EVIDENCE ON THE CARCINOGENICITY OF

4-METHYLQUINOLINE

FINAL

DECEMBER 2000

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity . . . if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board (22 CCR 12301).

4-Methylquinoline was assigned a final priority of 'high' carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on August 6, 1999. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced on August 6, 1999, in the *California Regulatory Notice Register*. This document reviews the available scientific evidence on the carcinogenic potential of 4-methylquinoline. It was released as the draft document *Evidence on the Carcinogenicity of 4-Methylquinoline* in August 2000.

At their November 16, 2000 meeting the Committee, by a vote of one in favor and five against, did not find that 4-methylquinoline had been "clearly shown through scientifically valid testing according to generally accepted principles to cause cancer."

The following is the final version of the document that was discussed by the Committee at their November 2000 meeting.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

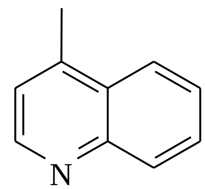
EXECUTIVE SUMMARY

4-Methylquinoline is an aza-arene compound and an environmental contaminant primarily associated with the use of hydrocarbons in shale oil and coal gasification and wood treatment processes. Exposure concern also stems from the identification of 4-methylquinoline in tobacco cigarette smoke and its presence in urban particulate matter.

There is evidence for the carcinogenicity of *4-methylquinoline*, with the development of liver tumors in male mice exposed as newborns via three intraperitoneal injections and the initiation of skin tumors in two studies in female mice. Further evidence of carcinogenic potential is provided by clear evidence of mutagenicity in short-term tests, induction of unscheduled DNA synthesis in rat hepatocytes *in vitro*, and by strong chemical structural analogy with a known carcinogen.

2 INTRODUCTION

2.1 Identity of 4-Methylquinoline



Molecular Formula:	$C_{10}H_9N$
Molecular Weight:	143.19
CAS Registry No.:	491-35-0
Chemical Class:	heterocyclic aromatic hydrocarbon; aza-arene
Synonym:	Lepidine; cincholepidine; γ-methylquinoline
Boiling point:	261-263°C (Chemfinder, 1997)
Melting point:	9-10°C (Chemfinder, 1997)

2.2 Occurrence and Use

4-Methylquinoline (4-MeQ) is an environmental contaminant primarily associated with the use of hydrocarbons in shale oil and coal gasification and wood treatment processes. These processes have resulted in the contamination of groundwater. Methylquinolines have been associated with the organic portion of urban particulate matter (Dong *et al.*, 1977).

4-MeQ has been identified as a component of tobacco smoke (Adams *et al.*, 1983). An evaluation of mainstream smoke from four brands of filtered cigarettes showed a range of 4-MeQ content from 67 to 420 ng 4-MeQ per cigarette. The mainstream smoke from one brand of nonfilter cigarettes was shown to contain 676 ng 4-MeQ per cigarette. 4-MeQ has also been identified as a pyrolysis product of nicotine (Schmeltz *et al.*, 1979).

The 1983 National Exposure Survey estimated 1557 employees (276 female) in the U.S. were potentially exposed to 4-MeQ (RTECS, 1997). In addition to occupational scenarios, widespread exposure concern also stems from the identification of 4-MeQ in tobacco cigarette smoke and its presence in urban particulate matter.

3 DATA ON 4-METHYLQUINOLINE CARCINOGENICITY

Two series of carcinogenicity studies have been reported: studies in mice in which 4-MeQ was administered intraperitoneally and studies in rats in which 4-MeQ was administered subcutaneously. Two initiation/promotion studies have also been reported with 4-MeQ administered as the initiator to female mice. 4-MeQ has also been tested for genotoxicity in multiple *Salmonella* reverse mutation assays and in a single *in vitro* test in mammalian cells.

3.1 Epidemiological Studies of Carcinogenicity in Humans

No data on long-term effects of human exposure to 4-methylquinoline were found in a recent search by OEHHA.

