EVIDENCE ON THE CARCINOGENICITY OF

2,4,5-TRIMETHYLANILINE AND ITS STRONG ACID SALTS

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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 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).

2,4,5-Trimethylaniline was discussed as a high priority candidate for Committee review during a public committee meeting held in Sacramento, California, on July 22, 1996 and at a public workshop held November 15, 1996. Public input was solicited on the priority of this chemical in two public comment periods, each of 60 days duration. Once the chemical was selected for Committee review, a public request for pertinent information was made.

This draft document *Evidence on the Carcinogenicity of 2,4,5-Trimethylaniline and Its Strong Acid Salts* was developed to provide the Committee with relevant information for use in its deliberations, and reviews the available scientific evidence on the carcinogenic potential of 2,4,5-trimethylaniline and its strong acid salts. A public meeting of the Committee to discuss this evidence is tentatively scheduled for September 25, 1997. Written public comment on the document should be submitted to OEHHA by August 20, 1997, in order to be considered by the Committee in advance of the meeting. During the September meeting, the public will have an opportunity to present verbal comments to the Committee.

1 EXECUTIVE SUMMARY

2,4,5-Trimethylaniline (CAS number 137-17-7) has been used as an intermediate for dye manufacture. The hydrochloride (CAS number 21436-97-5) and other strong acid salts are expected exist in a dissociated state in solution and *in vivo*, and thus are considered toxicologically equivalent to the free or protonated amine form. 2,4,5-Trimethylaniline has been demonstrated to induce liver carcinoma in male and female Fischer 344 rats and female $B6C3F_1$ mice in studies by the National Cancer Institute (NCI). In these studies liver carcinomas were also significantly elevated in male mice, but not to the same extent as for the female. Significant increases in lung tumors were also observed in treated rats of both sexes and female mice. Bile duct carcinoma, which rarely occurs spontaneously, was also observed in male rats, and a few tumors of this type were also noted in male mice; the results were statistically significant in the rat but not the mouse. While it has been noted that the NCI test substance contained an unidentified impurity, gas-liquid chromatography indicated a greater than 99.9% purity of 2,4,5-trimethylaniline, and an impurity that could account for study findings has not been hypothesized.

In a second series of studies using the hydrochloride (Weisburger *et al.*, 1978), the findings of statistically significant increases of liver tumors were repeated in HaM/ICR mice of both sexes. In addition, lung tumors were observed to be significantly elevated in male and female mice. Male Charles River rats were also tested in this series, and significant dose-response was not observed. Because of limitations in study reporting, the findings in mice for this second series are considered to provide supportive but not conclusive evidence of carcinogenicity.

2,4,5-trimethylaniline is mutagenic in the *Salmonella typhimurium* with metabolic activation, cultured rat fibroblasts, and *in vivo* in *Drosophila melanogaster*.

Based on the information reviewed in the preparation of this document, there is evidence for the carcinogenicity of *2,4,5-trimethylaniline and its strong acid salts* at multiple sites in two species. Observations of genetic toxicity and chemical structural analogies with known carcinogens contribute to the weight of evidence.

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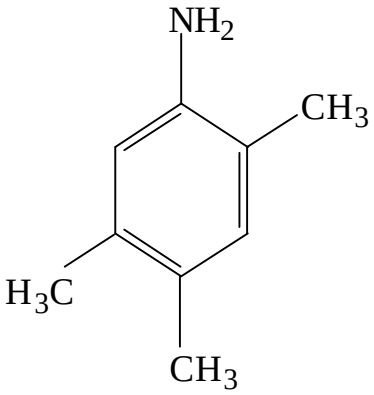
2 INTRODUCTION

2.1 Identity of 2,4,5-trimethylaniline



2,4,5-Trimethylaniline: $C_9H_{13}N$
Molecular Weight = 135.2 CAS Registry No. 137-17-7

Synonyms: 1-amino-2,4,5-trimethylbenzene; *psi*-cumidine; pseudo-cumidine; 1,2,4-trimethyl-5-aminobenzene

2,4,5-Trimethylaniline Hydrochloride: $C_9H_{13}N.HCl$
Molecular Weight = 171.7 CAS Registry No. 21436-97-5

Synonyms: 1-amino-2,4,5-trimethylbenzene hydrochloride; *psi*-cumidine hydrochloride; pseudo-cumidine hydrochloride; 1,2,4-trimethyl-5-aminobenzene hydrochloride

NTP (1979) described 2,4,5-trimethylaniline (the free amine) as a fine gray-white powder with a melting point of 64°C. The hydrochloride has a melting point of 235°C (Weisburger *et al.*, 1978).

