

**EVIDENCE ON THE CARCINOGENICITY OF**

# **PHENELZINE AND ITS ACID SALTS**

**DRAFT**

**September 2002**



**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) (referred to hereafter as the Committee).

Phenelzine and its acid salts were assigned a final priority of ‘high’ carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on November 5, 1999. A public request for information relevant to the assessment on the evidence on the carcinogenicity of this chemical was announced in the *California Regulatory Notice Register* on November 19, 1999. No information was received as a result of this request.

This draft document, *Evidence on the Carcinogenicity of Phenelzine and its Acid Salts*, was developed to provide the Committee with the available scientific evidence on the carcinogenic potential of these chemicals. A public meeting of the Committee to discuss this evidence is scheduled for December 17, 2002. Following discussion and Committee deliberation, the Committee will determine whether phenelzine and its acid salts have been “clearly shown through scientifically valid testing according to generally accepted principles to cause cancer.” Written public comment on the document should be submitted to OEHHA by November 19, 2002 in order to be considered by the Committee in advance of the meeting. During the December 2002 meeting, the public will have an opportunity to provide verbal comments to the Committee.

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## 1 EXECUTIVE SUMMARY

Phenelzine is used to produce phenelzine sulfate, a monoamine oxidase (MAO) inhibitor prescribed for the treatment of depression. Phenelzine sulfate is dispensed in tablets equivalent to 15 mg of phenelzine, with a typical starting dose for adults of 45 mg/day increasing to 60-90 mg/day. A maintenance dose is generally 15-45 mg every other day. In 1998, an estimated 1,200 patients received phenelzine sulfate in California.

Phenelzine sulfate has been tested for carcinogenicity in lifetime drinking water studies in male and female mice, and in a single diet study in male rats. Adenocarcinomas and adenomas of the lung and angiosarcomas and angiomas were induced in female mice receiving phenelzine sulfate in drinking water for life. In a parallel study in male mice, the incidence of lung adenoma was significantly higher in treated animals; the combined incidence of lung adenoma and lung adenocarcinoma in males was marginally statistically significant ( $p = 0.07$ ) when compared to controls. If the number of animals is adjusted to reflect only those alive at first occurrence of tumor (*i.e.*, effective number or number of animals "at risk"), the combined incidence of lung adenoma and lung adenocarcinoma in males is statistically significantly increased. No treatment-related increase in tumors was observed in male rats administered phenelzine sulfate in the diet for 87 weeks. The male rat study was limited by small group sizes ( $n < 15$ ), use of a single dietary phenelzine sulfate concentration, less than lifetime study duration, and incomplete reporting.

Phenelzine sulfate is genotoxic in a variety of short-term tests. Sister chromatid exchanges (SCE) were induced in the bone marrow of mice treated by intraperitoneal injection with phenelzine sulfate. DNA strand breaks were demonstrated in lungs and liver of mice treated by intraperitoneal injection or orally with phenelzine sulfate. A number of studies have demonstrated the mutagenic activity of phenelzine sulfate in *Salmonella typhimurium* strains that respond to base substitution mutations. In addition, DNA damage was induced by phenelzine sulfate in repair-deficient strains of *Escherichia coli*.

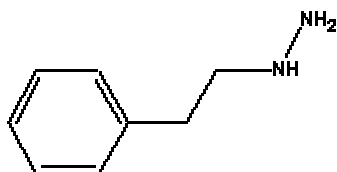
The mechanism of carcinogenicity of phenelzine sulfate is not well understood. Available evidence from *in vitro* mechanistic studies suggests that oxidation of phenelzine can lead to the formation of phenylethyl radicals and other radical species, and arenediazonium ions, which can react with DNA to form adducts of purine bases. The formation of DNA adducts may lead to mispairing, depurination and DNA strand breaks, eventually leading to cancer.

Phenelzine and its acid salts are structurally related to 1,2-diethylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, 1,2-diphenylhydrazine, hydrazine, hydrazine sulfate, methylhydrazine and its salts, and phenylhydrazine and its salts, all of which are listed under Proposition 65 as chemicals known to the State to cause cancer.

## 2 INTRODUCTION

### 2.1 Identity of Phenelzine and its Acid Salts

Figure 1. Structure of phenelzine.



Molecular Formula:  $C_8H_{12}N_2$

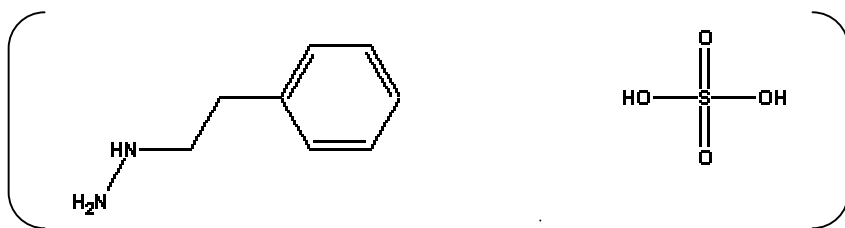
Molecular Weight: 136.2

CAS Registry No. 51-71-8

Chemical Class: Hydrazine derivative; monoamine oxidase inhibitor

Synonyms: Phenylethylhydrazine; 2-phenylethylhydrazine;  $\beta$ -phenylethylhydrazine; 1-hydrazino-2-phenylethane; phenalzine

Figure 2. Structure of phenelzine sulfate.



Molecular Formula:  $C_8H_{12}N_2 \cdot H_2SO_4$

Molecular Weight: 234.3

CAS Registry No. 156-51-4

### 2.2 Occurrence and Use

Phenelzine has been used to manufacture phenelzine sulfate and phenelzine hydrochloride (IARC, 1980). Phenelzine sulfate, a monoamine oxidase (MAO) inhibitor marketed under the trade name Nardil® for the treatment of depression, is the only phenelzine salt identified as being in use. Nardil® is typically used in depressed patients “clinically characterized as ‘atypical’, ‘nonendogenous’, or ‘neurotic’ ” (Physician’s Desk Reference, 2000). Such patients often experience mixed anxiety and depression with phobic or hypochondriacal symptoms. Nardil® is generally not the first drug prescribed, but is reserved for use in patients that fail to respond to the more common antidepressants. Nardil® treatment is undertaken in stages (Physician’s Desk Reference, 2000). The usual initial dose is one tablet (equivalent to 15 mg of phenelzine) three times per day. The dose is then increased rapidly to 60 mg/day, depending on

patient tolerance. A dose of 90 mg/day may be required to achieve a beneficial effect. Once the beneficial effect is observed, the dose is slowly decreased to a maintenance level, which typically is between 15 to 45 mg every other day. In 1998, approximately 1,200 patients in California were prescribed Nardil®, with approximately 12,000 patients receiving the drug nationwide (Warner-Lambert Company, 1999).

### **3 DATA ON CARCINOGENICITY OF PHENELZINE AND ITS ACID SALTS**

The carcinogenicity of phenelzine sulfate has been studied in male and female mice exposed via drinking water for life and in male rats exposed via diet to phenelzine sulfate for 87 weeks. Phenelzine sulfate has been tested in a variety of *in vivo* and *in vitro* short-term tests including assays for sister chromatid exchanges (SCE) in the bone marrow of mice, DNA damage in lungs and liver of mice and in repair-deficient strains of *Escherichia coli*, and mutagenic activity in *Salmonella typhimurium*. Phenelzine is structurally related to hydrazine and numerous hydrazine derivatives, most of which have been shown to be carcinogenic.

#### **3.1 Carcinogenicity Studies in Humans**

No epidemiological studies on phenelzine or its acid salts were located in the literature. Daneshmend *et al.* (1979) reported a case of a woman treated with phenelzine for at least six years who developed fatal angiosarcoma of the liver, a rare tumor. An occasional dose of diazepam was the only other regular medication. The woman had no history of exposure to thorium dioxide, arsenic or vinyl chloride, agents known to be associated with liver angiosarcoma.

#### **3.2 Carcinogenicity Studies in Animals**

Carcinogenicity studies in male and female mice and in male rats are discussed.