3.2 Carcinogenicity Studies in Animals

Mouse Intraperitoneal Exposure: LaVoie et al., 1988

Newborn CD-1 mice (n=57) were injected intraperitoneally with 0.25, 0.50, and 1.00 μmol 4-MeQ per mouse (in DMSO) on the first, eighth, and 15th days of life, respectively (total dose =1.75 μmol 4-MeQ; LaVoie *et al.*, 1988). Control animals (n=46) were treated with DMSO alone. At 21 days, mice were separated by sex and observed until week 52 at which time all surviving animals were killed. Gross lesions were examined histologically and included liver sectioning. Tumor incidences are presented in Table 1. Significant increases in liver adenomas and total liver tumors (*i.e.*, adenomas and hepatomas) were observed among treated male mice. The four liver tumors reported for the DMSO-treated (control) mice were identified as neither adenomas nor hepatomas. The histological category into which these tumors in control animals fall is therefore unclear. No liver tumors were observed among female mice in LaVoie *et al.* (1988) studies.

According to the authors who cited Prejean *et al.*, 1973, the spontaneous incidence of liver tumors in Swiss-Webster mice (CD-1 mice were derived from the Swiss strain) is generally less than 10%. Prejean *et al.* (1973) identified a single "hepatocytic adenoma" and a single "hemangioendothelial sarcoma" of the liver among 254 Swiss-Webster mice observed for 540 days (sex not stated). Sher *et al.* (1982) reported incidence ranges of 0-12% for liver adenomas and 0-8% for liver "adenocarcinomas" (presumably hepatocellular carcinomas) among 24 groups of control male CD-1 mice (1232 total mice) surviving 81-105 weeks in studies conducted at Merck, Sharp and Dohme Research Laboratories. The average incidence was 3% liver adenomas and 2% liver adenocarcinomas with an average combined incidence of 5%. A more recent evaluation of the incidence of spontaneous tumors in Swiss CD-1 mice at 21 months of age from three breeding facilities showed a range of 2.7-12.4% liver adenomas (overall incidence: 5.8% or 23/397) and 2.7-6.0% liver carcinomas (overall incidence: 4.3% or 17/397) (Engelhardt *et al.*, 1993). These authors stated that spontaneous liver adenomas and carcinomas were limited to male CD-1 mice.

No significant increases in tumors at sites other than the liver were observed in male or female mice. A single leukemia or lymphoma was observed in a 4-MeQ-treated male mouse. Two lung tumors were reported in each of the 4-MeQ treated male and female mouse groups. No lung tumors were reported in the control groups. This increase was not statistically significant (Fisher's exact test; males, p = 0.32; females, p = 0.33).

Table 1. Tumor incidence in CD-1 mice injected intraperitoneally with 4-methylquinoline as newborns surviving six months and sacrificed at one year (LaVoie *et al.*, 1988).

Treatment	Sex	Liver tumors *			Lung tumors *
		Total	Adenomas	Hepatomas	
4-MeQ	Male	23/28 **	20/28 **	3/28	2/28
	Female	0/29	0/29	0/29	2/29
Control (DMSO)	Male	4/21 ***	0/21	0/21	0/21
	Female	0/21	0/21	0/21	0/21

* Tumor incidences are expressed as number of tumor bearing animals / number of animals alive at six months.

** Significant increase in incidence relative to controls by Fisher's exact test ($p < 0.0001$).

*** Tumors seen in controls were neither adenomas nor hepatomas: histological classification for these tumors was not provided by the authors.

Rat Subcutaneous Exposure: LaVoie, 1988

Newborn Sprague-Dawley rats (n=50) were injected subcutaneously on the first day of life with 200 µmol 4-MeQ/kg_{bw}, subsequently weekly with 100 µmol/kg_{bw} during weeks two to seven, and finally at 200 µmol/kg_{bw} at the eighth week (LaVoie *et al.*, 1988). Injected concentrations were 0.1 M 4-MeQ in DMSO for weeks one through four, 0.3 M for weeks five and six, and 1.2 M for weeks seven and eight. Control animals (n=50) received 500 µl DMSO/kg_{bw} on the first day of life and weekly thereafter for eight weeks. Two percent of the 4-MeQ treated rat pups died within the first week of life. At four weeks, the animals were separated by sex and observed until the 78^{th} week at which time the animals were killed. Livers and macroscopic lesions were examined histologically. Liver tumor incidences are presented in Table 2. No significantly increased tumor incidences were observed in either male or female rats at any site.

