0	0.0039%
		Average dose (mg/day/animal)	
		0	0.4
Lung [2]	Adenoma	17/88	32/41***
	Adenocarcinoma	6/88	14/41***
	Adenoma and adenocarcinoma combined (week 40)	22/88	34/41***
Liver [2]	Hepatoma (hepatocellular adenoma)	2/88	9/41***
	Hepatocellular carcinoma	0/88	19/41***
	Hepatoma (hepatocellular adenoma) and carcinoma combined (week 50)	2/88	28/41***
Gallbladder	Adenoma (week 50) (r)	0/88	5/41**
Bile duct	Cholangioma (week 50) (r)	0/88	6/41***
Seminal vesicles	Adenoma (week 62) (r)	0/88	3/41*
Blood vessels [3]	Angioma and angiosarcoma combined (week 57)	5/88	4/41

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 40 weeks of age (denominator). In the case of liver, gallbladder, bile duct, seminal vesicle, and blood vessel tumors, the selection of 40 weeks instead of 50, 50, 50, 60, or 50 weeks, respectively, was based on poor survival in the treated group at 50 weeks of age. The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth et al. 1979). For liver, gallbladder, bile duct, seminal vesicles, and blood vessel tumor incidences, sensitivity analyses were performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B3 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth et al. 1979).

[2] The authors (Toth et al. 1979) reported the combined incidence of lung adenoma and adenocarcinoma and of hepatocellular adenoma and carcinoma for the control group. Tumor incidences in controls for lung adenoma, lung adenocarcinoma, hepatocellular adenoma, and hepatocellular carcinoma are as reported in Toth and Erickson (1978).

[3] Tumor incidence in controls for angioma and angiosarcoma combined of the blood vessels is as reported in Toth (1979).

r, rare tumor

Females

In females, survival in the 0.0039% MFH treated group was similar to controls until 60 to 70 weeks of age. In the treated animals, survival was 80% at 60 weeks and dropped to 58% by 70 weeks of age. In controls, survival was 91% at 60 weeks and 78% at 70 weeks of age. No animals in the MFH treated group survived to 100 weeks of age.

As shown in Table 6, statistically significant increases in tumors were observed in the lung, liver, gallbladder, and blood vessels in female mice treated with 0.0039% MFH in drinking water. The first occurrence of lung, liver, and blood vessel tumors occurred well before 60 weeks of age, when differences in survival between treated and control females were apparent, but not as pronounced as at 70 weeks of age.

In the lung, incidences of lung adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were statistically significantly increased.

In the liver, statistically significant increases in hepatocellular adenoma, carcinoma, and hepatocellular adenoma and carcinoma combined were observed.

In the gallbladder, there were statistically significant increases in rare tumors, specifically, adenoma, and adenoma and adenocarcinoma combined, compared to controls.

In the blood vessels of treated animals, statistically significant increases in angioma, angiosarcoma, and combined angioma and angiosarcoma were observed. Among the 0.0039% dosed animals, seven animals developed angioma (liver), and 10 animals had angiosarcoma (liver, lung or uterus). In the controls, as reported by Toth (1979), four animals developed angioma (liver or ovary), and four had angiosarcoma (liver, uterus or lymph nodes).

Table 6. Tumor incidence in female Swiss mice administered MFH in drinking water (Toth et al. 1979)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water	
		0	0.0039%
		Average dose (mg/day/animal)	
		0	0.4
Lung [2]	Adenoma	10/99	41/49***
	Adenocarcinoma	6/99	24/49***
	Adenoma and adenocarcinoma combined (week 39)	15/99	43/49***
Liver	Hepatoma (hepatocellular adenoma)	0/96	10/47***
	Hepatocellular carcinoma	0/96	8/47***
	Hepatoma (hepatocellular adenoma) and carcinoma combined (week 46)	0/96	18/47***
Gallbladder	Adenoma (r)	0/91	4/40**
	Adenocarcinoma (r)	0/91	1/40
	Adenoma and adenocarcinoma combined (week 62) (r)	0/91	5/40**
Blood vessels [2, 3]	Angioma	4/99	7/49*
	Angiosarcoma	4/99	10/49**
	Angioma and angiosarcoma combined (week 39)	8/99	17/49***

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 30 weeks of age for lung and blood vessel tumors, 40 weeks of age for liver tumors and 60 weeks of age for gallbladder tumors. The denominators were taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth et al. 1979). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

[1] Age when first tumor was observed is the “age at death”, as reported for each tumor type in Table 2 of the published study (Toth et al. 1979).

[2] The authors (Toth et al. 1979) reported the combined incidence of lung adenoma and adenocarcinoma and of blood vessel angioma and angiosarcoma for the control group. Tumor incidences in controls for lung adenoma and lung adenocarcinoma are as reported in Toth and Erickson (1978).

[3] Tumor incidences in controls for angioma and angiosarcoma of the blood vessels are as reported in Toth (1979).

r, rare tumor

Toth and Patil (1980b)

Male and female Swiss albino mice were administered 0, 0.001%, or 0.002% [w/v] MFH in drinking water starting at six weeks of age for their lifetime. In each study 100 animals were included as controls and 50 animals were included in each treatment group. The purity of MFH used in the studies was > 99%. The average daily intake of MFH was reported to be 0, 0.14, and 0.29 mg per animal for males and 0, 0.12, and 0.21 mg for females in the control, low-, and high-dose groups, respectively. The authors did not report information on body weights or body weight changes for any groups during the studies.

Males

In males, survival declined in the high-dose (0.002%) MFH group between 40 to 50 weeks of age, from 82% to 72%, and further declined to 60% at 60 weeks of age. Survival in the low-dose (0.001%) group at 40, 50 and 60 weeks of age was 86%, 84%, and 72%, respectively. Survival in controls at 40, 50 and 60 weeks of age was 88%, 80%, and 62%, respectively. No animals in the high-dose, two animals in the low-dose, and three animals in the control group survived to 100 weeks of age.

As shown in Table 7, statistically significant increases in tumors were observed in the lung, liver, gallbladder and blood vessels. Taking into account the age at first tumor occurrence and the decline in survival in the high-dose animals after 40 weeks of age, OEHHA presents the lung and gallbladder tumor incidence data as the number of tumor-bearing animals divided by the number of animals alive at first occurrence of tumor (i.e., 20 and 30 weeks of age, respectively), and the liver and blood vessel tumor incidence data as the number of tumor-bearing animals divided by the number of animals alive at 40 weeks of age.

In the lung, increases in adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were statistically significant in both the 0.001% and 0.002% dose groups by pairwise comparison with controls, all with dose-related trends.

In the liver, statistically significant increases in hepatocellular adenoma and carcinoma combined occurred in the low-dose (0.001%) group, and hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma and carcinoma combined occurred in the high-dose (0.002%) group, all with dose-related trends.

In the gallbladder, statistically significant increases in rare tumors, specifically adenoma, and adenoma and adenocarcinoma combined were observed in each dose group, each with a dose-related trend.

In blood vessels, statistically significant increases were observed in angiosarcoma (liver, subcutis, spleen) in the low-dose (0.001%) group and angioma and angiosarcoma

combined (liver, subcutis, spleen) in the low- (0.001%) and high-dose (0.002%) groups, with a dose-related trend. In the controls (as reported in Toth (1979)), angioma or angiosarcoma were observed at various sites (liver, anal gland, pararenal fat).

Table 7. Tumor incidence in male Swiss mice administered MFH in drinking water (Toth and Patil 1980b)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water			Trend test *p*-value
		0	0.001%	0.002%	
		Average dose (mg/day/animal)			
		0	0.14	0.29	
Lung [2]	Adenoma	17/98	31/49***	26/46***	< 0.0001
	Adenocarcinoma	6/98	21/49***	14/46***	< 0.0001
	Adenoma and adenocarcinoma combined (week 21)	22/98	36/49***	31/46***	< 0.0001
Liver [2]	Hepatoma (hepatocellular adenoma)	2/88	4/43	9/41***	< 0.001
	Hepatocellular carcinoma	0/88	2/43	5/41**	< 0.01
	Hepatoma (hepatocellular adenoma) and carcinoma combined (week 54)	2/88	6/43*	14/41***	< 0.0001
Gallbladder	Adenoma (r)	0/92	6/46**	7/43***	< 0.001
	Adenocarcinoma (r)	0/92	0/46	1/43	NS
	Adenoma and adenocarcinoma combined (week 38) (r)	0/92	6/46**	8/43***	< 0.0001
Blood vessels [2]	Angioma	3/88	0/43	4/41	NS
	Angiosarcoma	2/88	13/43***	3/41	NS
	Angioma and angiosarcoma combined (week 52)	5/88	13/43***	7/41*	< 0.05

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 20 weeks of age for lung tumors [based on first occurrence of these tumors], 30 weeks of age for gallbladder tumors [based on first occurrence of these tumors], and 40 weeks of age for liver and blood vessel tumors [based on decreases in survival in the high-dose group between 40 to 50 weeks of age] (denominator). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1980b). For liver and blood vessel tumor incidences, sensitivity analyses were performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B4 in Appendix B).

Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.
[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1980b).
[2] The authors (Toth and Patil 1980b) reported the combined incidence of lung, liver and blood vessel tumors for the control group. Tumor incidences in controls for lung adenoma, lung adenocarcinoma, hepatocellular adenoma, and hepatocellular carcinoma are as reported by Toth and Erickson (1978). Tumor incidences in controls for angioma and angiosarcoma of the blood vessels are as reported by Toth (1979).
NS, not significant; r, rare tumor

Females

In females, survival declined in the high-dose (0.002%) MFH group between 50 to 60 weeks of age, from 96% to 76%, and further declined to 50% at 70 weeks of age. Survival in the low-dose (0.001%) group at 50, 60 and 70 weeks of age was 88%, 84%, and 58%, respectively. Survival in controls at 50, 60 and 70 weeks of age was 95%, 91%, and 78%, respectively. One animal in the high-dose, two animals in the low-dose, and 28 animals in the control group survived to 100 weeks of age.

As shown in Table 8, statistically significant increases in tumors were observed in the lung, gallbladder, and blood vessels. The first occurrence of lung, gallbladder and blood vessel tumors occurred before 60 weeks of age, when differences in survival between treated and control females were apparent.

In the lung, incidences of adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were statistically significantly increased by pairwise comparison with controls in both dose groups, all with dose-related trends.

In the gallbladder, a statistically significant increase in rare adenoma and adenocarcinoma combined was observed in the high-dose group, with a dose-related trend.

In blood vessels, statistically significant increases were observed in angioma (liver, ovary, subcutis), and angiosarcoma (liver, ovary, spleen) in the high-dose (0.002%) group, and angioma and angiosarcoma combined in the low- (0.001%) and high-dose (0.002%) groups, all with dose-related trends. In the controls (as reported in Toth (1979)), angioma or angiosarcoma were observed at various sites (liver, ovary, uterus, lymph nodes).

Table 8. Tumor incidence in female Swiss mice administered MFH in drinking water (Toth and Patil 1980b)

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

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 30 weeks of age for lung tumors, and 50 weeks of age for gallbladder and blood vessel tumors). (denominator). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1980b). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1980b).