##### *Mice Drinking Water Studies: Toth (1976); Toth and Nagel (1976a); Toth and Shimizu (1974)*

Toth and coworkers (Toth, 1976; Toth and Nagel, 1976a; Toth and Shimizu, 1974) administered phenelzine sulfate in the drinking water at a level of 0.015% to groups of 50 male and 50 female Swiss albino mice for life. A range-finding study was conducted prior to the chronic study to select the phenelzine water concentration. Average daily water intake was 8.5 ml for the females and 15.6 ml for the males.

The Swiss albino mice were from a colony bred at the Toth laboratory since 1951. Because a large number of studies were being conducted concurrently, control groups of 100 male and 100 female Swiss albino mice were started every 1 to 1.5 years (Toth, personal communication, 2002). Tumor incidence data from a control group started close in time to the treated group were then used for statistical analysis. For the phenelzine sulfate studies, Toth (1976) cited Toth and



Shimizu (1974) as the source of the control data used to carry out the statistical comparisons. The male and female controls that were put on test at the same time as the phenelzine sulfate treatment groups and evaluated for tumorigenicity soon after the phenelzine sulfate studies were completed showed “types and incidences of neoplasms that are similar to those of previous [control] groups” (Toth, 1976). Historical control tumor incidence data from the Toth laboratory are discussed in greater detail below.

Survival was significantly reduced among treated animals compared to controls ( $p < 0.01$  for treated males and treated females compared to respective controls, based on the Kolmogorov-Smirnov test) (Toth, 1976). Males were more severely affected than females. Survival at 50 weeks was 84% for exposed females (compared to 91% in controls) and 54% for exposed males (compared to 88% in controls). At 80 weeks, survival was 32% for females (versus 71% in controls) and 18% for males (versus 65% in controls). All females in the treatment group died by 100 weeks, while all males died by 110 weeks.

Table 1 summarizes the tumor incidence data in female mice. The incidence data as reported by Toth and coworkers (Toth, 1976; Toth and Shimizu, 1974) are given along with the incidence data adjusted for early mortality (animals dying before the first occurrence of tumor in any group were excluded from the denominator, giving the effective number of animals at risk). In female mice, compared to controls, the unadjusted incidence of lung adenoma was more than three-fold greater (56% vs. 18%;  $p < 0.001$ , Fisher’s exact test), of lung adenocarcinoma was more than two-fold greater (10% vs. 4%;  $p = 0.14$ , Fisher’s exact test), and of combined lung adenoma and adenocarcinoma was more than two-fold greater (56% vs. 21%;  $p < 0.001$ , Fisher’s exact test). Similarly, the adjusted incidence for combined lung adenoma and adenocarcinoma was significantly greater in treated females (57% vs. 22%;  $p < 0.001$ , Fisher’s exact test). With regard to tumors of the vasculature in female mice, the unadjusted incidences of angiosarcoma (36% vs. 3%;  $p < 0.001$ , Fisher’s exact test) and of angioma and angiosarcoma combined (44% vs. 5%;  $p < 0.001$ , Fisher’s exact test) were greatly increased in exposed compared to unexposed animals. The adjusted incidence for combined angioma and angiosarcoma was more than 10-fold higher in treated females versus controls (69% vs. 6%;  $p < 0.001$ , Fisher’s exact test).

**Table 1. Tumor incidence data in female Swiss mice receiving 0.015% phenelzine sulfate via drinking water for life (Toth, 1976; Toth and Nagel, 1976a; Toth and Shimizu, 1974)**

Tumor Site and Type		Unadjusted incidence		Adjusted incidence (effective number <sup>1</sup> )	
		Untreated controls	Phenelzine sulfate	Untreated controls	Phenelzine sulfate
Lung	Adenoma	18/99	28/50 <sup>2</sup>	--	--
	Adenocarcinoma	4/99	5/50	--	--
	Combined	21/99	28/50 <sup>2</sup>	21/95	28/49 <sup>2</sup>
Blood vessel	Angioma	2/99	4/50	--	--
	Angiosarcoma	3/99	18/50 <sup>2</sup>	--	--
	Combined	5/99	22/50 <sup>2</sup>	5/88	22/32 <sup>2</sup>

<sup>1</sup> Effective number is the number of animals alive at first occurrence of tumor (week 48 for lung tumors, week 69 for blood vessel tumors). Effective number was determined only for the combined data, because Toth (1976) did not specify the type of tumor when reporting the week of first occurrence of tumor.

<sup>2</sup>  $p < 0.001$  for pairwise comparison between treated group and untreated controls (Fisher's exact test).

In male mice, the unadjusted incidence of lung adenoma was statistically significantly increased compared to controls (30% vs. 15%;  $p < 0.05$ , Fisher's exact test). When combined with the data for lung adenocarcinoma, the unadjusted incidence was marginally statistically significant compared to controls (36% vs. 23%;  $p = 0.07$ , Fisher's exact test). After adjusting for early mortality, the incidence of combined lung adenoma and lung adenocarcinoma was statistically significant (53% vs. 25%;  $p < 0.01$ , Fisher's exact test). The increase in lung adenoma accounts for the statistical significance of the combined incidence data. Toth (1976) suggested that the lower sensitivity of male mice might be attributable to survival problems resulting from the toxicity of phenelzine. If males had survived longer, progression of lung adenoma to adenocarcinoma may have been more likely. It is unknown whether better survival among the treated males would have resulted in the development of tumors at other sites. The tumor incidence data for male mice are summarized in Table 2.

**Table 2. Tumor incidence data in male Swiss mice receiving 0.015% phenelzine sulfate via drinking water for life (Toth, 1976; Toth and Nagel, 1976a; Toth and Shimizu, 1974)**

Tumor Site and Type		Unadjusted incidence		Adjusted incidence (effective number <sup>1</sup> )	
		Untreated controls	Phenelzine sulfate	Untreated controls	Phenelzine sulfate
Lung	Adenoma	15/99	15/50 <sup>2</sup>	--	--
	Adenocarcinoma	13/99	3/50	--	--
	Combined	23/99	18/50 <sup>3</sup>	23/93	18/34 <sup>4</sup>

<sup>1</sup> Effective number is the number of animals alive at first occurrence of tumor (week 42). Effective number was calculated only for the combined data, because Toth (1976) did not report whether the first tumor was an adenoma or adenocarcinoma.

<sup>2</sup>  $p < 0.05$  for pairwise comparison between treated group and untreated controls (Fisher's exact test).

<sup>3</sup>  $p = 0.07$  for pairwise comparison between treated group and untreated controls (Fisher's exact test).

<sup>4</sup>  $p < 0.01$  for pairwise comparison between treated group and untreated controls (Fisher's exact test).

Tumor incidence data for untreated controls published by Toth prior to and immediately following the phenelzine sulfate studies are shown in Table 3. These data are consistent with the control data (shown in bold in Table 3) that were used by Toth (1976) for statistical analysis.

**Table 3. Comparison of tumor incidence data in untreated Swiss albino mice controls from the Toth laboratory**

Sex	Lung tumors <sup>1</sup>	Blood vessel tumors <sup>1</sup>	References
Female	14/110 (13%)	4/110 (4%)	Toth and Shubik (1966); Toth and Rustja (1967); Toth (1969; 1972a; 1973); Toth and Wilson (1971); Toth and Shimizu (1973)
	<b>21/99 (21%)</b>	<b>5/99 (5%)</b>	Toth and Shimizu (1974; 1976); Nagel <i>et al.</i> (1975); Shimizu and Toth (1974); Shimizu <i>et al.</i> (1974 ; 1975); Toth <i>et al.</i> (1976) ; Toth and Nagel (1976b)
	15/100 (15%)	8/100 (8%)	Toth (1978; 1979); Toth <i>et al.</i> (1977; 1978) ; Toth and Erickson (1978); Toth and Nagel (1978; 1980); Toth and Patil (1980);
Male	11/110 (10%)	2/110 (2%)	See references for female
	<b>23/99 (23%)</b>	<b>6/99 (6%)</b>	See references for female
	22/100 (22%)	5/100 (5%)	See references for female

<sup>1</sup> Control data shown in bold were used by Toth (1976) for statistical analysis.