Table 2. Tumor incidence in Sprague-Dawley rats injected subcutaneously with 4-methylquinoline as newborns surviving nine months and sacrificed at 78 weeks (LaVoie *et al.*, 1988).

Treatment	Sex	Liver tumors*		
		Total	Adenomas	Hepatomas
4-MeQ	Male	1/26	0/26	1/26
	Female	2/20	2/20	0/20
Control (DMSO)	Male	5/27	3/37	2/27
	Female	1/22	1/22	0/22

* Tumor incidences are expressed as number of tumor bearing animals / number of animals alive at nine months.

Discussion of Carcinogenicity Studies in Animals

In summary, 4-MeQ induced liver tumors within one year following three intraperitoneal injections administered to neonatal male, but not female, CD-1 mice. Sprague-Dawley rats administered 4-MeQ subcutaneously as newborns and during the first eight weeks of life showed no carcinogenic effect. The ability of each of these studies to detect a carcinogenic effect of 4-MeQ was limited by the short dosing periods employed and the less-than-lifetime duration of the studies.

3.3 Other Relevant Data

3.3.1 Tumor Initiation/Promotion Studies

Mouse Dermal Initiation Study: LaVoie et al., 1983

Outbred female Hfd:SENCAR mice (n=25) aged 50-55 days were treated on their shaved backs with 0.1 ml of a 0.5% 4-MeQ solution in acetone 10 times, every other day, producing a total initiating dose of 5 mg 4-MeQ (LaVoie *et al.*, 1983). Negative and positive control animals (n=25) were treated with acetone alone or with benzo[a]pyrene (total dose = 0.03 mg B[a]P), respectively. Ten days following the last treatment with initiator, 2.5 μg tetradecanoyl phorbol acetate (TPA) was applied three times weekly for 20 weeks. Animals were monitored for skin tumors weekly during the promotion period. Skin tumor incidences after 20 weeks of promotion (<25 weeks after initial exposure to 4-MeQ or B[a]P) are presented in Table 3. Significant increases in skin tumors were observed in mice initiated with 4-MeQ and with B[a]P relative to the negative control group.

Spontaneous skin tumors are rare in the SENCAR mouse strain. Among 223 untreated female SENCAR mice observed until their natural death (50% survival to 24 months), one skin papilloma and no squamous cell carcinomas were observed (Conti *et al.*, 1985). In another study of untreated female SENCAR mice observed until their natural death (median survival to approximately two years), no skin tumors were reported in one group of 78 mice and one skin sarcoma and no papillomas were reported among another group of 41 mice (Melchionne *et al.*, 1986).

Table 3. Skin tumor incidence in SENCAR mice treated dermally with 4-methylquinoline in an initiation / promotion protocol evaluated at approximately 32 weeks of age (LaVoie *et al.*, 1983).

Treatment	Skin Tumors*	Avg. # Skin Tumors/Animal
4-MeQ	11/25 **	0.56
B[a]P	18/24 **	1.79
Control (acetone)	1/24	0.04

* Tumor incidences are expressed as number of tumor bearing animals / number of animals examined.

** Statistically significant increase relative to acetone controls by Fisher's exact test ($p < 0.005$).

Mouse Dermal Initiation Study: LaVoie et al., 1984

Outbred female Hfd:SENCAR mice (n=30) aged 50-55 days were treated on their shaved backs with 0.1 ml of a 0.75% 4-MeQ solution in acetone 10 times, every other day, producing a total initiating dose of 7.5 mg 4-MeQ (LaVoie *et al.*, 1984). Negative and positive control animals (n=40) were treated with acetone alone or with benzo[a]pyrene (total dose = 0.03 mg B[a]P), respectively. Ten days following the last treatment with initiator, 2.0 μg TPA was applied twice weekly for 18 weeks. Animals were monitored for skin tumors weekly during the promotion period. Skin tumor incidences after 18 weeks of promotion (<23 weeks after exposure to 4-MeQ or B[a]P) are presented in Table 4. Significant increases in skin tumors were observed in mice initiated with 4-MeQ and with B[a]P relative to the negative control group.

Table 4. Skin tumor incidence in SENCAR mice treated dermally with 4-methylquinoline in an initiation/promotion protocol evaluated at approximately 30 weeks of age (LaVoie *et al.*, 1984).