[2] The authors (Toth and Patil 1980b) reported the combined incidence of lung adenoma and adenocarcinoma and of blood vessel angioma and angiosarcoma for the control group. Tumor incidences in controls for lung adenoma and lung adenocarcinoma are as reported in Toth and Erickson (1978). Tumor incidences in controls for angioma and angiosarcoma of the blood vessels in the controls are as reported in Toth (1979).

NS, not significant; r, rare tumor

Toth and Patil (1982)

Male and female Swiss albino mice were administered 0, 0.00025%, or 0.0005% [w/v] MFH in drinking water starting at six weeks of age for their lifetime. In each study there were 100 animals in the control group and 50 animals in the treated groups. The purity of MFH used in these studies was > 99%. The average daily intake of MFH was reported to be 0, 0.03, and 0.06 mg per animal for males, and 0, 0.02, and 0.05 mg per

animal for females in the control, low-, and high-dose groups, respectively. The authors did not report information on body weights or body weight changes for any groups during the studies.

Males

In males, survival declined in the high-dose (0.0005%) group from 94% at 40 weeks of age to 86% at 50 weeks of age, to 54% at 60 weeks of age. Survival in the low-dose (0.00025%) group at 40, 50 and 60 weeks of age was 90%, 78%, and 52%, respectively. Survival in controls at 40, 50 and 60 weeks of age was 83%, 80%, and 70%, respectively. Three animals in the high-dose, none in the low-dose, and 8 in the control group survived to 100 weeks of age.

As shown in Table 9, statistically significant increases in tumors were observed in the lung, forestomach, glandular stomach, and subcutis.

In the lung, there were statistically significant increases by pairwise comparison with controls in both dose groups for adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined, all with dose-related trends.

In the forestomach, squamous cell carcinoma was statistically significantly increased in the high dose (0.0005%) group, with a significant trend. Spontaneous occurrence of forestomach squamous cell carcinoma is rare in male mice, with a rate 0.1% (2 out of 1355 animals) in male B6C3F1 mice (Haseman et al. 1999). Data collected from four published studies from the Toth laboratory indicate no occurrence of squamous cell carcinoma of the forestomach among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

In the glandular stomach, a statistically significant increase in polypoid adenoma was observed in the low-dose (0.00025%) group. Spontaneous occurrence of polypoid adenoma of the glandular stomach is uncommon in male mice (Leininger et al. 1999), and rare in male Swiss mice, based on data collected from four published studies from the Toth laboratory, with no such tumors observed among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

In the subcutis, a statistically significant increase in fibrosarcoma was observed in the low-dose (0.00025%) group.

Table 9. Tumor incidence in male Swiss mice administered MFH in drinking water (Toth and Patil 1982)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water			Trend test *p*-value
		0	0.00025%	0.0005%	
		Average dose (mg/day/animal)			
		0	0.03	0.06	
Lung	Adenoma	13/83	20/45***	18/47**	< 0.01
	Adenocarcinoma	9/83	14/45**	11/47*[2]	< 0.05
	Adenoma and adenocarcinoma combined (week 42)	19/83	27/45***	24/47**	< 0.001
Forestomach	Squamous cell carcinoma (week 54) (r)	0/80	0/39	3/43*	< 0.05
Glandular stomach	Polypoid adenoma (week 57) (r)	0/80	3/39*	0/43	NS
Subcutis	Fibrosarcoma (week 45)	1/83	5/45*	0/47	NS

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 40 weeks of age for lung and subcutis tumors, and 50 weeks of age for forestomach and glandular stomach tumors). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1982). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1982).

[2] The *p* value is 0.051.

NS, not significant; r, rare tumor.

Females

In females, survival declined in the high-dose (0.0005%) group from 94% at 50 weeks of age to 76% at 60 weeks of age. Survival in the low-dose (0.00025%) group at 50 and 60 weeks of age was 96% and 90%, respectively. Survival in controls at 40, 50 and 60 weeks of age was 94%, 88%, and 83%, respectively. Six animals in the high-dose, none in the low-dose, and 13 in the control group survived to 100 weeks of age.

As shown in Table 10, statistically significant increases in lung adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were observed in both dose groups by pairwise comparisons with the control, all with dose-related trends.

Table 10. Tumor incidence in female Swiss mice administered MFH in drinking water (Toth and Patil 1982)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water			Trend test *p*-value
		0	0.00025%	0.0005%	
		Average dose (mg/day/animal)			
		0	0.02	0.05	
Lung	Adenoma	20/94	23/48**	21/48**	< 0.01
	Adenocarcinoma	14/94	23/48***	22/48***	< 0.0001
	Adenoma and adenocarcinoma combined (week 49)	29/94	31/48***	32/48***	< 0.0001

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 40 weeks of age). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1982). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1982).

4.1.2 Subcutaneous (s.c.) injection studies with lifetime observation in male and female Swiss mice

Single *s.c.* injection studies (Toth and Patil 1980a)

Groups of 6-week-old male and female Swiss albino mice were subcutaneously injected once with MFH and observed for their lifetime. In each study there were 100 untreated animals in the control group and 50 animals in each treatment group. MFH was synthesized in the laboratory according to List and Luft (1967), with a purity > 99%. The male mouse study included two treatment groups, one injected once subcutaneously with 100 micrograms (µg) per gram (g) body weight [equivalent to 100 milligrams per kilogram body weight (mg/kg-bw)] of MFH, and the other injected once subcutaneously with 120 µg/g-bw [120 mg/kg-bw] of MFH. The female mouse study included a single treatment group that received a single *s.c.* injection of 180 µg/g-bw [180 mg/kg-bw]. The average body weight reported at the age of *s.c.* injection was 32 g for males and 24 g for females.

Males

In males, survival in the high-dose (120 mg/kg-bw) group was reduced at 10 weeks of age to 80%, and continued to decline thereafter (e.g., 70% and 64% at 20 and 30 weeks of age, respectively). Survival in the low-dose (100 mg/kg-bw) group at 10, 20 30, 40 and 50 weeks of age was 96%, 92%, 86%, 80%, and 66%, respectively. Survival

in controls at 10, 20, 30, 40, and 50 weeks of age was 100%, 98%, 92%, 88%, and 80%, respectively. No animals in the high-dose, two in the low-dose, and three in the control group survived to 100 weeks of age. Given the early and significant reduction in survival in the high-dose group, OEHHA presents tumor incidence data from this study as the number of tumor-bearing animals divided by the number of animals alive at 10 weeks of age.

As shown in Table 11, statistically significant increases in tumors of the preputial gland were observed in male mice after a single *s.c.* injection of MFH in both the low- (100 mg/kg-bw) and high-dose (120 mg/kg-bw) groups. Specifically, statistically significant increases in preputial gland squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma and carcinoma combined were observed in both dose groups by pairwise comparisons with the control, all with dose-related trends. Tumors of specialized sebaceous glands, such as the preputial gland, are extremely uncommon in mice, with a spontaneous rate of 0.03% [15/46152] for preputial gland tumors in the B6C3F1 mouse (Radovsky et al. 1999). Data collected from four published studies from the Toth laboratory indicate no occurrence of preputial gland tumors among 399 untreated male Swiss mice (Toth and Nagel 1978; Toth et al. 1980; Toth and Patil 1982; Toth and Shimizu 1974).

Table 11. Incidence of tumors in male Swiss mice administered MFH via a single *s.c.* injection (Toth and Patil 1980a)

Tumor site	Tumor type (age when first tumor was observed) [1]	Administered dose (mg/kg-bw)			Trend test *p*-value
		0	100	120	
Reproductive system	Preputial squamous cell papilloma (r)	0/100	3/48*	3/40*	< 0.01
	Preputial squamous cell carcinoma (r)	0/100	3/48*	3/40*	< 0.01
	Preputial papilloma and carcinoma combined (51 weeks) (r)	0/100	6/48***	6/40***	< 0.001

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 10 weeks of age (denominator). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1980a). A sensitivity analysis was performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B5 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. Exact trend test conducted by OEHHA.
[1] Age when first tumor was observed is the “age at death”, as reported for each tumor in Table 2 of the published study (Toth and Patil 1980a).
r, rare tumor.

Females

In females, survival declined in the treated group (180 mg/kg-bw) from 86% at 60 weeks of age to 72% at 70 weeks of age. Survival in controls at 60 and 70 weeks of age was 91% and 78%, respectively. Six animals in the treated group and 28 in the control group survived to 100 weeks of age.

As shown in Table 12, lung tumors were observed in female mice after a single *s.c.* injection of 180 mg/kg-bw MFH. Specifically, statistically significant increases in lung adenoma, adenocarcinoma, and adenoma and adenocarcinoma combined were observed by pairwise comparison with the control.

Table 12. Incidence of tumors in female Swiss mice administered MFH via a single *s.c.* injection (Toth and Patil 1980a)

Tumor site	Tumor type (age when first tumor was observed) [1]	Administered dose (mg/kg-bw)	
		0	180
Lung	**Adenomas**	10/95	15/45**
	Adenocarcinoma	6/95	13/45***
	Adenomas and adenocarcinomas combined (59 weeks)	15/95	20/45***

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 50 weeks of age). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1980a). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor in Table 2 of the published study (Toth and Patil 1980a).

Multiple *s.c.* injection studies (Toth and Patil 1983)

Groups of 6-week-old male and female Swiss albino mice were subcutaneously injected once per week for 40 weeks with MFH and observed for their lifetime. In each study there were 100 untreated animals in the control group and 50 animals in the treatment group. The same male and female control mice were used as controls for the drinking water studies reported in Toth and Patil (1982). MFH was synthesized in the laboratory with a purity > 99%. The reported administered doses were 10 µg/g-bw [10 mg/kg-bw] per injection in the male mouse study and 20 µg/g-bw [20 mg/kg-bw] per injection in the female mouse study.

Males

In males, survival declined in the treated group (10 mg/kg-bw) from 96% at 20 weeks of age to 88% and 76% at 30 and 40 weeks of age, respectively. Survival in controls at 20, 30, and 40 weeks of age was 92%, 86%, and 83%, respectively. Two animals in the treated group and eight in the control group survived to 100 weeks of age. Given the reduction in survival in treated males between 30 to 40 weeks of age, OEHHA presents tumor incidence data from this study as the number of tumor-bearing animals divided by the number of animals alive at 30 weeks of age.

As shown in Table 13, an increase in lung tumors was observed in male mice administered 40 weekly *s.c.* injections of 10 mg/kg-bw MFH. Statistically significant increases in lung adenoma, and adenoma and adenocarcinoma combined were observed by pairwise comparison with the control. The increase in lung adenocarcinoma was marginally significant.

Table 13. Tumor incidence in male Swiss mice administered MFH via 40 weekly *s.c.* injections (Toth and Patil 1983)

Tumor site	Tumor type (age when first tumor was observed) [1]	Administered dose (mg/kg-bw)	
		0	10
Lung [2]	**Adenoma**	13/86	16/44**
	Adenocarcinoma	9/86	10/44[3]
	Adenoma and adenocarcinoma combined (47 weeks)	19/86	20/44**

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at 30 weeks of age (denominator). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1983). A sensitivity analysis was performed to examine the impact of alternative denominators (i.e., number of animals alive at or just prior to the occurrence of the first tumor) (see Table B6 in Appendix B).
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.
[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1983).
[2] Tumor incidence in controls for lung adenoma and adenocarcinoma is as reported in Table 2 of Toth and Patil (1982).
[3] p= 0.0560 from pairwise comparison with control.