Phenelzine sulfate in aqueous solution has been shown to degrade to hydrazine (Matsui *et al.*, 1981). Hydrazine has also been measured in the drug raw material at levels up to 0.2% (by weight relative to phenelzine), and in the tablet formulation at levels up to 0.5% (Matsui *et al.*, 1981; Lovering *et al.*, 1983). Toth (1976) prepared a 0.015% solution of powdered phenelzine sulfate (Toth, personal communication, 2002) in water three times weekly and kept the solution in brown bottles to minimize potential degradation via light exposure. A gas chromatographic analysis of the solution after 48 hours at room temperature showed that 99% of the phenelzine sulfate was present unchanged. No data were provided on the fate of the 1% phenelzine sulfate that was lost, nor were any impurities or breakdown products identified in the solution. The simplest degradation reaction for phenelzine sulfate in solution is postulated to involve cleavage of the carbon nitrogen bond, forming hydrazine and ethylbenzene, both of which induce tumors in animals.

In the absence of data on the identity of contaminants or breakdown products present in the phenelzine sulfate solution administered via drinking water in the Toth (1976) studies, a “worst-case” analysis was performed, assuming that 1% of the phenelzine sulfate solution administered in the Toth (1976) studies had broken down to form hydrazine and ethylbenzene. Administration in drinking water of hydrazine or hydrazine sulfate to Swiss albino mice induced lung tumors, but not blood vessel tumors (Toth, 1969; 1972b). Administration of ethylbenzene via inhalation to B6C3F<sub>1</sub> mice induced lung tumors in males and liver tumors in females (NTP, 1999). The upper 95% confidence bounds on cancer potency in female and male mice were determined for hydrazine (in the presence of sulfate, since sulfate would be in solution with hydrazine after the degradation of phenelzine sulfate) based on Toth (1969) and ethylbenzene based on NTP (1999). The concentrations of hydrazine and ethylbenzene in a 0.015% phenelzine sulfate drinking water solution as a result of the breakdown of one percent of the phenelzine sulfate were predicted based on the assumption that one mole of phenelzine sulfate would degrade to one mole each of hydrazine and ethylbenzene. Intakes of hydrazine and ethylbenzene were calculated by multiplying the estimated concentrations of hydrazine and ethylbenzene by the volume of water consumed by female and male mice in the Toth (1976) studies. Default mouse body weights from Gold and Zeiger (1997) were applied to estimate dose. Hypothetical risks were calculated by multiplying the cancer potency estimates in female and male mice by the appropriate dose estimates. The number of animals with tumors in groups of 50 female and 50 male mice were predicted by multiplying the hypothetical risks by 50. In both female and male mice receiving 0.015% phenelzine sulfate in drinking water, the predicted lung tumor incidence associated with the postulated exposure to hydrazine was less than one in 50. The predicted lung tumor incidence associated with the postulated exposure to ethylbenzene was much less than one in 50 for both male and female mice. This “worst-case” analysis suggests that neither the postulated hydrazine contamination nor the postulated ethylbenzene contamination could be responsible for the observed increased incidence of lung tumors in female and male Swiss albino mice in the Toth (1976) phenelzine sulfate drinking water studies. Thus, the lung tumor findings of Toth (1976) can be reasonably attributed to phenelzine sulfate.

#### *Rat Diet Study (Gershbein and Rao, 1992)*

Gershbein and Rao (1992) conducted a study of the effect of several hydrazine drugs (phenelzine sulfate, hydralazine and isoniazid) on the carcinogenicity of 1,2-dimethylhydrazine. A group of 13 male Sprague-Dawley rats received phenelzine sulfate at a level of 0.020% in the diet for 87 weeks. Another group of 10 male Sprague-Dawley rats fed 0.020% phenelzine sulfate in the diet were also exposed to 9.0 mg/kg 1,2-dimethylhydrazine via subcutaneous injection one to two times per week for a total of 23 injections. An untreated control group of 14 male rats was also maintained. Gershbein and Rao described the processing of animals as follows: “Autopsies were performed on all dead rats. Moribund rats were killed and autopsied. Survivors at the conclusion of tests were killed (under ether), incised, and sections taken for microscopic examination (hematoxylin-eosin stained, among other techniques). The entire GI tract was carefully opened, then all lesions were located, measured, and sampled for histological review.” The authors did not state which tissues were sectioned and to what extent tissues other than those of the GI tract were examined for tumors. The authors tabulated intestinal and subcutaneous tumors, and mentioned lesions of the head and ear, and peritoneal soft tissue, but the comprehensiveness of the pathology assessment is unclear. In animals exposed to phenelzine

sulfate alone via diet, the authors stated that “tumors were absent throughout the gastrointestinal tract.” The authors noted that two subcutaneous tumors developed in the phenelzine sulfate exposed male rats versus one tumor in the control group (number of animals with tumors were not reported). In rats exposed to 1,2-dimethylhydrazine, 100% (14/14) developed colon tumors and 79% (11/14) developed tumors of the small intestine. In animals exposed to both phenelzine sulfate and 1,2-dimethylhydrazine, the colon tumor incidence was comparable (9/10; 90%) to the group receiving only 1,2-dimethylhydrazine, while the incidence of tumors of the small intestine was significantly reduced (3/10;  $p < 0.05$ , Fisher’s exact test). This study is limited by the small number of animals per group, use of a single dietary phenelzine sulfate concentration, less than lifetime study duration, and inadequate reporting.

### 3.3 Other Relevant Data

Other relevant data related to the possible carcinogenicity of phenelzine and phenelzine sulfate include studies of genetic toxicity and structure-activity comparisons. The metabolism of phenelzine is discussed. Toth’s description of the pathology of tumors induced in mice exposed to phenelzine sulfate is provided (Toth, 1976; Toth and Shimizu, 1974; Toth *et al.*, 1975).

#### 3.3.1 Genetic Toxicology

Phenelzine has been tested for the ability to induce sister chromatid exchanges (SCE) in mice (Brambilla *et al.*, 1982), DNA strand breaks in the lungs and livers of mice (Brambilla *et al.*, 1982; Parodi *et al.*, 1981), unscheduled DNA synthesis in mouse and rat hepatocytes in culture (Mori *et al.*, 1988), damage by inactivation of transforming bacterial DNA (Freese *et al.*, 1968; Rosenkranz and Carr, 1971), and DNA damage in repair deficient strains of *Escherichia coli* (Brambilla *et al.*, 1982). Strand breakage in purified DNA (Augusto *et al.*, 1984; Netto *et al.*, 1987; Leite and Augusto, 1989; Runge-Morris *et al.*, 1994; Yamamoto and Kawanishi, 1992) and alkylation of purified DNA (Leite and Augusto, 1989) have been reported. Phenelzine has also been tested for mutagenic activity in *Salmonella typhimurium* (Shimizu *et al.*, 1978; Parodi *et al.*, 1981; De Flora, 1981; De Flora and Mugnoli, 1981; Brambilla *et al.*, 1982; De Flora *et al.*, 1984).

#### *Clastogenic damage*

Brambilla *et al.* (1982) reported weak induction of SCE in bone marrow cells of mice treated by intraperitoneal injection with phenelzine sulfate and implanted subcutaneously with a 5-bromodeoxyuridine tablet. A phenelzine sulfate dose at the LD<sub>50</sub><sup>1</sup> resulted in a 34% increase ( $p < 0.05$ ) in SCE in bone marrow from treated mice.

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<sup>1</sup> LD<sub>50</sub> of phenelzine sulfate after a single dose in mice was reported by Brambilla *et al.* (1982) to be 168 mg/kg via i.p. administration and 156 mg/kg via oral administration.