Treatment	Skin Tumors*	Avg. # Skin Tumors/Animal
4-MeQ	13/29 **	0.90
B[a]P	25/39 **	2.1
Control (acetone)	3/39	0.08

* Tumor incidences are expressed as number of tumor bearing animals / number of animals examined.

** Statistically significant increase relative to acetone controls by Fisher's exact test ($p < 0.0005$).

3.3.2 Genetic Toxicology

The experimental evidence regarding the genotoxicity of 4-MeQ is somewhat limited, although that which is available indicates mutagenic properties. Other than multiple testing in several strains of *Salmonella typhimurium*, the only available genotoxicity test examined the effects of 4-MeQ on unscheduled DNA synthesis (UDS) in rat hepatocytes.

4-MeQ has consistently tested positive for mutagenicity in *Salmonella* assays in the presence, but not the absence, of metabolic activating systems. Positive reverse mutation tests were reported in *Salmonella* strains TA98 and TA100. In a test of 33 substituted quinolines with strain TA100, 4-MeQ produced the highest rate of reversion (Debnath *et al*., 1992). These authors noted that, in their hands, quinoline compounds were "inactive or very weakly active" in assays with strain TA98 (data not shown). *Salmonella* strain TA98 provides evidence of frameshift mutations to DNA, while strain TA100 provides evidence of base-pair mutations. A positive forward mutation test was reported in *Salmonella* strain TM677. The results from the testing of 4-MeQ in *Salmonella* are presented below in Table 5.

Table 5. Results of mutagenicity tests of 4-methylquinoline in several *Salmonella typhimurium* strains.

Reverse mutation assays				
Strain	**Treatment**	**Result**	**Compound/Notes**	**Reference**
TA98	+ S-9	+	Source/purity not stated	Sugimura *et al.*, 1976
TA100	+ S-9	+		
TA98	+ S-9	+	Commercial sample; "purest grade"	Nagao *et al.*, 1977
	– S-9	–		
TA100	+ S-9	+		
	– S-9	–		
TA100	+ S-9	+	Commercial sample; further purified by LC or HPLC; 11% survival	Dong *et al.*, 1978
TA98	+ S-9	+	Commercial sample; 500 µg/plate	Hashimoto *et al.*, 1979
	– S-9	–		
TA100	+ S-9	+		
	– S-9	–		
TA98	– S-9	–	Commercial sample; "highest grade"; mutagenicity data not shown	Ogawa *et al.*, 1987
TA100				
TA1537				
TA2637				
TA100	+ S-9	+	Source/purity not stated; 0-2.5 µmol/plate	Takahashi *et al.*, 1988
	– S-9	–		
TA100	+ S-9	+	Commercial sample; >99% pure by HPLC	Debnath *et al.*, 1992
TA100	+ S-9	+	Commercial sample; purity not stated	Saeki *et al.*, 1996

Forward mutation assay				
Strain	**Treatment**	**Result**	**Compound/Notes**	**Reference**
TM677	+ S-9	+	Commercial sample; 70-700 µM 4-MeQ	Kaden *et al.*, 1979

Primary cultures of hepatocytes isolated from male Sprague-Dawley rats were treated with 4-MeQ at concentrations of 0.1 and 1.0 mM 4-MeQ in a standard UDS assay (LaVoie *et al.*, 1991). 4-MeQ was considered positive in the induction of UDS relative to DMSO and untreated control cultures.

No *in vivo* tests for the genotoxicity of 4-MeQ were identified.

3.3.3 Structure-Activity Comparisons

Quinoline and several other quinoline derivatives have been tested for carcinogenic properties. Quinoline itself is the most extensively studied compound in this category, with bioassays showing the induction of vascular tumors of the liver in male rats (Hirao *et al.*, 1976; Shinohara *et al.*, 1977; Hasegawa *et al.*, 1989). Quinoline showed a similar spectrum of results compared to 4-MeQ in intraperitoneal injection studies in neonatal mice and subcutaneous injection studies in neonatal rats (LaVoie *et al.*, 1987; LaVoie *et al.*, 1988). That is, male mice, but not female mice or male or female rats, developed liver tumors following three injections of quinoline. Like 4-MeQ, quinoline has also demonstrated skin tumor-initiating activity in the dermal exposure studies reported by LaVoie *et al.* (1983; 1984) and has tested positive in multiple *Salmonella* reverse mutation assays (Dong *et al.*, 1978; Hollstein *et al.*, 1978; Nagao *et al.*, 1977; LaVoie *et al.*, 1991; Willems *et al.*, 1992). Quinoline and its strong acid salts were listed as “causing cancer” under Proposition 65 on October 24, 1997, based upon a determination by the state’s qualified experts.