Females

In females, survival declined in the treated group (20 mg/kg-bw) from 92% at 50 weeks of age to 78% at 60 weeks of age. Survival in controls at 50, and 60 weeks of age was 88% and 83%, respectively. Eight animals in the treated group and 13 in the control group survived to 100 weeks of age.

As shown in Table 14, an increase in lung tumors was observed in female mice administered 40 weekly *s.c.* injections of 20 mg/kg-bw MFH. Statistically significant increases in lung adenoma, and adenoma and adenocarcinoma combined were observed by pairwise comparison with the control.

Table 14. Tumor incidence in female Swiss mice administered MFH via 40 weekly *s.c.* injections (Toth and Patil 1983)

Tumor site	Tumor type (age when first tumor was observed) [1]	Administered Dose (mg/kg-bw)	
		0	20
Lung [2]	**Adenoma**	25/94	23/46*
	Adenocarcinoma	19/94	15/46
	Adenoma and adenocarcinoma combined (49 weeks)	29/94	28/46**

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 40 weeks of age). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1983). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study of Toth and Patil (1983).

[2] Tumor incidence in controls for lung adenoma and adenocarcinoma is as reported in Table 2 (Toth and Patil 1982)

4.2 Carcinogenicity Studies of MFH in Hamsters

4.2.1 Lifetime drinking water studies in male and female Syrian golden hamsters

Toth and Patil (1979)

Male and female Syrian golden hamsters, a strain randomly bred in the Toth laboratory since 1959, were administered 0 or 0.0078% [w/v] MFH in drinking water, starting at six weeks of age, throughout the lifespan. In each study 100 animals were included as controls and 50 animals were included in the treated group. The purity of MFH used in the studies was > 99%. The average daily intake was calculated by the study authors, based on average daily water consumption per animal, with estimates of 1.35 mg per animal for treated males and 1.37 mg per animal for treated females. The authors did not report information on body weights or body weight changes for any groups during the studies.

Males

The authors reported a difference in survival after about one year of age between the treated and control male hamsters, in which the treated group demonstrated a higher survival rate than the control group. Survival in the MFH treated males at 40, 50, and 60 weeks of age was 92%, 86%, and 74%, respectively. Survival in controls at 40, 50, and 60 weeks of age was 88%, 67%, and 36%, respectively. The authors did not provide a biological explanation for the decreased survival in control compared to treated males. Historical reports from the Eppley Institute in Omaha, NE, where the study was conducted, reported an average survival of 73 weeks for male hamsters from the same colony (Pour et al. 1976a).

As shown in Table 15, increases in liver hepatocellular tumors, liver malignant histiocytoma (Kupffer cell sarcoma), gallbladder tumors, and bile duct tumors were observed in male hamsters treated with 0.0078% MFH in drinking water. As discussed below, each of these tumors is considered rare, and none of these tumors were observed in the control group. These increases were statistically significant for each tumor site and cell type when incidence was expressed as the number of tumor-bearing animals divided by the number of animals alive at the first occurrence of the tumor.

In the liver, incidences of hepatocellular adenoma,[2] hepatocellular carcinoma, and hepatocellular adenoma and carcinoma combined were statistically significantly increased by pairwise comparison with the control. Hepatocellular carcinoma is rare in untreated male Syrian hamsters (Moore et al. 1996). The reported spontaneous occurrence of liver tumors (of any type) in males in the Eppley Institute colony was below 1% (Pour et al. 1976a).

The incidence of liver malignant histiocytoma, also known as Kupffer cell sarcoma, was statistically significantly increased by pairwise comparison with controls. This tumor arises from either resident liver macrophages (Kupffer cells) associated with sinusoidal endothelial cells or circulating macrophages (Thoolen et al. 2010). As noted above, spontaneous occurrence of liver tumors is rare in untreated male hamsters from the Eppley Institute colony, with an incidence below 1% (Pour et al. 1976a).

In the gallbladder, statistically significantly increased incidences of papillary adenomas and adenomas and adenocarcinomas combined were observed in treated males. In untreated hamsters, the spontaneous incidence of gallbladder tumors is particularly rare (Moore et al. 1996). Pour et al. (1976a, c) reported an incidence of 0% (0/162) for gallbladder tumors in untreated male hamsters from the Eppley Institute colony.

[2] Reported as hepatomas, an older term for hepatocellular adenoma. The term hepatocellular adenoma is now preferred (Moore et al. 1996).

In the bile ducts, statistically significantly increased incidences of cholangiosarcoma, as well as cholangioma and cholangiosarcoma combined, were observed in treated males by pairwise comparison with the control group. Spontaneous occurrence of cholangioma or cholangiosarcoma is rare in untreated male hamsters from the Eppley Institute, with a reported incidence of 0% (Pour et al. 1976c).

Several additional rare tumors were observed in male hamsters treated with MFH, although the increases were not statistically significant compared to controls. Specifically, there was one liver angiosarcoma (spontaneous rate: 0.6% [1/154]; Pour et al. 1976a, b), three anal gland adenomas (spontaneous rate: 0% [0/112]; Pour et al. 1976a, c), one polypoid adenoma of the cecum (spontaneous rate: 0% [0/112]; Pour et al. 1976a, c), and one kidney adenoma (spontaneous rate: 0% [0/166]; Pour et al. 1976a, b).

Table 15. Incidence of tumors in male Syrian golden hamsters administered MFH in drinking water (Toth and Patil 1979)

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

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 50 weeks of age for hepatocellular tumors, 60 weeks of age for gallbladder tumors, and 70 weeks of age for malignant histiocytoma of the liver and for bile duct tumors). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1979). A sensitivity analysis was performed to examine the impact of alternative denominators (i.e., number of animals alive at the start of the study) (see Table B7 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1979).

r, rare tumor.

Females

Similar to males, the treated group of females demonstrated a higher survival rate compared to untreated animals after one year of age. Survival in MFH treated females at 40, 50, and 60 weeks of age was 82%, 72%, and 70%, respectively, while survival in controls at 40, 50, and 60 weeks of age was 79%, 51%, and 26%, respectively. Historical controls from the same colony reported an average survival of 63 weeks for females (Pour et al. 1976a).

As shown in Table 16, statistically significantly increased incidences in liver hepatocellular tumors and liver malignant histiocytoma (Kupffer cell sarcoma) were observed in female hamsters treated with 0.0078% MFH in drinking water.

Incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma and carcinoma combined were statistically significantly increased by pairwise comparison with the control. The reported spontaneous occurrence of liver tumors (of any type) in female hamsters in the Eppley Institute colony was below 1% (Pour et al. 1976a).

The incidence of liver malignant histiocytoma (Kupffer cell sarcoma) was statistically significantly increased in treated females. As noted above, the reported spontaneous occurrence of liver tumors (of any type) in female hamsters in the Eppley Institute colony was below 1% (Pour et al. 1976a).

Several additional rare tumors were observed in female hamsters treated with MFH, although the increases were not statistically significant compared to controls. Specifically, in the gallbladder two papillary adenomas (spontaneous rate: 0% [0/139]; Moore et al. 1996; Pour et al. 1976a, b), and one adenocarcinoma was observed (spontaneous rate: 0% [0/139]; Moore et al. 1996; Pour et al. 1976a, b). In the bile ducts one cholangioma was observed (spontaneous rate: 0% [0/139]; Moore et al. 1996; Pour et al. 1976a, b). In the liver, three angiosarcomas were observed (spontaneous rate: 0.7% [1/139]; Pour et al. 1976a, c), and in the ovary one ovarian myxoma was observed (spontaneous rate: 0.9% [1/114]; Pour et al. 1976a, b).

Table 16. Incidence of tumors in female Syrian golden hamsters administered MFH in drinking water (Toth and Patil 1979)

Tumor site	Tumor type (age when first tumor was observed) [1]	Concentration in drinking water (%)	
		0	0.0078
		Average dose (mg/day/animal)	
		0	1.37
Liver	Hepatoma (hepatocellular adenoma) (r)	0/79	9/41***
	Hepatocellular carcinoma (r)	0/79	10/41***
	Hepatoma (hepatocellular adenoma) and carcinoma combined (47 weeks) (r)	0/79	19/41***
	Malignant histiocytoma (Kupffer cell sarcoma) (76 weeks) (r)	0/13	24/31***

Tumor incidence is expressed as the number of tumor-bearing animals (numerator) divided by the number of animals alive at first occurrence of the tumor (denominator) (i.e., 40 weeks of age for hepatocellular tumors, and 70 weeks of age for malignant histiocytoma of the liver). The denominator was taken from the survival data reported in 10-week intervals in Table 1 of the published study (Toth and Patil 1979). A sensitivity analysis was performed to examine the impact of alternative denominators (i.e., number of animals alive at the start of the study) (see Table B8 in Appendix B). Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.
[1] Age when first tumor was observed is the "age at death", as reported for each tumor type in Table 2 of the published study (Toth and Patil 1979).
r, rare tumor.

4.3 Summary of Animal Carcinogenicity Studies of MFH

Carcinogenicity studies of MFH have been conducted in both sexes of Swiss mice in drinking water and subcutaneous injection studies and in both sexes of Syrian golden hamsters in drinking water studies, with increases in tumors observed in all studies. Statistically significant tumor findings are summarized in Table 17, organized by species, sex, and exposure route.

As shown in Table 17, MFH consistently induced lung tumors in male and female mice when administered via drinking water or repeated *s.c.* injections. Lung tumors were also induced in female mice administered a single *s.c.* injection of MFH. At higher concentrations (0.001% to 0.0156%) of MFH in drinking water, tumors were often also observed in the liver, gallbladder (rare), and blood vessels of male and female mice, and in the bile duct (rare) and seminal vesicles (rare) in male mice. At lower concentrations (0.0005% or 0.00025%) of MFH in drinking water, tumors of the

forestomach (rare), glandular stomach (rare), and subcutis were also observed in male mice. In male mice administered a single *s.c.* injection of MFH, tumors of the preputial gland (rare) were observed.

In hamsters exposed to MFH via drinking water, multiple types of rare tumors were observed in males and females, including liver hepatocellular tumors and liver malignant histiocytoma (Kupffer cell sarcoma). In addition, rare tumors of the bile duct and gallbladder were also observed in males.