### *DNA damage and repair*

Brambilla *et al.* (1982) reported increases in DNA fragmentation using the alkaline elution technique in the lungs and liver of mice treated by intraperitoneal (i.p.) injection or orally with phenelzine sulfate. A statistically significant ( $p < 0.05$ ) increase in the rate of liver DNA elution, indicative of an increase in DNA single strand breaks, was observed in the group that received a single oral dose at double the LD<sub>50</sub> and were sacrificed one hour after treatment. No increase was observed when mice received the same dose via i.p. injection. Increases in the rates of liver DNA elution were observed in the groups receiving a single i.p. or oral dose at the LD<sub>50</sub> or five i.p. or oral doses of one third the LD<sub>50</sub> and sacrificed six hours after treatment was completed, but the increases were not statistically significant. A statistically significant ( $p < 0.05$ ) increased rate of lung DNA elution was observed in the group receiving five i.p. doses at one third the LD<sub>50</sub> and sacrificed six hours after treatment. No increase was observed when mice received the dose orally. Increases in the rates of lung DNA elution were observed in the groups receiving a single oral dose at double the LD<sub>50</sub> (sacrifice at one hour), a single i.p. dose at the LD<sub>50</sub> (sacrifice at one or six hours) or a single oral dose at the LD<sub>50</sub> (sacrifice at six hours), but the increases were not statistically significant.

In a second paper published by the same group using the same protocol, Parodi *et al.* (1981) reported in the text and in one summary table that there were no statistically significant increases in DNA elution in any group treated with phenelzine sulfate. However, the data table in the paper (Table 2) indicated that the group receiving five i.p. doses of 0.24 mmol/kg phenelzine sulfate had a statistically significant ( $p < 0.05$ ) increase in lung DNA elution rate over controls, a finding which is consistent with the study of Brambilla *et al.* (1982).

Mori *et al.* (1988) investigated the ability of hydrazine derivative compounds to elicit unscheduled DNA repair in mouse and rat hepatocytes harvested and grown in culture. In this assay phenelzine sulfate did not induce unscheduled DNA synthesis in either mouse or rat hepatocytes. However, this assay system may not be suitably sensitive for evaluation of hydrazine derivatives because, of the 24 compounds tested with reported carcinogenicity, only six were positive for inducing DNA repair in rat hepatocytes and an additional four were positive in mouse hepatocytes.

Early investigations by Freese *et al.* (1968) demonstrated the ability of phenelzine to react with DNA *in vitro* and cause damage that diminished the transforming ability of DNA introduced into *Bacillus subtilis*. Subsequent to this finding, Rosenkranz and Carr (1971) demonstrated that phenelzine decreased the survival of *Escherichia coli* strains deficient in *polA* mediated DNA repair in the spot test assay. The authors concluded that the decrease in viability of phenelzine sulfate treated *polA*<sup>-</sup> bacteria was due to DNA damage.

Brambilla *et al.* (1982) reported antibacterial activity of phenelzine sulfate toward repair-deficient *Escherichia coli* strains in the spot test, which provided evidence for phenelzine's ability to damage bacterial DNA and elicit various DNA repair mechanisms. The *Escherichia coli* strains were deficient in a variety of repair mechanisms including *uvrA*, *polA*, *recA*, or *lexA*.

*In vitro* studies utilizing purified DNA demonstrated the ability of phenelzine to induce DNA strand breaks in the presence of physiological oxidizing agents such as oxyhemoglobin (Augusto

*et al.*, 1984 ; Netto *et al.*, 1987; Leite and Augusto 1989; and Runge-Morris *et al.*, 1994). Single strand breaks were introduced into supercoiled PBR322 plasmid DNA by treatment with phenelzine in the presence of oxyhemoglobin (Augusto *et al.*, 1984). Similar studies by Runge-Morris *et al.* (1994) utilizing  $\phi$ X174 replicative form (closed circular supercoiled) DNA demonstrated a time- and concentration- dependent strand scission of the DNA substrate in the presence of oxyhemoglobin. See Section 3.4 (Mechanism) for a detailed discussion of the mechanism of DNA strand breakage mediated by heme groups such as oxyhemoglobin or by liver microsomes.

Yamamoto and Kawanishi (1992) demonstrated cleavage at the thymine residue of the sequence 5'-GTC by phenelzine sulfate in the presence of Cu(II) *in vitro*. Oxygen radical scavengers had little effect on Cu(II)-mediated DNA damage by phenelzine, indicating that the  $\cdot$ OH species was not likely to be responsible for the observed DNA damage.

Leite and Augusto (1989) also demonstrated that phenelzine, in the presence of oxyhemoglobin, was able to alkylate DNA as shown by electrophoretic studies with plasmid DNA and by experiments with 2-[ $^3$ H]-phenelzine in which the  $^3$ H label was incorporated into DNA. The phenelzine metabolites were further shown to have a preference for attacking guanine residues as was demonstrated by the use of DNA sequencing techniques using  $^{32}$ P-labeled DNA probes (Leite and Augusto, 1989).

#### *Mutagenesis in bacteria*

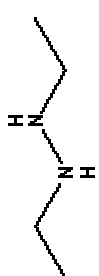
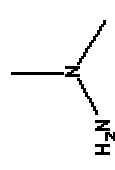
The mutagenic activity of phenelzine sulfate has been demonstrated in multiple studies in *Salmonella typhimurium* (Shimizu *et al.*, 1978; Parodi *et al.*, 1981; De Flora, 1981; De Flora and Mugnoli, 1981; Brambilla *et al.*, 1982; De Flora *et al.*, 1984). Shimizu *et al.* (1978) demonstrated positive results for phenelzine sulfate in the Ames test with *Salmonella* strains TA100 and TA1535, with or without the addition of S-9 mix, although the activity appeared to be less in the presence of S-9 mix. Five *Salmonella* strains (TA1535, TA1537, TA1538, TA98, TA100) were tested in the presence and absence of metabolic activation by the spot test, the plate incorporation test and the spiral test (Parodi *et al.*, 1981; Brambilla *et al.*, 1982). Phenelzine sulfate was mutagenic in strains TA1535 and TA100, strains responsive to agents which induce base substitution, under all three protocols. A dose-dependent response was observed using the plate incorporation test. Addition of mouse lung S-9 fraction reduced the mutagenicity of phenelzine sulfate; the same effect was seen to a lesser degree with rat or mouse liver preparations. When NADP was removed from the S9 preparation, the mutagenicity of phenelzine sulfate was no longer diminished. These results suggest that enzymatic reactions in which NADP is a cofactor reduce the mutagenicity of phenelzine sulfate (Brambilla *et al.*, 1982). Additional mutagenicity studies in *Salmonella typhimurium* by De Flora (1981), De Flora and Mugnoli (1981) and De Flora *et al.* (1984) also suggested that phenelzine sulfate induces mutations by a base substitution mechanism. These latter studies further confirmed the findings of Parodi *et al.* (1981) and Brambilla *et al.* (1982) that mutagenicity of phenelzine sulfate in sensitive *Salmonella typhimurium* tester strains was decreased by metabolic activation with S-9 microsome preparations.

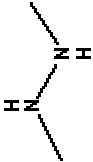
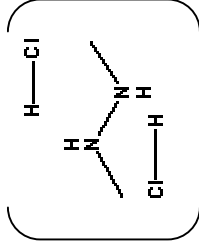


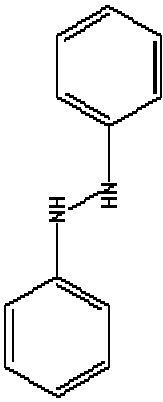

### ***3.3.2 Structure-Activity Comparisons***

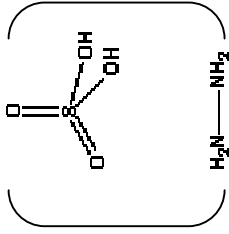
Phenelzine is a hydrazine derivative, structurally related to a number of known carcinogens. 1,2-Diethylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, 1,2-diphenylhydrazine, hydrazine, hydrazine sulfate, methylhydrazine and its salts, and phenylhydrazine and its salts are listed under Proposition 65 as chemicals known to the State to cause cancer. Table 4 summarizes the tumor sites observed in cancer bioassays of the hydrazine derivatives listed under Proposition 65. Several induced tumors in a pattern similar to phenelzine sulfate. 1,1-Dimethylhydrazine and 1,2-dimethylhydrazine induced blood vessel and lung tumors in female and male Swiss albino mice. Hydrazine, hydrazine sulfate, methylhydrazine and