8-Methylquinoline (8-MeQ) has also been tested in the same bioassay series as 4-MeQ reported by LaVoie *et al.* (1988). Among male CD-1 mice injected intraperitoneally three times with 8-MeQ as neonates, an increase in liver tumors (all adenomas) was observed at one year, although this increase was not statistically significant (8/28, treated *vs.* 4/21 controls). No carcinogenic effect was observed in female mice or in a subcutaneous injection study in Sprague-Dawley rats (LaVoie *et al.*, 1988). 8-MeQ demonstrated skin tumor-initiating activity in the dermal exposure study reported by LaVoie *et al.* (1984). 8-Methylquinoline has not been listed as “causing cancer” nor have any authoritative bodies under Proposition 65 reviewed it.

Table 6 below summarizes the available information concerning the carcinogenicity of several compounds that are structurally related to 4-MeQ. Positional isomers of 4-MeQ vary greatly in their carcinogenic and/or mutagenic properties, with only 8-MeQ exhibiting some carcinogenic potential in tumor initiation and subcutaneous injection assays.

A potential metabolite of 4-MeQ, 4-methylquinoline-N-oxide (see below), bears structural resemblance to 4-nitroquinoline-N-oxide, a potent genotoxic agent and contact carcinogen which induces squamous cell carcinomas of the mouth in exposed rodents (Wong and Wilson, 1983; Steidler and Reade, 1984; NTP, 2000).

Table 6. Carcinogenic and related properties of compounds structurally similar to 4-methylquinoline.

Compound	Evidence of Tumorigenicity	Tumor Initiation	Genotoxicity	References
4-MeQ	++	++	++	See text
Quinoline	++	++	++	See text
2-MeQ	–	–	±	LaVoie *et al.*, 1984; Debnath *et al.*, 1992
3-MeQ	ND	–	+	LaVoie *et al.*, 1984
5-MeQ	ND	–	ND	LaVoie *et al.*, 1984
6-MeQ	–	–	+	Fukushima *et al.*, 1981; LaVoie *et al.*, 1984
7-MeQ	ND	–	+	LaVoie *et al.*, 1983; LaVoie *et al.*, 1984
8-MeQ	+	++	±	See text; Debnath *et al.*, 1992
4-Ethyl-quinoline	ND	ND	–	Saeki *et al.*, 1996
4-Methoxy-quinoline	ND	ND	–	Saeki *et al.*, 1996