Table 17. Summary of statistically significant tumor findings in MFH animal studies

Dose or Concentration \ Tumor Site	Male						Female				Reference
	Lung	Liver	Gall-bladder	Bile duct	Blood vessel	Other	Lung	Liver	Gall-bladder	Blood vessel	
Mouse - drinking water											
0.0156% [w/v][1]	-	-	-	-	-	-	-	√	-	-	Toth and Nagel (1978)
0.0078% [w/v]	√	√	√ r	√ r	-	-	√	√	√ r	-	Toth and Nagel (1978)
0.0039% [w/v]	√	√	√ r	√ r	-	√ 2, r	√	√	√ r	√	Toth et al. (1979)
0.0020% [w/v]	√	√	√ r	-	√	-	√	-	√ r	√	Toth and Patil (1980b)
0.0010% [w/v]	√	√	√ r	-	√	-	√	-	-	√	Toth and Patil (1980b)
0.0005% [w/v]	√	-	-	-	-	√ 3, r	√	-	-	-	Toth and Patil (1982)
0.00025% [w/v]	√	-	-	-	-	√ 4, r	√	-	-	-	Toth and Patil (1982)
Mouse - *s.c.* injection											
20 mg/kg-bw (40 weekly injections)	NT	NT	NT	NT	NT	NT	√	-	-	-	Toth and Patil (1983)
10 mg/kg-bw (40 weekly injections)	√	-	-	-	-	-	NT	NT	NT	NT	Toth and Patil (1983)
180 mg/kg-bw (single injection)	NT	NT	NT	NT	NT	NT	√	-	-	-	Toth and Patil (1980a)
120 mg/kg-bw (single injection)	-	-	-	-	-	√ 5, r	NT	NT	NT	NT	Toth and Patil (1980a)
100 mg/kg-bw (single injection)	-	-	-	-	-	√ 5, r	NT	NT	NT	NT	Toth and Patil (1980a)
Syrian golden hamster - drinking water											
0.0078% [w/v]	-	√ 6, r	√ r	√ r	-	-	-	√ 6, r	-	-	Toth and Patil (1979)

[1] Very high mortality was observed at this drinking water concentration after 30 weeks of age (84% mortality in males, 78% mortality in females).
[2] Seminal vesicle adenoma (rare)
[3] Forestomach squamous cell carcinoma (rare)
[4] Glandular stomach polypoid adenoma (rare), and subcutis fibroadenoma
[5] Preputial gland tumors (rare; papilloma, carcinoma, and papilloma and carcinoma combined)
[6] Liver tumors included rare hepatocellular tumors (adenoma, carcinoma, and adenoma and carcinoma combined), and rare malignant histiocytoma (Kupffer cell sarcoma)
√, statistically significantly increased tumors; "-", no statistically significant tumor findings; r, rare tumor; NT, not tested.

4.4 Carcinogenicity Studies of *Gyromitra esculenta* Mushrooms in Mice

Gyromitra esculenta mushrooms contain multiple hydrazine and hydrazone compounds, in addition to MFH. These include methylhydrazine (MMH) and gyromitrin, both of which are Proposition 65 carcinogens, as well as propanal methylformylhydrazone (MFHO), butanal MFHO, 3-methylbutanal MFHO, pentanal MFHO, hexanal MFHO, octanal MFHO, and 2-octenal MFHO (Toth et al. 1992). Carcinogenicity studies of *Gyromitra esculenta* mushrooms thus do not provide direct evidence on the carcinogenicity of MFH, and information on these studies is included here only for context and interest.

4.4.1 Lifetime feeding studies in male and female Swiss mice

Toth et al. (1992)

Male and female Swiss albino mice were fed raw *Gyromitra esculenta* mushrooms *ad libitum* for three days followed by four days of a semisynthetic diet starting from six weeks of age for the life span of the animals. In each study there were 50 animals in the control group and 50 animals in the treated group. The mushrooms were collected in southern and eastern Finland, and were frozen after collection and thawed before being given to the mice. Concentrations of gyromitrin and MFH measured in samples of these mushrooms were 44 and 4 mg/kg, respectively.

Statistically significant increases in tumors of the lung, nasal cavity, blood vessels, forestomach, glandular stomach, and cecum were observed in male and female mice fed *Gyromitra esculenta* mushrooms.

Tumors at some of these sites were also observed in mouse carcinogenicity studies of MFH, including lung and blood vessel tumors (in males and females), and forestomach and glandular stomach tumors (in males).

5. MECHANISTIC CONSIDERATIONS AND OTHER RELEVANT DATA

5.1 Pharmacokinetics and Metabolism

5.1.1 Overview

Few, if any, data exist on the absorption, distribution, metabolism, and excretion (ADME) of MFH. However, there are a number of studies that provide relevant information. For example, information on MFH's ADME may be inferred from reports on the onset and subsidence of poisoning symptoms in humans following the ingestion of

Gyromitra esculenta mushrooms. Information may also be inferred from studies investigating MFH's toxicity, as well as from studies investigating the toxicity and metabolism of its parent compound, gyromitrin, and its hydrolysis metabolite, MMH.

MFH hydrolyzes to MMH and forms formaldehyde, acetaldehyde, methane, and carbon dioxide in downstream reactions, as well as free radicals and DNA adducts. Formaldehyde, acetaldehyde, and MMH ("methylhydrazine") are each listed under Proposition 65 as carcinogens.

Below we describe the ADME of MFH. Since humans and animals share many of the reported metabolic pathways and metabolites, data from animal studies are included when human data are unavailable or incomplete, when data from animal studies are useful to complement human data, and when route, species, and gender differences are observed.

5.1.2 Absorption

There is indirect evidence in humans for absorption of MFH by the oral route, following consumption of wild edible *Gyromitra* mushrooms, although inferences are complicated by the co-occurrence of MFH with gyromitrin, MMH, and other hydrazine and hydrazone compounds in these mushrooms (Larsson and Eriksson 1989; List and Luft 1969; Pyysalo et al. 1979; Toth et al. 1991), and by the formation of MFH and MMH from ingested gyromitrin (Andary et al. 1985; Gouvinhas et al. 2024; Nagel et al. 1977). Consideration of rodent LD_{50} values for gyromitrin, MFH, and MMH, effects of MFH on intestinal enzymes, and early symptoms of poisoning from these mushrooms in humans provide indirect evidence consistent with absorption of ingested MFH. MFH and its metabolite, MMH, have lower LD_{50} values compared to gyromitrin, suggesting that they may be responsible for, or contribute to, the onset of poisoning symptoms (Horowitz et al. 2024; von Wright et al. 1978b). MFH may directly contribute to gastrointestinal distress (an early poisoning symptom), as it is a non-competitive inhibitor of intestinal diamine oxidase, an enzyme involved in the metabolism of histamines (Biegański et al. 1984). Low or reduced diamine oxidase can lead to accumulation of histamines, which in turn can cause a range of gastrointestinal symptoms (Jochum 2024). Initial *Gyromitra* mushroom poisoning symptoms, including gastrointestinal distress, occur approximately 5–12 hours post mushroom ingestion, suggesting absorption of MFH within a matter of hours (Horowitz et al. 2024; Michelot and Toth 1991; Spencer and Kisby 2021).

There is also evidence in humans for absorption of MFH by the inhalation route. Both MFH and MMH have been identified as volatile components in the steam condensate of *Gyromitra esculenta* mushrooms (Pyysalo et al. 1979). Steam containing MFH can be generated during certain mushroom preparation methods, such as boiling. The inhalation of, or contact with, cooking steam, as well as regular breathing while handling

fresh mushrooms in an enclosed area, have been associated with inflammation, swelling of eye and respiratory tissues, as well as gastrointestinal distress in workers from three different mushroom processing plants in Europe (Franke et al. 1967).

Direct evidence in animals that MFH is absorbed by the oral and subcutaneous injection routes includes the toxicity and tumor findings observed in the carcinogenicity studies of MFH in mice and hamsters (See Section 4).

5.1.3 Distribution

There are no studies of the distribution of MFH; however, studies on the distribution of gyromitrin and/or its metabolites in rabbits, rats, and mice suggest that MFH is likely to be widely distributed throughout the body.

A study in rabbits examined the distribution of radioactivity following oral gavage or intravenous administration of ^{14}C-labeled gyromitrin (Savolainen et al. 1978). Four hours post oral administration, levels of radioactivity were highest in the stomach, followed by duodenum, liver, kidney, spleen and thyroid (equal), lungs, blood, thymus, heart, brain, skeletal muscle, and bile. No radioactivity was found in abdominal fat, skin, adrenal gland, or bone marrow. By 24 hours most levels of radioactivity had decreased, except for liver and bile where levels had increased. Four hours post intravenous administration levels of radioactivity were highest in skeletal muscle, followed by lower levels in liver, blood, skin, kidney, duodenum, stomach and thymus, and even lower levels in other organs. At the 24-hour timepoint, levels of radioactivity had declined in all organs except the liver. While gyromitrin metabolites were not identified, the study found that free gyromitrin was only present in the liver and at very small amounts (0.03 ppm) but not present in other organs, suggesting extensive metabolism.

A study in rats reported levels of radioactivity of [^{3}H]gyromitrin six hours post oral administration as follows: 5.1–5.4% in whole blood, 2–3% in the liver, 0.3–0.5% in the kidney and 0.1–0.3% in the lung (Meier-Bratschi et al. 1983).

A study in mice found that gyromitrin, given via gavage, was rapidly absorbed and metabolized to MFH and MMH (von Wright et al. 1978c). Both metabolites were observed in the peritoneal fluid as early as one hour post gavage and up to three hours post administration. The parent compound, gyromitrin, was detected in peritoneal fluid at the 2- and 3-hour intervals but not at the 1-hour time point.

5.1.4 Elimination

Indirect information on the elimination of MFH in humans comes from mild to moderate poisoning cases following consumption of wild edible *Gyromitra* mushrooms. A recovery period of two to five days in most patients from poisoning symptoms suggests that

residual gyromitrin, MFH and downstream metabolites are largely excreted within that time frame (Horowitz et al. 2024; Michelot and Toth 1991).

No animal studies of the elimination of MFH were identified; however, studies on the elimination of radiolabeled gyromitrin in rabbits and rats, and of radiolabeled MMH in rats and mice suggest that MFH or its metabolites may be eliminated in the urine, feces, and expired air.

Excretion of radioactivity following oral administration of ^{14}C-labeled gyromitrin in rabbits occurred predominantly via urine in the first four hours post administration, with urinary excretion declining over the next 20 hours and fecal excretion becoming the main excretory pathway at 24 hours (Savolainen et al. 1978). A study in rats that orally administered [^{3}H]gyromitrin reported that 16–18% of the radioactivity was excreted in the urine within six hours (Meier-Bratschi et al. 1983).

Excretion of radioactivity following intraperitoneal (*i.p.*) injection of [^{14}C]MMH, the MFH hydrolysis metabolite, was detected in expired air and in urine in rats (Dost et al. 1966). Two metabolites, CO_2 and methane, were identified in expired air, accounting for approximately 45% of the administered dose, and of that, CO_2 accounted for approximately 20–25% of the total respiratory radioactivity. Expiration was rapid, with the peak of radioactivity occurring approximately two hours post injection and nearing completion around eight hours at the low dose (5.5 mg/kg) and about 16 hours at the high dose (22 mg/kg). Urinary excretion of radioactivity 27 hours post administration accounted for 36–46% of the total administered radioactivity at the low dose and 20–25% at the high dose.

In studies of [^{14}C]MMH administered by *s.c.* injection in mice, about 7% of the administered radioactivity was excreted in expired air as CO_2 over a 24-hour period, and about 21–36% was excreted in the urine (Hawks and Magee 1974).