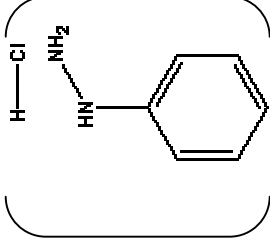
**Table 4. Tumor sites observed in cancer bioassays of hydrazine derivatives listed under Proposition 65 (from Gold and Zeiger [1997] unless otherwise noted)**

Chemical	Structure	Species	Sex	Strain	Tumor Sites
1,2-Diethylhydrazine		Rat	Not specified	BD	Brain, olfactory bulb, mammary gland, liver <sup>1</sup>
		Rat	F (pregnant)	BD	In offspring: Brain, spinal cord, peripheral nervous system <sup>1</sup>
1,1-Dimethylhydrazine		Mouse	F	Swiss albino	Lung, blood vessel
		Mouse	M	Swiss albino	Lung, blood vessel, liver, kidney
		Hamster	F	Syrian Golden	Cecum
		Hamster	M	Syrian Golden	Blood vessel, liver, cecum
		Mouse	F	CD1	Lung, blood vessel

Chemical	Structure	Species	Sex	Strain	Tumor Sites
		Mouse	M	CD1	Lung, blood vessel
		Rat	F	F344	Liver, pituitary (adenoma)
		Rat	M	F344	No significant findings
1,2-Dimethylhydrazine		Rat	M	Sprague-Dawley	Colon, intestine <sup>2</sup> small
1,2-Dimethylhydrazine dihydrochloride		Mouse	F	Swiss albino	Lung, blood vessel
		Mouse	M	Swiss albino	Lung, blood vessel
		Hamster	F	Syrian Golden	Blood vessel, liver, cecum
		Hamster	M	Syrian Golden	Blood vessel, liver
		Mouse	M	B6C3F <sub>1</sub>	No significant findings
		Rat	F	F344	Mammary gland, liver
		Rat	M	F344	Liver, Zymbal's gland

Chemical	Structure	Species	Sex	Strain	Tumor Sites
1,2-Diphenylhydrazine (hydrazobenzene)		Mouse	F	B6C3F <sub>1</sub>	Liver
Hydrazine		Mouse	F	Swiss	Lung
		Mouse	M	Swiss	Lung, lymphoma
		Mouse	F	C57BL/6	Lung
		Mouse	F	Bor:NMRI, SPF-bred NMRI	No significant findings
		Mouse	M	Bor:NMRI, SPF-bred NMRI	No significant findings
		Hamster	M	Syrian Golden	Nasal cavity
		Rat	F	F344	Nasal cavity
		Rat	M	F344	Nasal cavity

Chemical	Structure	Species	Sex	Strain	Tumor Sites
Hydrazine sulfate		Mouse	F	Swiss albino	Lung
		Mouse	M	Swiss albino	Lung
		Mouse	Both	Swiss	Lung
		Mouse	F	AKR	No significant findings; exposed until sacrifice at 69 weeks
		Mouse	M	AKR	No significant findings; exposed until sacrifice at 69 weeks
		Mouse	F	C3H	Lung <sup>3</sup>
		Mouse	F	ICRC×C3h (Jax)	Lung
		Mouse	M	ICRC× C3h (Jax)	Lung <sup>3</sup>
		Hamster	F	Syrian Golden	Cecum
		Hamster	M	Syrian Golden	Liver, cecum
		Rat	F	Cb/Sc	Lung
		Rat	M	Cb/Sc	Lung, liver

Chemical	Structure	Species	Sex	Strain	Tumor Sites
Methylhydrazine		Mouse	F	Swiss albino	Lung, liver, bile duct
		Mouse	M	Swiss albino	Lung (adenoma), liver, bile duct
		Mouse	F	C57BL/6	Lung, blood vessel, liver, nasal, duodenum <sup>4</sup>
		Hamster	F	Syrian Golden	Liver, cecum
		Hamster	M	Syrian Golden	Liver, cecum
Methylhydrazine sulfate		Mouse	F	Swiss albino	Lung
		Mouse	M	Swiss albino	Lung, malignant lymphoma
Phenylhydrazine hydrochloride		Mouse	F	Swiss albino	Blood vessel
		Mouse	M	Swiss albino	Blood vessel
		Mouse	Both	BALB/c/Cb/Se	Lung <sup>5</sup>

<sup>1</sup> From IARC (1974).

<sup>2</sup> From Gershbein and Rao (1992).

<sup>3</sup> Lung tumors considered treatment-related by authors, although the incidence was not statistically significant.

<sup>4</sup> From Kinkead *et al.* (1985).

<sup>5</sup> From Clayson *et al.* (1966).

methylhydrazine sulfate induced lung tumors in Swiss albino mice. Phenylhydrazine hydrochloride induced blood vessel tumors in Swiss albino mice. The hydrazine derivatives listed under Proposition 65 that have a more complete carcinogenic bioassay dataset, including 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, hydrazine, hydrazine sulfate, 1,2-diphenylhydrazine, and methylhydrazine, induced tumors in other species (hamster and rat).

Toth (1982) reported that 55 hydrazine derivatives were shown to cause cancer in animals as of that publication date. Based on data in the Carcinogenic Potency Database (Gold and Zeiger, 1997), several of these compounds induced blood vessel and lung tumors in both female and male mice: N-acetyl-4-(hydroxymethyl)phenylhydrazine, N-ethyl-N-formylhydrazine, and N-methyl-N-formylhydrazine. Others showed the same pattern of tumorigenicity as phenelzine sulfate, with induction of blood vessel and lung tumors in female mice and induction of lung tumors only in male mice: allylhydrazine hydrochloride, ethylhydrazine hydrochloride, and n-pentylhydrazine hydrochloride. Numerous additional hydrazine derivatives tabulated in Gold and Zeiger (1997) induced a variety of tumors in other strains of mice and other species.

### **3.3.3 Pharmacokinetics and Metabolism**

Baker *et al.* (1999) reviewed the available information on the pharmacokinetics and metabolism of phenelzine. Phenelzine is rapidly absorbed in humans, reaching a maximum plasma concentration within two to four hours of initial treatment and having a plasma elimination half-life of 1.5 to four hours (Robinson *et al.*, 1985). After chronic treatment with phenelzine sulfate for six to eight weeks, steady-state levels in plasma slowly rise. This increase may be attributable to an inhibiting effect of phenelzine sulfate or its metabolites on the metabolism of the parent drug (Baker *et al.*, 1999).

Phenelzine is an inhibitor and substrate of MAO-A and MAO-B. Phenylacetic acid, formed from the action of MAO on phenelzine, is a major metabolite. Phenylacetic acid is further converted to *p*-hydroxyphenylacetic acid. Robinson *et al.* (1985) reported that 66 to 79% of a phenelzine dose administered to human volunteers was excreted as phenylacetic acid or *p*-hydroxyphenylacetic acid in the urine within 96 hours. Suggested intermediate metabolites along the MAO pathway include 1-(2-phenylethyl)diazene and phenylethylidene hydrazine (Baker *et al.*, 1999).

Phenelzine is metabolized to  $\beta$ -phenylethylamine in rats (Dyck *et al.*, 1985).  $\beta$ -Phenylethylamine is also a substrate of MAO, so the relative importance of direct formation of  $\beta$ -phenylethylamine from phenelzine versus buildup of  $\beta$ -phenylethylamine as a result of phenelzine's inhibition of MAO is unclear (Baker *et al.*, 1999).

Because of the similarity of phenelzine to other drugs that are known to be acetylated, studies were carried out to examine the effect of acetylator status on clinical outcome of phenelzine treatment. Conflicting results were reported. Current information indicates that acetylation of phenelzine occurs, but is likely a minor pathway (Baker *et al.*, 1999).