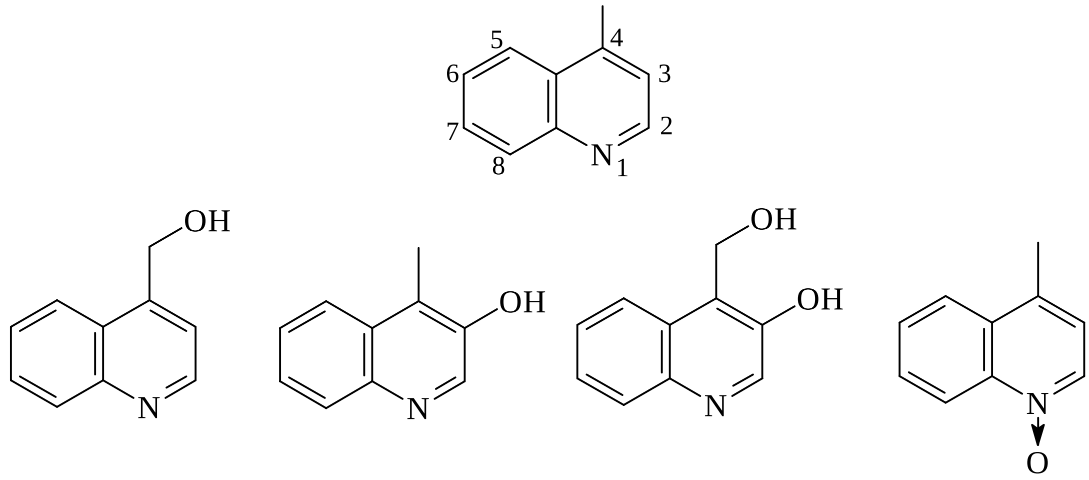
ND = no data available

3.3.4 Pharmacokinetics and Metabolism

The metabolism of 4-MeQ has not been investigated *in vivo*; however, some metabolites have been identified from *in vitro* studies. Saeki *et al.* (1996) subjected 4-MeQ to metabolism for 60 minutes by S-9 microsomal fraction prepared from 3-methylcholanthrene-induced rat liver. Metabolites were characterized by high-performance liquid chromatography (HPLC) and ultraviolet spectroscopy. The primary metabolites identified in an acetonitrile-soluble fraction of the reaction mixture were 4-hydroxymethylquinoline (4-HMeQ: 38.7%), 3-hydroxy-4-hydroxy-methylquinoline (3-OH-4-MeQ: 8.0%), 4-methylquinoline N-oxide (4-MeQO: 3.6%), and 3-hydroxy-4-methylquinoline (3-OH-4-MeQ: 1.6%) (see Figure 1 below). There was no evidence for the presence of a 5,6-diol metabolite. Approximately 48% of the metabolites were unidentified; the authors commented that “no other particular intense peaks were observed on the HPLC profile,” suggesting that the remaining material comprised numerous compounds.

No data have been located regarding the pharmacokinetics of 4-MeQ.

Figure 1. Metabolites of 4-methylquinoline identified from *in vitro* studies (Saeki *et al.,* 1996).