5.1.5 Metabolism

There is no direct evidence of step-by-step metabolic pathways for MFH. However, based on the integration of *in vitro* and *in vivo* studies of MFH, MMH, and gyromitrin, it has been proposed that metabolism of MFH mainly involves non-enzymatic hydrolysis, followed by oxidation, resulting in the formation of reactive species that can ultimately yield DNA and protein adducts (Michelot and Toth 1991; Nagel et al. 1977; von Wright et al. 1978c).

From *in vitro* experiments, it is proposed that gyromitrin is converted to MFH which is then hydrolyzed to MMH (Nagel et al. 1977). In their work, Nagel and colleagues monitored MMH formation at pH 1 by measuring the disappearance of gyromitrin and MFH, since MMH readily decomposes during chromatography. MMH was also detected in the contents of excised mouse stomachs after oral administration of gyromitrin; MFH

did not appear to be measured (Nagel et al. 1977). Additionally, both MFH and MMH have been detected *in vivo* in the peritoneal cavity of mice treated orally (gavage) with gyromitrin (von Wright et al. 1978c).

Microsome-mediated oxidation, leading to the formation of the aldehyde products formaldehyde and acetaldehyde (Figure 3), has been demonstrated in rat liver microsomes treated with MFH (Gannett et al. 1991). *In vivo* metabolic studies with ^{14}C-MMH administered to rats *i.p.* showed that the ^{14}C was respired as ^{14}C-methane and ^{14}C-carbon dioxide during the 24 hours following exposure (Dost et al. 1966). Based on the occurrence of an oxidation reaction and the observed products (free radicals and alkyl substances), several intermediates have been suggested but not measured, such as diazenium ion, diazene (Gannett et al. 1991), and nitrosamide compounds (Braun et al. 1980). In addition to the enzymatically formed formaldehyde, acetaldehyde, methane, and carbon dioxide, chemical oxidation of MFH yields tetrazene, and the hydrazones derived from the condensation of methane and acetaldehyde, all of which are consistent with the formation of diazenium ion and diazene intermediates (Gannett et al. 1991).

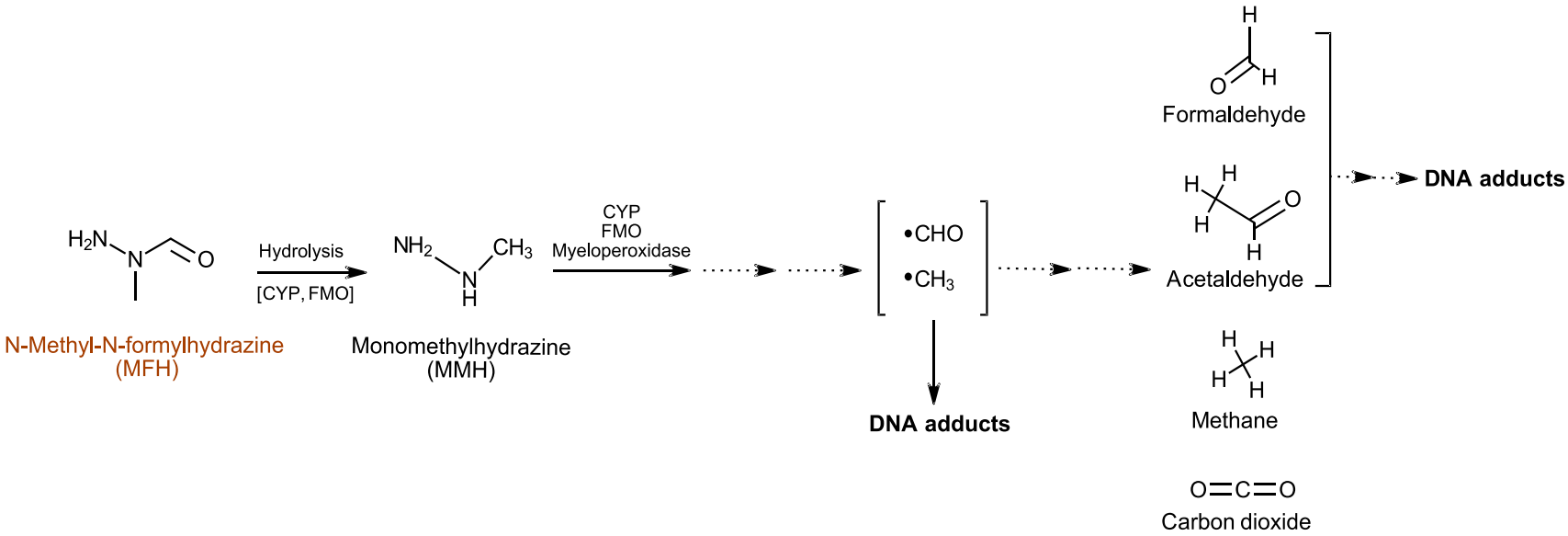


Figure 3. Proposed metabolism of MFH.

MFH can be hydrolyzed to MMH. MMH undergoes enzymatic oxidation by CYP, FMO, or myeloperoxidase to generate methyl and formyl radicals via unconfirmed reactive intermediate(s). These radicals can then abstract hydrogen and form formaldehyde, acetaldehyde, methane, and carbon dioxide. Dashed arrows indicate unconfirmed or theoretical reactions and brackets indicate theoretical reactions and intermediates. See text for details.

Enzymes capable of metabolizing MMH include cytochrome P-450 monooxygenases (CYP), flavin adenine dinucleotide-containing monooxygenases (FMO), and myeloperoxidases. Oxidative metabolism of MMH by the CYP and FMO systems has been investigated using various inhibitors of mixed function oxidase systems (Albano et al. 1989; Diaz Gomez and Castro 1986; Tomasi et al. 1987). In studies looking at biotransformation of MMH to CO_2, a non-specific CYP inhibitor and several FMO inhibitors reduced the amount of CO_2 detected in rat liver slices (Diaz Gomez and Castro 1986). In rat liver microsomes, a different FMO inhibitor, methimazole, showed no effect on the metabolism of MMH and production of free radicals, whereas all CYP inhibitors tested reduced the formation of free radicals (Albano et al. 1989; Tomasi et al. 1987). In rats *in vivo*, Braun et al. (1980) observed that administration of MFH reduced CYP protein content in the liver, compared to levels in untreated controls, and suggested that MFH itself is a substrate of the mixed function oxidase systems. In a study of rat neutrophils, the myeloperoxidase system was shown to be involved in the metabolism of MMH to form methyl free radicals (Gamberini and Leite 1993). In this study, addition of the reactive oxygen scavengers superoxide dismutase (SOD) and catalase, or the myeloperoxidase inhibitor, azide, to activated neutrophils inhibited free radical formation (Gamberini and Leite 1993).

Formation of free radicals has been observed in the presence of spin-trapping agents when MMH was incubated with isolated hepatocytes (Albano et al. 1989; Tomasi et al. 1987), liver microsomes (Albano et al. 1989; Tomasi et al. 1987), and neutrophils (Gamberini and Leite 1993). The radicals produced from MMH in enzymatic oxidation systems are the same as those produced with chemical oxidizing agents, as confirmed by comparable electron spin resonance (ESR) spectra (Tomasi et al. 1987). Similar findings were reported by Albano et al. (1989), who was able to report that the spectra of the products derived from MMH corresponded to those reported for the methyl radical. Similarly, the oxidation of MMH by rat neutrophils led to the formation of what the authors describe as carbon-centered radicals (Gamberini and Leite 1993), which could include a methyl radical.

Reactive species generated during the metabolism of MFH and its hydrolysis metabolite MMH (e.g., methyl radicals, formaldehyde, and acetaldehyde) can react with nucleic acids to form DNA and RNA adducts (See Section 5.2.1).

Little is known about possible detoxification pathways for MFH and its hydrolysis metabolite MMH. Data suggest that glutathione (GSH) is able to effectively scavenge reactive species, as suggested by studies in isolated rat hepatocytes and liver microsomes treated with MMH (Albano et al. 1989). It is also likely that liver acetylation plays a role in the detoxification of MFH (Braun et al. 1981; Horowitz et al. 2024). Several review papers discuss the likelihood of acetylation based on the varying severity and onset of adverse effects of *Gyromitra esculenta* poisonings where it is

suggested that greater severity occurs in slow acetylators (Lagrange et al. 2021; Vohra et al. 2024). This hypothesis remains to be assessed in humans. However. in studies in rabbits exposed to two different hydrazine compounds, isoniazid and its metabolite, monoacetylhydrazine, slow acetylators were found to exhibit greater susceptibility to the lethal central nervous system effects of those exposures than the rapid acetylators (Hein and Weber 1984).

5.1.6 Summary

In summary, MFH appears to be readily absorbed, widely distributed, and readily metabolized to the hydrolysis metabolite MMH, and ultimately to formaldehyde, acetaldehyde, methane, and carbon dioxide, during which free radicals and DNA adducts are formed. Studies with gyromitrin and MMH show that excretion occurs via expired air, urine, and feces.

5.2 Key Characteristics of Carcinogens

A comprehensive review of the more than 100 agents known to cause cancer in humans identified 10 key characteristics (KCs) of carcinogens (Samet et al. 2020; Smith et al. 2016) (Table 18). KCs are characteristics of agents that cause cancer, in contrast to the hallmarks of cancer (Hanahan and Weinberg 2000, 2011), which are properties of cancer cells and neoplasms, and also in contrast to modes of action, which are sequences of key events that transform normal cells into malignant tumors. Mode of action analysis depends on prior knowledge sufficient to hypothesize how an agent might cause cancer, knowledge that too often is incomplete. The KCs can encompass many types of mechanistic endpoints and are not constrained to previously formulated hypotheses, allowing a broader consideration of multiple mechanistic pathways and hypotheses.

Table 18. Key characteristics of carcinogens

Key characteristic	Example of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (*e.g.,* epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (*e.g.,* CAs, MN)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (*e.g.,* topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (*e.g.,* DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor inactivation/activation (*e.g.,* ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Source: Smith et al. (2016) and Samet et al. (2020)
AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (*e.g.*, oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

OEHHA used the KCs of carcinogens to systematically identify, organize, and summarize mechanistic information from studies of MFH. Data for two of the 10 KCs, namely KC1 and KC2, were identified and summarized in the following sections.

5.2.1 KC1. Is electrophilic or can be metabolically activated

Electrophiles are reactive, electron-seeking molecules capable of binding to electron-rich cellular macromolecules including DNA, RNA, lipids, and proteins, thereby forming covalent adducts. The measurement of covalent adducts on DNA and proteins is the most common method of assessing electrophilic activity (Smith et al. 2020).

As discussed in the metabolism section (Section 5.1.5), MFH can be hydrolyzed to MMH, which is metabolized to additional electrophilic species that react with DNA, RNA, and proteins. No studies of direct MFH exposure and subsequent formation of DNA and/or protein adducts have been conducted; however, such studies have been conducted with MMH. These studies of direct exposure to MMH demonstrate the formation of DNA, RNA, and protein adducts. These studies are summarized below.