Ring hydroxylation has not been demonstrated for phenelzine, but there is evidence to suggest it may occur (Baker *et al.*, 1999). Phenelzine is structurally related to amphetamine, which

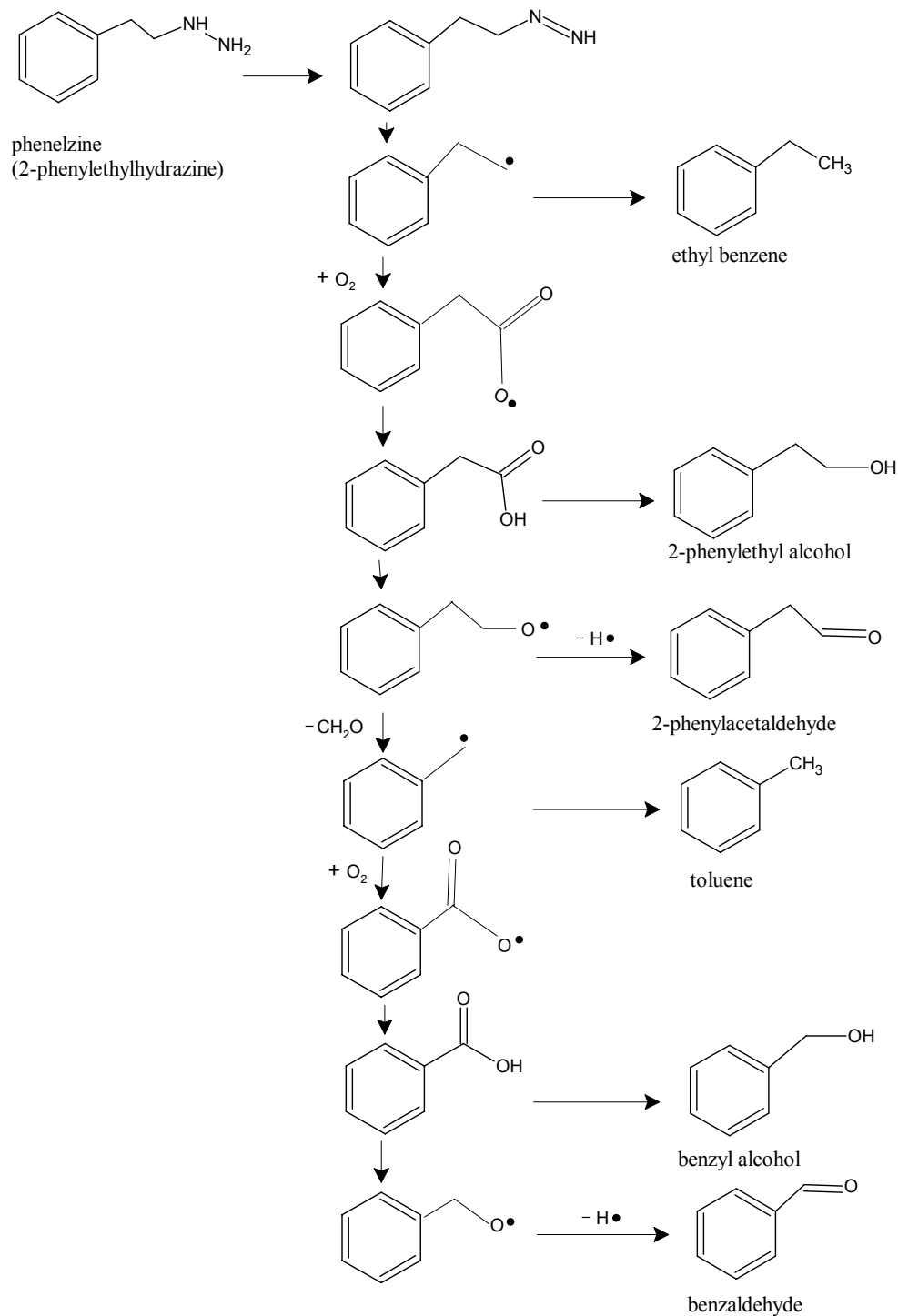
undergoes ring hydroxylation. *p*-Hydroxyphenylacetic acid, a major metabolite of phenelzine, may be formed from phenylacetic acid, another major metabolite of phenelzine, but may also be formed from *p*-hydroxyphenelzine or from *p*-tyramine. *p*-Hydroxyphenelzine would arise directly from ring hydroxylation of phenelzine, while *p*-tyramine would be a product of ring hydroxylation of  $\beta$ -phenylethylamine, formed from biotransformation of phenelzine. A peak with the same retention time as a standard of *p*-hydroxyphenelzine was observed using gas chromatography on urine samples from patients treated with phenelzine, although the identification was not confirmed by gas chromatography-mass spectrometry (Baker *et al.*, 1999). N-Methylation of phenelzine has been observed *in vitro* (Baker *et al.*, 1999).

Danielson *et al.* (1984) identified ethylbenzene as a metabolite of phenelzine in a preliminary mass spectrometric analysis of expired air from Sprague-Dawley rats exposed to the drug. IARC (2000) has classified ethylbenzene as a Group 2B carcinogen (possibly carcinogenic to humans) based on sufficient evidence of carcinogenicity in animals, including development of lung tumors in male mice. Danielson *et al.* (1984) noted the significance of their finding given the fact that phenelzine sulfate also causes lung tumors in mice. *In vitro* studies of phenelzine metabolism by phenobarbital pre-treated rat liver microsomes confirmed ethylbenzene as a metabolite and also identified benzaldehyde, benzylalcohol, 2-phenylacetaldehyde, 2-phenylethanol, and toluene as metabolites (Ortiz de Montellano and Watanabe, 1987) [for metabolic pathway see Figure 3]. The oxidation reactions leading to the above mentioned metabolites may be mediated by the microsomal enzymes cytochrome P450 and flavin adenine dinucleotide containing amino oxidases, and by auto-oxidation reactions with heme and other metal complexes to form carbon centered radicals (Ortiz de Montellano and Watanabe, 1987). Ortiz de Montellano and Watanabe (1987) suggested that *in vitro* metabolism of phenelzine by rat microsomes proceeds primarily via the 2-phenylethyl radical. Evidence for this pathway comes from experiments using spin-trapping techniques in which 2-phenylethyl radicals were isolated and characterized by mass spectrometry (Ortiz de Montellano *et al.*, 1983). In addition, Augusto *et al.* (1984), Netto *et al.* (1987) and Leite and Augusto (1989) demonstrated by spin-trapping techniques that phenylethyl radicals were produced by oxidation of phenelzine sulfate in the presence of oxyhemoglobin. The phenylethyl radical is a potent electrophile that is capable of reacting with strong nucleophiles found on proteins and nucleic acids and is thought to account for the observed DNA adducts (see Section 3.4 Mechanism).

Phenelzine sulfate has been shown to degrade to hydrazine in aqueous solution (Matsui *et al.*, 1981). Hydrazine, a chemical listed as known to the State to cause cancer under Proposition 65, has been proposed as a metabolite of phenelzine (Lovering *et al.*, 1983). Given the evidence for the formation of the 2-phenylethyl radical discussed above, it seems likely that hydrazine could be produced as another reaction product.



**Figure 3. Proposed metabolic pathway of phenelzine oxidation by liver microsomes resulting in six primary metabolites (adapted from Ortiz de Montellano and Watanabe, 1987).**



### 3.3.4 Pathology

Angiomas and angiosarcomas are considered to be related in origin, and angiomas can progress to angiosarcomas. These tumor types are therefore appropriately combined for carcinogen identification and risk assessment purposes. Toth (1976) described the pathology of the blood vessel tumors: “Often the blood vessel tumors were observed s.c. on the neck and interscapular regions of living animals, exhibiting round shape and sizes up to 50 mm in diameter. The neoplastic lesion sometimes obliterated the entire dermis and, in nearly all cases, the brown adipose tissue of the fat pad. Sometimes in the liver a large area was replaced by vascular endothelial cell growths. Also, in a few instances, the neoplastic vascular tissue was clearly evident in the lungs. The malignant vascular tissue exhibited the characteristic appearance of vascular spaces and clefts which were lined by the endothelial cells. These cells were anaplastic, elongated, or round shaped, and showed disorientation and bizarre growth patterns. Otherwise these tumors were similar to those found earlier with other hydrazine treatments, and were described and illustrated in detail...”