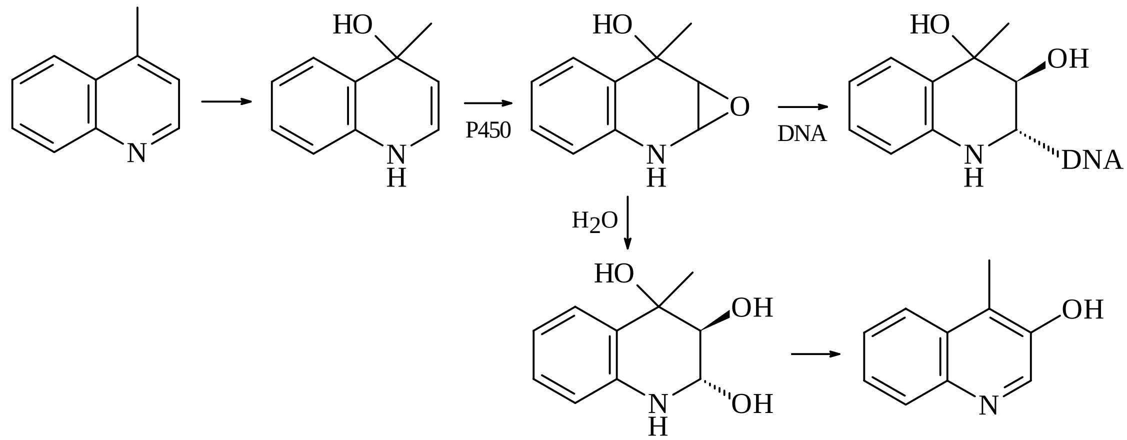


4-HMeQ **3-OH-4-MeQ** **3-OH-4-HMeQ** **4-MeQO**

Saeki *et al.* (1996) conducted *in vitro* metabolism studies not only with 4-MeQ, but also with the more weakly mutagenic isomers, 3-MeQ and 2-MeQ (see description of methods above). The profile of metabolites generated from these two other isomers of 4-MeQ showed that the epoxidation at the 5,6 position was the primary product generated by the metabolic activation system leading to the production of MeQ-5,6-dihydro-5,6-diol compounds. Since this epoxide does not form as a product of the metabolism of 4-MeQ, it was surmised that steric hindrance leads 4-MeQ to be primarily metabolized by another pathway. In a scenario based upon the observed metabolites, ring hydroxylation at the methyl group followed by oxidation by cytochrome P450 at the 2,3 position could lead to a product which could hypothetically undergo either reaction with DNA or hydrolysis to form 3-OH-4-MeQ (see Figure 2 below). Deuteration of the hydrogens on the 4-methyl group as well as at the 2-position (selective deuteration could not be achieved experimentally) led to a considerable reduction in metabolism to the 4-hydroxymethyl metabolite with a concomitant increase in 3-hydroxy- and 3-hydroxy-4-hydroxymethyl metabolites. This finding, coupled with a higher relative mutagenicity of the deuterated compound, suggests that hydroxylation of the methyl group serves to detoxify 4-MeQ, insofar as mutagenicity is concerned. The pathway proposed in Figure 2 is also supported by the lack of mutagenicity of 3-chloro-4-methylquinoline, with the presence of the chlorine at the three position blocking the formation of the hydroxy compound which could interact with DNA. Further studies with mono- and di-substitution of fluorine on the 4-MeQ molecule reinforce the concept that the availability of the 2-position is important in 4-MeQ's mutagenicity (Kato *et al.*, 2000). Fluorine substitution at the 2-position (2-F-4-MeQ, 2,6-diF-4-MeQ, and 2,7-diF-4-MeQ) eliminated mutagenicity, whereas substitution at other sites resulted in little change in mutagenicity (7-F-4-MeQ) or a moderate attenuation of mutagenicity (6-F-4-MeQ).

No studies of the *in vivo* metabolism of 4-MeQ have been published; similarly, no studies investigating the potential for 4-MeQ or its metabolites to form DNA adducts have been reported.

Figure 2. Proposed metabolic scheme of 4-methylquinoline leading to mutagenic intermediates (adapted from Saeki *et al.*, 1996).



3.3.5 Pathology

The liver tumors identified in the LaVoie *et al.* (1988) mouse bioassays were termed either liver adenomas or "hepatomas." Because of the authors' use of these distinct classifications, it is assumed that those tumors termed "hepatoma" are, in fact, malignant tumors. It is generally considered that liver adenomas and malignant hepatomas are related in origin, and that the liver adenomas may progress to a malignant phenotype (Frith *et al.*, 1994). They are therefore usually aggregated for carcinogen identification and risk assessment purposes.

The identity of the skin tumors formed in the initiation/promotion studies in SENCAR mice (LaVoie *et al.*, 1983; LaVoie *et al.*, 1984) was not stated in the studies' findings. Skin tumors observed in this SENCAR mouse initiation/promotion model, such as those observed following exposure to benzo[a]pyrene, are typically squamous cell papillomas, which frequently progress to squamous cell carcinomas (Buhler *et al.*, 1982; Slaga, 1986; Bogovski, 1994).

3.3 Mechanism

The genotoxicity of 4-MeQ demonstrated in a number of *in vitro* tests is consistent with the hypothesis that 4-MeQ increases the incidence of tumors by a genotoxic mechanism. The *in vitro* investigations of Saeki *et al.* (1996) have identified metabolites and plausible intermediates that may react covalently with DNA (see Section 3.3.4 Pharmacokinetics and Metabolism above).

4 SUMMARY AND CONCLUSIONS

4.1 Summary of Evidence

In neonatal male mice treated intraperitoneally with three doses, 4-MeQ induced liver tumors within one year. Studies in female mice and male and female rats did not produce significant increases in tumor incidence, although the limited dosing and duration of the experiments may have resulted in a limited ability to detect a carcinogenic effect in these cases. In two studies in female mice in which 4-MeQ was administered as an initiating agent followed by promotion with TPA, significant increases in skin tumors were observed within six months. Genotoxicity data on 4-MeQ indicate that the compound causes mutational changes in *Salmonella typhimurium* and induces unscheduled DNA synthesis in rat hepatocytes. 4-MeQ also shows a structural analogy to quinoline, a known carcinogen.

4.2 Conclusion

There is evidence for the carcinogenicity of 4-MeQ, with the development of liver tumors in male mice receiving three intraperitoneal injections as neonates. Two initiation/promotion studies in female mice have also demonstrated the initiating activity of 4-MeQ. Further evidence includes observations of genotoxicity in short-term tests in bacteria and mammalian cells, and by structural analogy with a known carcinogen.

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