In vivo studies of direct exposure to MMH: DNA and RNA adducts

Mice

- Formation of N7-methylguanine (N7MeGu) was reported in liver and large intestine DNA, as well as in liver, large intestine and kidney RNA six hours after subcutaneous administration of 15 mg/kg-bw [^{14}C]MMH in MNRI female mice (Hawks and Magee 1974).
- N7MeGu and O^6-methylguanine (O^{6}MeGu) was detected in liver DNA of male Swiss Webster mice one hour after oral gavage with 15 mg/kg-bw MMH (Bosan and Shank 1983).
- N7MeGu and O^{6}MeGu was detected in liver and kidney DNA of male BALB/c mice administered MMH in drinking water for two weeks (13 mg/kg/day). The level of N7MeGu in the kidney was reported as ‘trace’; neither DNA adduct was detected in lung tissue (Bergman and Hellenäs 1992).
- No N7MeGu or O^{6}MeGu was detected in liver, kidney, or lung DNA of male BALB/c mice six hours after an *i.p.* injection of 7.5 mg/kg [^{14}C]MMH (Bergman and Hellenäs 1992).

Hamsters

- No N7MeGu or O^{6}MeGu was detected in liver DNA of male Syrian golden hamsters 24 hours after oral gavage with MMH at doses ranging from 11.3 to 27.5 mg/kg-bw (Bosan and Shank 1983).

Rats

- N7MeGu was detected in the liver DNA of a male Sprague-Dawley rat six hours after the last of five daily *i.p.* injections of 7.3 mg/kg [^{14}C]MMH. N7MeGu was not detected in kidney or lung DNA, and O^{6}MeGu was not detected in liver, kidney, or lung DNA of this rat (Bergman and Hellenäs 1992).
- No N7MeGu or O^{6}MeGu was detected in liver, kidney, or lung DNA of a male Sprague-Dawley rat six hours after an *i.p*. injection of 6.4 mg/kg [^{14}C]MMH (Bergman and Hellenäs 1992).

In vitro studies of direct exposure to MMH: DNA and protein adducts

- Rat liver slices – MMH binds covalently to nucleic acids extracted from liver slices exposed to 0.15 mM [^{14}C]MMH (Godoy et al. 1983).
- Rat microsomes and S9 (9000xg supernatant) – MMH binds covalently to proteins. Non-enzymatic covalent binding to heat-inactivated microsomal and S9 proteins was observed under both aerobic and anaerobic conditions, while increased covalent binding mediated by microsomal enzymatic activity (but not S9 enzymatic activity) was observed under aerobic conditions (Godoy et al. 1983).

Studies demonstrating free radical formation in vitro or in cell-free systems

- Free radical species, most likely methyl radicals, were detected by electron spin resonance (ESR) spectroscopy in rat hepatocytes and liver microsomes incubated with 1 mM of MMH for 30 min (Tomasi et al. 1987). Cytochrome P450 inhibitors and glutathione (GSH) reduced the ESR signal, indicating that enzymatic oxidation of MMH results in free radical formation.
- Carbon-centered methyl radical formation was detected by ESR in rat hepatocytes and liver microsomes incubated with 1 mM of MMH for 30 min. Free radical formation was dependent on cytochrome P450 activity and reduced by the addition of GSH (Albano et al. 1989).
- Methyl radical formation was identified by electron paramagnetic resonance (EPR) in rat neutrophils incubated with MMH at concentrations ranging from 0.1 to 5 mM for 1 hour. The yield of methyl radicals increased with increasing MMH concentrations and was mediated by myeloperoxidases in this cell type (Gamberini and Leite 1993).

In summary, the available evidence for the electrophilicity of MFH is based on several studies of its hydrolysis metabolite, MMH, that report the formation of DNA, RNA, or protein adducts by MMH (or subsequent metabolites, e.g., methyl radicals, formaldehyde, acetaldehyde). Additionally, ESR and EPR studies identified methyl

radical formation after incubation of MMH with rat cells (hepatocytes, neutrophils) and liver microsomes. Methyl radicals may alkylate DNA via a substitution nucleophilic (SN) reaction, reacting with the highly nucleophilic guanine ring (Chatterjee and Walker 2017).

5.2.2 KC2. Is genotoxic

Genotoxicity refers to the ability of a chemical or other type of agent or biological process to damage DNA or induce changes in the DNA sequence. The link between genotoxicity and carcinogenesis is well established (Smith et al. 2016; Smith et al. 2020). Changes in the DNA sequence include gene or point mutations such as base substitutions, frameshifts, and small deletions or insertions, and chromosomal effects such as chromosomal aberrations, micronuclei, and aneuploidy. Examples of DNA damage include DNA adducts, DNA strand breaks, and oxidative damage to DNA.

The genotoxicity of MFH has been investigated in a limited number of experimental systems (i.e., *in vitro* studies with rat and mouse hepatocytes, and bacteria) for just two endpoints, i.e., unscheduled DNA synthesis (UDS) and point mutations (See Table 19). The genotoxicity of MFH's hydrolysis metabolite, MMH, has been investigated in these same experimental systems, as well as in additional systems (See Table 20 and Table 21). These studies are summarized below.

Genotoxicity studies of direct exposure to MFH

As shown in Table 19, MFH did not induce UDS *in vitro* in rat or mouse hepatocytes (Mori et al. 1988). MFH induced mutations in a single strain of *Salmonella typhimurium* (TA100), in one study (von der Hude and Braun 1983). A dose dependent increase in mutations was observed in the presence of metabolic activation at MFH concentrations of 0.04 to 0.76 mmol/plate, and a 2–3-fold increase in mutations was also observed with MFH in the absence of metabolic activation (von der Hude and Braun 1983). MFH was not mutagenic in other *Salmonella* strains (TA98, TA1950[3], TA1535 and 1537) in either the presence or absence of metabolic activation (Rogan et al. 1982; von der Hude and Braun 1983; von Wright et al. 1978a).

[3] TA1950 was only tested in the absence of metabolic activation.

Table 19. MFH genotoxicity studies in rodent cells *in vitro* and in bacteria

Endpoint	Test System	Concentrations/Doses Tested	Results	Comments	Reference
UDS	*In vitro* Mouse hepatocytes	0.01, 0.1, and 1 mM	-		Mori et al. (1988)
	In vitro Rat hepatocytes		-		
Mutation	Bacteria *S. typhimurium* TA100	0.04, 0.08, 0.15, 0.38, 0.76, 1.14 and 1.51 mmol/plate	+	With and without metabolic activation (rat S9)	von der Hude and Braun (1983)
	TA98		-	With and without metabolic activation (rat S9)	
Mutation	Bacteria *S. typhimurium* TA1950	2, 5 and 10 µmol/plate	-	No metabolic activation (spot test)	von Wright et al. (1978a)
	TA98		-		
	TA100		-		
	TA100	100, 500, 1000 and 2000 µg/plate	-	Metabolic activation (mouse S9)	
Mutation	Bacteria *S. typhimurium* TA1535	100, 200, 500 and 1000 µg/plate	-	With and without metabolic activation	Rogan et al. (1982)
	TA1537		-		

"+" denotes positive results; "-" denotes negative results.

Genotoxicity studies of direct exposure to MMH

Table 20 presents findings from *in vivo* studies of MMH assessing UDS in rats and dominant lethal mutations in rats and mice, each of which were negative. In other *in vivo* studies, MMH has been shown to form DNA adducts in mouse and rat liver and kidney

(summarized in Section 5.2.1 KC1). As shown in Table 21, MMH has been tested for genotoxicity *in vitro* in human and rodent experimental systems, and in bacteria and yeast, for several different endpoints. Positive findings include increased UDS *in vitro* in mouse and rat hepatocytes (Mori et al. 1988), and, as reviewed by US EPA, increased forward mutations in Chinese hamster lung fibroblasts, reverse mutations in *S. typhimurium*, and SOS induction and DNA repair in *E. coli* (US EPA 2010). MMH also formed DNA adducts in rat liver slices *in vitro* (See Section 5.2.1 KC1).

Table 20. **MMH genotoxicity studies *in vivo*** (adapted from US EPA 2010)

Endpoint	Species	Dose and route	Results	**Reference** (as cited in US EPA 2010)
UDS (in liver)	Rat	30 mg/kg-bw, oral gavage	-	Beije and Olsson (1990) (abstract)
Dominant lethal mutation	Mouse	0.26-26 mg/kg-bw, *i.p.* injection	-	Brusick and Matheson (1976) (unpublished)
Dominant lethal mutation	Rat	0.22-22 mg/kg-bw, *i.p.* injection	-	

"-" denotes negative results

Table 21. **MMH genotoxicity studies in human and rodent cells *in vitro*, bacteria, and yeast** (adapted from US EPA 2010)

Endpoint	Test System	Concentrations/ Doses Tested	Results	Comments	**Reference** (as cited in US EPA 2010)
UDS	Human fibroblasts	0.1-1.0 µL /mL[a]	-	Without metabolic activation	Brusick and Matheson (1976) (unpublished)
		0.1-0.5 µL/mL[a]	-	With metabolic activation	
UDS	Mouse hepatocytes	0.01, 0.1, and 1 mM	+		Mori et al. (1988)
	Rat hepatocytes		+	Positive at all doses tested; cytotoxic at 1 mM	
Forward mutation	Mouse lymphoma cells	0.0005– 0.1 µL/mL[a]	-	With and without metabolic activation	Brusick and Matheson (1976) (unpublished)
Forward mutation	Mouse lymphoma cells	0.1–5 mM	-	With and without metabolic activation	Rogers and Back (1981)

Endpoint	Test System	Concentrations/ Doses Tested	Results	Comments	Reference (as cited in US EPA 2010)
Forward mutation	Chinese hamster lung fibroblasts	Not reported	+	Weak response with or without metabolic activation (cytotoxicity not reported)	Kuszynski et al. (1981) (abstract)
Reverse mutation	Bacteria *S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	0.0001–5.0 µL/plate[a] (plate assay)	-	Without metabolic activation	Brusick and Matheson (1976) (unpublished)
	TA1535	1–5 µL/mL[a] (suspension assay)	+	Without metabolic activation (cytotoxicity not reported)	
Reverse mutation	Bacteria *S. typhimurium* TA100	1–3 µmol	-	With and without metabolic activation.	von Wright and Tikkanen (1980b)
Reverse mutation	Bacteria *S. typhimurium* TA1535, 1537	100–1000 µg/plate	+	Positive with or without metabolic activation (positive only at cytotoxic concentrations, i.e. ≥200µg/plate)	Rogan et al. (1982)
Reverse mutation	Bacteria *S. typhimurium* TA1535, TA1537, TA97, TA98, TA100	1–100 µg/plate	-	With and without metabolic activation	Mortelmans et al. (1986)
Reverse mutation	Bacteria *S. typhimurium TA100, TA102*	Up to 2 µmol for TA100 and 10 µmol for TA102 (suspension assay)	+	With metabolic activation (cytotoxicity not reported)	Matsushita et al. (1993)
Reverse mutation	Bacteria *S. typhimurium* TA102	0.5–2.0 µmol/plate	+	With metabolic activation (cytotoxicity not reported)	Poso et al. (1995)
Reverse mutation	Bacteria *E. coli WP2 try, hcr*	5–20 µg/mL	+	Without metabolic activation	von Wright et al. (1977)

Endpoint	Test System	Concentrations/ Doses Tested	Results	Comments	Reference (as cited in US EPA 2010)
SOS induction	Bacteria *E. coli WP2B/r trp; WP2B/r uvrA, trp; CM871 uvrA,recA,lexA ,trp*	0.5–2.0 µmol/plate (spot test); 0.5–1.0 µmol/mL[a] (liquid incubation test)	+ (greater response in repair-deficient strains)	Without metabolic activation (cytotoxicity not reported)	von Wright and Tikkanen (1980a)
DNA repair	Bacteria *E. coli WP2uvrA-*	0.0001– 5.0 µL/plate[a]	-	With and without metabolic activation	Brusick and Matheson (1976) (unpublished)
DNA repair	Bacteria *E. coli WP2 and CM871*	Not reported	+ (for repair-deficient CM871)	With metabolic activation (cytotoxicity not reported)	Poso et al. (1995)
DNA repair	Bacteria *E. coli pol A1+, pol A1-, WP2 try,hcr, B/r WP2try*	0.5–1.0 mg	+	Without metabolic activation (cytotoxicity not reported)	von Wright et al. (1977)
Gene recombination	Yeast *Saccharomyces cerevisiae D4*	0.000–5.0 µL/plate[a]	-	With and without metabolic activation	Brusick and Matheson (1976) (unpublished)

[a] dose/concentration as reported by US EPA (2010).
"+" denotes positive results; "-" denotes negative results.
Cytotoxicity defined as survival < 50% by US EPA (2010).