Lung adenomas and lung adenocarcinomas as described by Toth and coauthors (Toth and Shimizu, 1974; Toth *et al.*, 1975) are considered to be related in origin. Lung adenomas can progress to lung adenocarcinomas, therefore, these tumor types are combined for purposes of carcinogen identification and risk assessment. Toth (1976) indicated that the lung adenomas observed were similar to those described by Toth and Shimizu (1974) in a previous experiment:

“Grossly, the lesions were multiple, well-circumscribed, whitish nodules from 1 to 5 mm in diameter. Most were subpleural, but tumors deep inside the lungs were also seen...At a later stage, well-developed adenomas were observed. In these, the neoplastic cells showed adenoid structures. Usually the lesion was clearly demarcated from the surrounding lung tissue. The constituent cells were cuboidal or columnar, and nonciliated, with dark nuclei and acidophilic cytoplasm. They were supported by thin fibrous stroma and small blood vessels. Transmission electron microscopy revealed that the lesions consisted of alveolar cells, type B, or great alveolar cells. The tumor cells formed rows with free cell surfaces covered with numerous microvilli. The cytoplasm was rich in organelles. The nuclei were usually highly indented and often the chromatin was attached to the nuclear membrane. No virus or virus-like particles were observed. The free cell surfaces were covered with microvilli which were essentially cytoplasmic extensions covered by plasma membranes. In the cytoplasm of the alveolar cells, type B, in the treated and control mice, 5 distinctive characteristics could be summarized as follows: a) Lamellar bodies: The dense strata and the separating spaces varied considerably; they were irregular and distinct. b) Osmiophilic bodies: They were round or oval, sometimes fusiform, and were surrounded by membranes. Their electron densities varied and some had vacuoles. c) Membrane-bound structures containing gray homogenous material: Occasional features of the cells were lakelike dilations with deep indentations. Between them many free ribosomes and mitochondria were seen. d) Aggregations of dense particles: They exhibited fine structure and were in a homogenous matrix; their borders were clearly visible. e) Myelin-like figures: In a few cases, myelin figures consisting of multiple circular

laminae around a central core were observed. The scanning electron microscope revealed the cell-surface characteristics of the tumor cells. The irregular growth pattern and the protruding and invading cells were distinct. The cell surfaces were covered by microvilli; in some areas fibrinous materials were visible. The microvilli were nodular; their sizes and shapes varied substantially, and they were dispersed irregularly on the cell surfaces.”

The diagnostic criteria for a lung adenocarcinoma were provided in Toth *et al.* (1975): “In case of adenocarcinoma, the composing cells showed irregular growth pattern, they usually invaded the surrounding tissues including blood vessels and bronchi. The sizes and shapes of the cells varied, often many mitoses were seen.”

### 3.4 Mechanism

The mechanism of phenelzine carcinogenesis is not well understood. Two mechanisms have been proposed. The first involves the oxidation of phenelzine by heme groups (Augusto *et al.*, 1984, Leite and Augusto, 1989; Runge-Morris *et al.*, 1994) or microsomal enzymes (Ortiz de Montellano *et al.*, 1983; Ortiz de Montellano and Watanabe, 1987), producing carbon-centered radicals that alkylate DNA. An alkali labile site is formed leading to strand scission. DNA repair processes may result in mutations in critical genes leading to abnormal cell growth and ultimately tumorigenesis. In the second proposed mechanism, oxidation of phenelzine leads to the formation of an arenediazonium ion that reacts with nucleophilic centers of the DNA bases adenine and guanine to produce azo aryl adducts linked through the amino side chains of the DNA bases or at the C8 position of the purine ring (Powell and Gannett, 2002).

The ability of phenelzine to induce DNA strand breaks in the presence of physiological oxidizing agents such as oxyhemoglobin has been demonstrated in several studies, including Augusto *et al.* (1984), Netto *et al.* (1987), Leite and Augusto (1989), and Runge-Morris *et al.* (1994). Single strand breaks were introduced into supercoiled PBR322 plasmid DNA by treatment with phenelzine in the presence of oxyhemoglobin (Augusto *et al.*, 1984). Spin-trapping studies established a clear correlation between the yield of 2-phenylethyl radicals and DNA strand scission (Augusto *et al.*, 1984; Netto *et al.*, 1987). The same correlation was obtained in the presence of reactive oxygen species scavengers or when the carbon radical was generated under anaerobic conditions or by chemical oxidation of phenelzine by ferricyanide. In addition to DNA damage, the phenelzine turnover by oxyhemoglobin promoted destruction of the hemoprotein catalyst into unidentified products (Augusto *et al.*, 1984).

Similar studies by Runge-Morris *et al.* (1994) utilizing  $\phi$ X174 replicative form (closed circular supercoiled) DNA demonstrated a time- and concentration- dependent strand scission of the DNA substrate in the presence of oxyhemoglobin. Runge-Morris *et al.* (1994) also demonstrated that the damage resulted from organic free radicals rather than reactive oxygen free radical species because the antioxidants dimethylfuran, dimethyl sulfoxide, and dimethylthiourea failed to inhibit DNA damage while the organic free radical spin trap reagent dimethylpyrrolidin-*N*-oxide, and the free radical scavenger *N*-acetylcysteine inhibited phenelzine induced DNA damage. Leite and Augusto (1989) demonstrated that the 2-phenylethyl radical generated by oxyhemoglobin metabolism of phenelzine was able to alkylate DNA as shown by electrophoretic

studies with plasmid DNA and by experiments with 2-[<sup>3</sup>H]-phenelzine in which the <sup>3</sup>H label was incorporated into DNA. The carbon centered radical was further shown to have a preference for attacking guanine residues as was demonstrated by the use of DNA sequencing techniques using <sup>32</sup>P-labeled DNA probes (Leite and Augusto, 1989).

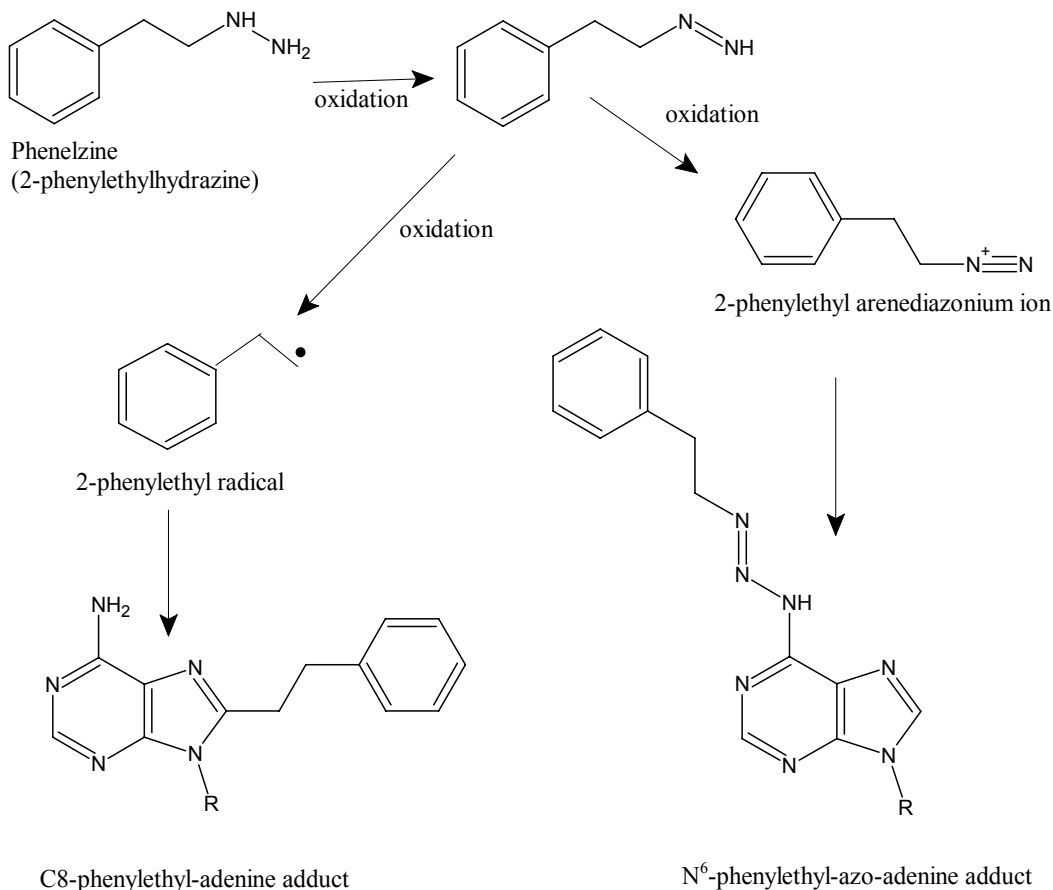
Supporting studies by Ortiz de Montellano *et al.* (1983) and Ortiz de Montellano and Watanabe (1987) demonstrated that carbon radicals were formed during the metabolism of phenelzine by rat liver microsomes. As discussed above, phenelzine is metabolized *in vitro* by phenobarbital pre-treated rat liver microsomes to yield ethylbenzene, 2-phenylethanol, 2-phenylacetaldehyde, benzaldehyde, benzylalcohol and toluene (Ortiz de Montellano and Watanabe, 1987). A carbon radical was formed during the oxidative metabolism of phenelzine that reacts with the prosthetic heme group of cytochrome P450 and irreversibly inactivates the enzyme. The carbon radical was spin trapped, isolated and shown by mass spectrometry to be the 2-phenylethyl radical (Ortiz de Montellano *et al.*, 1983).