In summary, the available evidence for the genotoxicity of MFH comes from a limited number of studies on MFH, with one positive finding of mutations, and a larger number of studies on its hydrolysis metabolite MMH, with some positive findings of UDS, mutations, DNA repair and SOS responses. Specifically, MFH induced mutations in the *Salmonella* reverse mutation assay in one strain, TA100, when tested at doses ≥ 0.04 mmol/plate, but not in other studies using lower doses or in other *Salmonella* strains, and did not induce UDS in mouse or rat hepatocytes *in vitro*. In the limited number of *in vivo* genotoxicity assays conducted on MMH, no induction of UDS (rat liver) or dominant lethal mutations (rats, mice) was observed. In *in vitro* studies, MMH induced UDS in mouse and rat hepatocytes, but not human fibroblasts, and forward mutations in

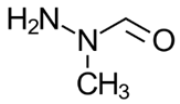
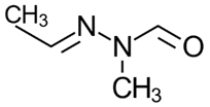
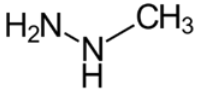
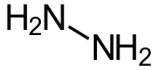
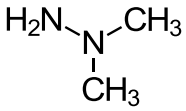
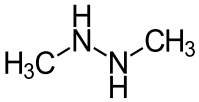
Chinese hamster lung fibroblasts, but not mouse lymphoma cells. MMH induced mutations in the *Salmonella* reverse mutation assay in strains TA100 and TA102, and in some, but not all assays in strains TA1535 and 1537. MMH induced reverse mutations and SOS response in *E. coli*, DNA repair in all but one *E. coli* strain, and did not induce gene recombination in *S. cerevisiae*. In addition, two other metabolites of MFH, formaldehyde and acetaldehyde, are genotoxic carcinogens.

5.3 Structure Activity Considerations

Hydrazines are characterized by a N-N covalent bond. MFH shares structural similarities with other carcinogenic hydrazines, including its metabolite MMH, and hydrazine, 1,1-dimethylhydrazine (1,1-DMH), and 1,2-dimethylhydrazine (1,2-DMH). The structures of these chemicals, as well as that of the hydrazone gyromitrin, which is hydrolyzed to MFH, are shown in Table 22. Information on the tumor findings from cancer bioassays conducted in mice and hamsters are also summarized in Table 22.

Many of the tumor types that are increased in mouse and hamster studies of MFH are also increased in those same species in studies of the comparator chemicals (Table 22). Specifically, in the mouse all the comparator chemicals also increased the incidence of lung tumors, three (MMH, hydrazine, 1,1-DMH) also increased the incidence of liver hepatocellular tumors, and three (MMH, 1,1-DMH, 1,2-DMH) also increased the incidence of blood vessel tumors. In the hamster two of the comparator chemicals (hydrazine, 1,2-DMH) also increased the incidence of liver hepatocellular tumors and one (MMH) also induced liver malignant histiocytoma (Kupffer cell sarcoma).

Table 22. Comparison of target tumor sites in mice and hamsters for MFH and structurally related hydrazines and the hydrazone gyromitrin

Chemical [Cancer classification]	Structure	Tumor sites	
		Mouse	Hamster
N-Methyl-N-formylhydrazine (MFH)[1]		Lung, liver hepatocellular, gallbladder, bile duct, blood vessel, seminal vesicles, forestomach, glandular stomach, subcutis, preputial gland	Liver hepatocellular, liver malignant histiocytoma (Kupffer cell sarcoma), gallbladder, bile duct
Gyromitrin (acetaldehyde methylformylhydrazone)[2] [P65, IARC Group 3]		Lung, forestomach, preputial gland, clitoral gland	Not tested
Monomethylhydrazine (MMH)[3] [P65]		Lung, liver hepatocellular, blood vessel, nasal, duodenum (adenoma)	Liver malignant histiocytoma (Kupffer cell sarcoma), large intestine, nasal
Hydrazine[4] [P65, IARC Group 2A, NTP RA]		Lung, liver hepatocellular, myeloid leukemia and lymphoma	Liver hepatocellular, nasal cavity, colon, thyroid
1,1-Dimethylhydrazine (1,1-DMH, unsymmetrical dimethylhydrazine, UDMH)[5] [P65, IARC Group 2B, NTP RA]		Lung, liver hepatocellular, blood vessel, kidney	Large intestine, peripheral nerve sheath
1,2-Dimethylhydrazine (1,2-DMH)[6] [P65, IARC Group 2A]		Lung, blood vessel, colon, small intestine	Liver hepatocellular, blood vessel, large intestine

P65, Proposition 65 carcinogen; IARC, International Agency for Research on Cancer; IARC Group 2A, probably carcinogenic to humans; IARC Group 2B, possibly carcinogenic to humans; IARC Group 3, Not classifiable as to its carcinogenicity to humans; NTP RA, NTP Report on Carcinogens *Reasonably anticipated to be a human carcinogen.*

[1] Tumor findings for MFH as reported in Section 4 of this HID

[2] Tumor findings for gyromitrin as reported in IARC (1987) and Gold and Zeiger (1997)

[3] Tumor findings for MMH as reported in NIOSH (1978), OEHHA (2001), and Gold and Zeiger (1997)

[4] Tumor findings for hydrazine as reported in IARC (1987), IARC (1999), IARC (2018), and NTP (2021)

[5] Tumor findings for 1,1-DMH as reported in IARC (1974), IARC (1999), and NTP (2021)

[6] Tumor findings for 1,2-DMH as reported in IARC (1974), IARC (1999), and Gold and Zeiger (1997)

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APPENDIX A: LITERATURE SEARCH ON THE CARCINOGENICITY OF N-METHYL-N-FORMYLHYDRAZINE (MFH)

Literature searches on the carcinogenicity of N-methyl-N-formylhydrazine (MFH) were initiated in December 2024. The goal was to identify peer-reviewed journal articles, print and digital books, reports, and gray literature that potentially reported toxicological and epidemiological information on the carcinogenicity of this chemical.

As described below, we used an approach similar to that recommended by the National Toxicology Program (NTP) Handbook for Preparing Report on Carcinogens (RoC) Monographs (NTP 2015; https://ntp.niehs.nih.gov/ntp/roc/handbook/roc_handbook_508.pdf).

The search was comprised of three approaches:

- Primary searches in major biomedical databases, conducted by OEHHA librarian Nancy Firchow, MLS
- Searches in other data sources, including authoritative reviews and reports, and databases or web resources, conducted by OEHHA scientists
- Additional focused searches, conducted by OEHHA scientists

Primary Search Process

1) Data Sources

Table A1 lists the data sources that were searched to find information on MFH. The list is adapted from the recommendation by the NTP Handbook for Preparing Report on Carcinogens (RoC) Monographs (NTP 2015), based on availability and suitability for this topic.

Table A1. Biomedical literature and other databases used in primary literature search

PubMed (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pmc/)
Embase (https://www.embase.com/)
SciFinder-n (https://scifinder-n.cas.org/)
Google Scholar (scholar.google.com)
ToxPlanet (www.toxplanet.com)

2) Search Term Identification

The US EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) was used to identify synonyms for MFH. The PubMed MeSH database

(https://www.ncbi.nlm.nih.gov/mesh/) was used to find subject headings and other index terms related to the chemical.

3) Primary Search Execution

Searches were executed in PubMed, Embase, SciFinder-n, Google Scholar, and ToxPlanet in December 2024. Because evidence on this chemical is limited, only one search using the chemical name and synonyms was performed in each database. There were no limits on language, date, species or end points.

The search was run first in PubMed. Then the search terms and syntax were tailored according to the search features unique to Embase. The Emtree subject heading list was searched to identify equivalent terms to replace the MeSH terms used in the PubMed searches. The translated search was then run in Embase.

The PubMed search strategy is shown in Table A2.

Table A2. PubMed search strategy

Search step	Search Terms	Concept
#1	N-methyl-N-formylhydrazine[nm] OR "N-methyl-N-formylhydrazine"[tiab] OR "N-formyl-N-methylhydrazine"[tiab] OR 758-17-8[rn] OR "hydrazine, 1-formyl-1-methyl-"[tiab] OR "N-methylformohydrazine"[tiab] OR "acetaldehyde N-methyl-N-formylhydrazone"[tiab] OR gyromitra[tiab] OR gyromitrin*[tiab] OR "false morel*"[tiab] OR ((monomethylhydrazine[tiab] OR MMH[tiab] OR monomethylhydrazine[mh] or MFH[tiab]) AND (mushroom*[tiab] OR gyromitra[tiab] OR gyromitrin*[tiab]))	MFH terms

Next, searches were run in SciFinder-n, Google Scholar, and ToxPlanet. Each of these resources requires unique search strategies, but all were limited to chemical name, synonym, and CAS registry number as appropriate.

Results from all databases were uploaded to EndNote and duplicates were removed. The total retrieval of the primary searches for MFH are shown in Table A3.

Table A3. MFH primary search results

Database	Total Unique Results After Deduplication
PubMed	116
Embase	67
SciFinder-n	6
Google Scholar	9
ToxPlanet	3

In addition to the primary searches listed above, focused searches were conducted by OEHHA scientists on the potential use and exposure of MFH, animal pathology, and structurally related chemicals.

Additional relevant literature was identified from citations of individual articles.

Introduction (Sections 1.2, 1.3, and 1.4)

Limited information on MFH's use was available from the primary searches. Additional focused searches of the worldwide web were conducted to identify information on the use of MFH, and on *Gyromitra spp* mushroom consumption.

Animal carcinogenicity studies - tumor pathology (Section 4)

Focused searches of the literature on tumor pathology of the mouse and hamster were conducted using:

- Pathology of the Mouse: edited by Maronpot RR, Boorman GA and Gaul BW. Cache River Press. 1999.
- Tumours of the Mouse in Pathology of Tumours in Laboratory Animals Vol. II: edited by Turusov VS and Mohr U. In IARC scientific publication No. 111. IARC, Lyon, France, 1994.
- Tumours of the Hamster in Pathology of Tumours in Laboratory Animals Vol. III: edited by Turusov VS and Mohr U. In IARC scientific publication No. 126. IARC, Lyon, France, 1996.
- Additional relevant literature was identified from citations of individual articles (e.g., McConnell et al. 1986).

Structure-activity considerations (Section 5.3)

Focused searches on animal carcinogenicity studies of chemicals structurally related to MFH were conducted, utilizing resources such as the IARC Monographs series, the

Handbook of Carcinogenic Potency and Genotoxicity Databases (edited by Gold and Zeiger, 1997), and additional relevant literature cited in these reviews.

Literature Screen Processes

Use of Health Assessment Workspace Collaborative (HAWC)

HAWC (https://hawcproject.org/about/) was used as a tool to screen and tag the literature on the carcinogenicity of MFH, following the guidance provided in the NTP RoC Handbook (NTP 2015).

Importing the EndNote library into HAWC

Citations retrieved from the literature searches for MFH were uploaded to an EndNote library, and duplicates were removed. Next, the EndNote library was uploaded to HAWC for multi-level screening using specific inclusion and exclusion criteria.

Screening and tagging references

In Level 1 screening, each citation was first screened by at least one OEHHA scientist, based solely on titles and abstracts, to eliminate studies or articles that do not contain information on MFH on any of the key topics covered in this cancer hazard identification document, such as cancer studies in humans and animals, toxicokinetics, metabolism, genotoxicity, or other cancer-associated mechanisms. The Level 1 screen was intended to identify all studies deemed to have a reasonable possibility of containing information that could be useful for the review process. Papers identified for inclusion during Level 1 screening were tagged in HAWC according to key topics. A paper can be assigned (or tagged) to one or more of the key topic(s). A positive response by only one of the reviewers was sufficient to pass a publication on to the next review level.

In Level 2 screening, the full papers were obtained for all citations that passed the Level 1 screen. These full papers were screened independently by at least one OEHHA scientist, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgments about the relevance of the citations because they were reviewing the full text of the articles, in addition to the title and abstract.

Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC. Level 1 and 2 screenings were conducted and HAWC search results were updated if additional relevant studies in addition to those cited in the original set of publications (“secondary citations”) were identified.

See Figure A1 for the overview of the HAWC literature screening results (literature tag tree) for the MFH HAWC project.

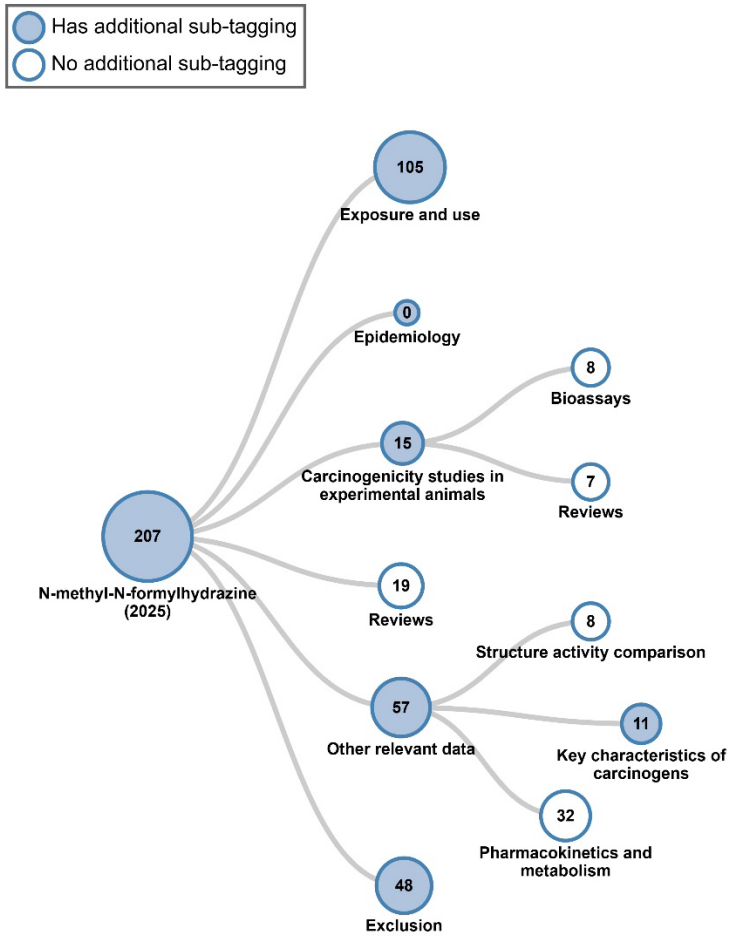

Figure A1. Overview of HAWC literature screening results for MFH.
The number of publications is indicated in each node of the literature tag tree.

Summary

More than 200 references, including peer-reviewed journal articles and government reports, were identified through these search strategies. Among these, over 90 references were cited in this document. See Figure A1 for the HAWC literature screening results.

APPENDIX B. SENSITIVITY ANALYSES: IMPACT OF ALTERNATIVE DENOMINATORS USED IN REPORTING TUMOR INCIDENCE DATA

In each of the analyses reported below, treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls, as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table B1. Bile duct tumor incidence using alternative denominators in male Swiss mice administered MFH in drinking water (Toth and Nagel 1978)

Tumor site	Tumor type	Concentration in drinking water		
		0	0.0078%	0.0156%
Bile duct	Cholangioma (r)	0/92	2/29	0/8
	Cholangiocarcinoma (r)	0/92	3/29*	0/8
	Cholangioma and cholangiocarcinoma combined (week 33) (r)	0/92	5/29***	0/8

The denominators for bile duct tumors were the number of animals alive in each group at week 30.

Table B2. Liver, gallbladder, and bile duct tumor incidences using alternative denominators in female Swiss mice administered MFH in drinking water (Toth and Nagel 1978)

Tumor site	Tumor type	Concentration in drinking water		
		0	0.0078%	0.0156%
Liver	Hepatocellular adenoma	0/99	12/43***	3/11***
	Hepatocellular carcinoma	0/99	10/43***	0/11
	Hepatocellular adenoma and carcinoma combined (week 35)	0/99	22/43***	3/11***
Gallbladder	Adenoma (week 59) (r)	0/95	4/23*	1/5*
Bile duct	Cholangioma and cholangiocarcinoma combined (week 31) (r)	0/99	2/43	1/11

The denominators for liver and bile duct tumors were the number of animals alive in each group at week 30, and for gallbladder tumors, the denominators were the number of alive animals in each group at week 50.

Table B3. Liver, gallbladder, bile duct, seminal vesicles, and blood vessel tumor incidences using alternative denominators in male Swiss mice administered MFH in drinking water (Toth et al. 1979)

Tumor site	Tumor type	Concentration in drinking water	
		0	0.0039%
Liver	Hepatocellular adenoma	2/80	9/30***
	Hepatocellular carcinoma	0/80	19/30***
	Hepatocellular adenoma and carcinoma combined (week 50)	2/80	28/30***
Gallbladder	Adenoma (week 50) (r)	0/80	5/30**
Bile duct	Cholangioma (week 50) (r)	0/80	6/30***
Seminal vesicles	Adenoma (week 62) (r)	0/60	3/24*
Blood vessels	Angioma and angiosarcoma combined (week 57)	5/80	4/30

The denominators for liver, gallbladder, bile duct and blood vessel tumors were the number of animals alive in each group at week 50, and for seminal vesicular tumors the denominators were the number of animals alive in each group at week 60.

Table B4. Liver and blood vessel tumor incidences using alternative denominators in male Swiss mice administered MFH in drinking water (Toth and Patil 1980a)

Tumor site	Tumor type	Concentration in drinking water		
		0	0.001%	0.002%
Liver	Hepatocellular adenoma	2/80	4/42	9/36***
	Hepatocellular carcinoma	0/80	2/42	5/36**
	Hepatocellular adenoma and carcinoma combined (week 54)	2/80	6/42*	14/36***
Blood vessels	Angioma	3/80	0/42	4/36
	Angiosarcoma	2/80	13/42***	3/36
	Angioma and angiosarcoma combined (week 52)	5/80	13/42***	7/36*

The denominators for liver and blood vessel tumors were the number of animals alive in each group at week 50.

Table B5. Preputial gland tumor incidence using alternative denominators in male Swiss mice administered MFH via a single *s.c.* injection (Toth and Patil 1980b)

Tumor site	Tumor type	Administered dose (mg/kg-bw)		
		0	100	120
Reproductive system - Preputial gland	Squamous cell papilloma (r)	0/80	3/33*	3/26*
	Squamous cell carcinoma (r)	0/80	3/33*	3/26*
	Papilloma and carcinoma combined (51 weeks) (r)	0/80	6/33***	6/26***

The denominators for preputial tumors were the number of animals alive in each group at week 50.

Table B6. Lung tumor incidence using alternative denominators in male Swiss mice administered MFH via 40 weekly *s.c.* injections (Toth and Patil 1983)

Tumor site	Tumor type	Administered dose (mg/kg-bw)	
		0	10
Lung	Adenoma	13/83	16/38**
	Adenocarcinoma	9/83	10/38*
	Adenoma and adenocarcinoma combined (week 47)	19/83	20/38**

The denominators for lung tumors were the number of animals alive in each group at week 40.

Table B7. Liver, gallbladder, and bile duct tumor incidences using alternative denominators in male Syrian golden hamsters administered MFH in drinking water (Toth and Patil 1979)

Tumor site	Tumor type	Concentration in drinking water (%)	
		0	0.0078
Liver	Hepatoma (hepatocellular adenoma) (r)	0/100	16/50***
	Hepatocellular carcinoma (r)	0/100	8/50***
	Hepatoma (hepatocellular adenoma) and carcinoma combined (r) (week 53)	0/100	24/50***
	Malignant histiocytoma (Kupffer cell sarcoma) (r) (week 73)	0/100	10/50***
Gallbladder	Papillary adenoma (r)	0/100	5/50**
	Adenocarcinoma (r)	0/100	3/50*
	Papillary adenoma and adenocarcinoma combined (r) (week 65)	0/100	8/50***
Bile duct	Cholangioma (r)	0/100	1/50
	Cholangiocarcinoma (r)	0/100	6/50**
	Cholangioma and cholangiocarcinoma combined (r) (week 73)	0/100	7/50**

The denominators were the number of animals alive in each group at the start of the study.

Table B8. Liver tumor incidence using alternative denominators in female Syrian golden hamsters administered MFH in drinking water (Toth and Patil 1979)

Tumor site	Tumor type	Concentration in drinking water (%)	
		0	0.0078
Liver	Hepatoma (hepatocellular adenoma) (r)	0/100	9/50***
	Hepatocellular carcinoma (r)	0/100	10/50***
	Hepatoma (hepatocellular adenoma) and carcinoma combined (r) (week 47)	0/100	19/50***
	Malignant histiocytoma (Kupffer cell sarcoma) (r) (week 76)	0/100	24/50***

The denominators were the number of animals alive in each group at the start of the study.