An alternative mechanism resulting in DNA adduct formation from oxidation of aryl hydrazines such as phenelzine has been proposed by Powell and Gannett (2002). In this model, phenelzine is oxidized to its most terminal form, an arenediazonium ion (R—N≡N<sup>+</sup>), a potent electrophile that reacts with nucleophilic centers of the DNA bases adenine or guanine to produce azo aryl adducts linked through amino side chains of the DNA bases. Support for this mechanism comes from studies of *in vitro* reactions of arenediazonium ions with nucleic acids or nucleotides. It has been known since 1965 that arenediazonium ions could react with nucleotides to produce highly colored products (Kossel, 1965 as cited in Powell and Gannett, 2002). Numerous studies have demonstrated aryl hydrazine adduction to guanine or adenine at the C-8 position of the purine ring via the hydrazine moiety (Hung and Stock, 1982; Chin *et al.*, 1981; Kikugawa *et al.*, 1992). Patel and Brown (1967) reported that hydrazine and aryl hydrazines attacked and replaced amino groups on nucleosides including N<sup>6</sup>-adenine and N<sup>2</sup>-guanine.

Although DNA adduct formation by phenelzine has not been investigated *in vivo*, based on the studies of Leite and Augusto (1989) and studies of other aryl hydrazines, one can predict that phenelzine would also form adenine and guanine adducts through an analogous mechanism. Figure 4 illustrates these two pathways for the postulated adduction of phenelzine metabolites to adenine. Similar C-8 and N<sup>2</sup> adducts for guanine are not pictured. Each of these substitutions might be expected to interfere with hydrogen bonding of DNA bases resulting in mispairing and mutation. Depurination may also occur during the reaction of the aryl radical with the purine and represents another form of DNA damage potentially caused by aryl hydrazines and their metabolites. The DNA repair systems activated by hydrazines have not been studied and how aryl hydrazine induced DNA damage is repaired is not known (Powell and Gannett, 2002).

Yamamoto and Kawanishi (1992) demonstrated cleavage at the thymine residue of the sequence 5'-GTC by phenelzine sulfate in the presence of Cu(II) *in vitro*. Oxygen radical scavengers had little effect on Cu(II)-mediated DNA damage by phenelzine, indicating that the <sup>•</sup>OH species was not likely to be responsible for the observed DNA damage. Yamamoto and Kawanishi (1992) suggested a mechanism of DNA cleavage via a copper-oxygen complex.

**Figure 4. Proposed pathways for reaction of phenelzine with DNA.**



## 4 OTHER REVIEWS

The International Agency for Research on Cancer (IARC, 1980) reviewed the carcinogenicity of phenelzine and phenelzine sulfate and concluded that “there is limited evidence that phenelzine sulfate is carcinogenic in experimental animals.” IARC (1980) recommended further studies on the carcinogenicity of phenelzine sulfate based on the evidence in experimental animals, the mutagenicity of the compound, and the case report of an angiosarcoma of the liver in a patient taking phenelzine sulfate. In 1987, IARC categorized phenelzine sulfate as being “not classifiable as to its carcinogenicity in humans” (Group 3), based on the studies included in the 1980 review and an additional set of genotoxicity studies by Parodi *et al.* (1981). Other studies relevant to the carcinogenicity of phenelzine that were not reviewed by IARC (1980, 1987) include: Augusto *et al.* (1984), Brambilla *et al.* (1982), Danielson *et al.* (1984), De Flora (1981), De Flora *et al.* (1984), DeFlora and Mugnoli (1981), Gershbein and Rao (1992), Leite and Augusto (1989), Mori *et al.* (1988), Netto *et al.* (1987), Ortiz de Montellano *et al.* (1983), Ortiz

de Montellano and Watanabe (1987), Runge-Morris *et al.* (1994), and Yamamoto and Kawanishi (1992).

The carcinogenic activity of phenelzine and its acid salts does not appear to have been evaluated by the National Toxicology Program, the U.S. Environmental Protection Agency, the National Institutes of Occupational Safety and Health, or the U.S. Food and Drug Administration.

## **5 SUMMARY AND CONCLUSIONS**

### **5.1 Summary of Evidence**

Phenelzine sulfate administered in the drinking water induced increases in lung adenoma, lung adenoma/adenocarcinoma combined, angiosarcoma, and angioma/angiosarcoma combined in female Swiss albino mice. Phenelzine sulfate also induced increases in lung adenoma in male Swiss albino mice exposed via drinking water, as well as lung adenoma/adenocarcinoma combined when the incidence was expressed in terms of effective number. No treatment-related increase in tumors was reported in male rats administered phenelzine sulfate in the diet at one dose level for 87 weeks; however, this study was limited by small group sizes ( $n < 15$ ), less than lifetime study duration, and incomplete reporting.

Phenelzine sulfate is genotoxic in a variety of short-term tests. Sister chromatid exchanges were induced in the bone marrow of mice treated by intraperitoneal injection with phenelzine sulfate. DNA strand breaks were demonstrated in lungs and liver of mice treated by intraperitoneal injection or orally with phenelzine sulfate. A number of studies have demonstrated the mutagenic activity of phenelzine sulfate by the Ames test in *Salmonella typhimurium* strains that respond to base substitution mutations. In addition, DNA damage was induced by phenelzine sulfate in repair-deficient strains of *Escherichia coli*.

Phenelzine likely exerts its carcinogenic effects through a genotoxic mode of action. Although the precise mechanism is not well understood, available evidence from *in vitro* mechanistic studies suggests that oxidation of phenelzine can lead to the formation of phenylethyl radicals and other radical species, and arenediazonium ions, which can react with DNA to form adducts of purine bases. The formation of DNA adducts may lead to mispairing, depurination and DNA strand breaks, eventually leading to cancer.

Phenelzine is structurally related to hydrazine and hydrazine derivatives, which are known to induce similar carcinogenic effects in Swiss albino mice and additional carcinogenic effects in other strains and species.

### **5.2 Conclusion**

There is evidence for the carcinogenicity of phenelzine and its acid salts, based on studies of phenelzine sulfate showing induction of malignant and benign tumors of the blood vessels and malignant and benign tumors of the lung in mice. One limited study in male rats administered phenelzine sulfate in the diet did not report any treatment-related increase in tumors. A

mechanism of DNA damage via oxidation of phenelzine to an aryl radical or an arenediazonium ion that leads to formation of DNA adducts, mispairing, depurination and DNA strand breaks has been proposed. Additional evidence comes from studies of phenelzine showing chromosomal alterations and DNA damage *in vivo* in experimental animals and mutations in bacterial test strains, and the structural similarity of phenelzine to a number of hydrazine derivatives known to induce carcinogenic effects in animals.

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