2.2 Occurrence and use

2,4,5-Trimethylaniline is one of the structural isomers of trimethylaniline present in the amine mixture formerly manufactured in the US as an intermediate for the synthesis of the dye Ponceau 3R (C.I. Food Red 6; 3-hydroxy-4-[(2,4,5-trimethylphenyl)azo]-2,7-naphthalenedisulphonic acid, disodium salt). This mixture also contained methyl-, dimethyl- and

trimethylanilines, including 2,4,6-trimethylaniline. The hydrochloride (CAS number 21436-97-5) and other strong acid salts are expected to exist in dissociated in form in solution and *in vivo*, and thus are toxicologically equivalent to the free and protonated amine.

Ponceau 3R dye was formerly used in foods and cosmetics, but approval of these uses has been canceled on the basis of animal toxicity studies (IARC, 1975). It has also been used to dye wool. No evidence of 2,4,5-trimethylaniline manufacture in the US since the 1960s was found, but it is unclear whether it might be present in imported products, or as an environmental contaminant in areas of former manufacture or storage.

3 DATA ON 2,4,5-TRIMETHYLANILINE CARCINOGENICITY

Two series of carcinogenicity studies have been reported, in each of which the compound was administered in the diet to rats or mice. There are data indicating that 2,4,5-trimethylaniline has genetic toxicity: mutational effects in *Salmonella, Drosophila* and mammalian cells in culture have been reported.

3.1 Epidemiological studies of carcinogenicity in humans.

No data on long-term effects of human exposure to 2,4,5-trimethylaniline were found in an earlier search by the International Agency for Research on Cancer (IARC, 1982) or more recently by OEHHA.

3.2 Carcinogenicity studies in animals.

In two series of animal carcinogenicity studies (Weisburger *et al.*, 1978; NCI, 1979) both rats and mice received 2,4,5-trimethylaniline in the diet. A clear increase in liver tumors was observed in treated mice and rats of both sexes in one series (NCI, 1979), and in treated mice in the second series (Weisburger *et al.*, 1978). Tumors were also observed at sites other than the liver (in mice, lung and vascular tumors [NCI, 1979; Weisburger *et al.*, 1978]; in rat lung [NCI, 1979] and subcutaneous fibromas and fibrosarcomas [Weisburger *et al.*, 1978; marginally significant]).

The NCI (1979) found as follows: "It is concluded that under the conditions of this bioassay, 2,4,5-trimethylaniline was carcinogenic for male and female F344 rats and female B6C3F_1 mice …" On the other hand IARC (1982)

described the overall evidence of carcinogenicity in animals as limited. The IARC reviewers did not state their reasons for this divergence of opinion with the NCI in detail, although they expressed concerns for the small control groups, the possibility of cross-contamination between this and other concurrent studies in the experimental suite, and purity of the test compound. The limitations of the design and reporting of the studies by NCI (1979) and Weisburger (1978) are described below, along with study findings. No other studies have been reported.

Rat Dietary Exposure: NCI, 1979

Groups of 50 male and 50 female Fischer 344 rats received 200 ppm or 800 ppm 2,4,5-trimethylaniline (free amine) in the diet for 101 weeks. Matched control groups for each sex, containing 20 animals, received plain diet. Data from additional concurrent untreated groups of 20 animals of each sex, and historical and pooled control data were also reviewed.

The design of this study, one of the early series conducted by NCI, is not consistent with the standard protocol used by National Toxicology Program. The matched control groups had 20 rather than 50 animals, and the test animals were housed in the same room as animals exposed to several other potentially carcinogenic test materials. Thus studies run under current protocol would have greater power than the NCI studies.

NCI reported that gas chromatography (detector type not specified) indicated two components, one of which was > 99.9 %, and provided other identity data including mass spectrometric identification of the major component as 2,4,5-trimethylaniline. IARC (1982) on the other hand asserted that the purity of the test compound had not been established and noted the identification of at least one impurity.

Hepatocellular carcinomas and neoplastic nodules, and lung adenomas and carcinomas were observed in both males and females. Survival in control and exposed groups was close to 100% throughout most of the study, and was at or above 70% at study termination. Tumor incidences in exposed and control animals are reported in detail in Table 1. The dose-related trend for liver tumors was significant ($p < 0.001$, Cochran-Armitage trend test) in both males and females, and for rare (Eustis *et al.*, 1990) bile duct carcinoma in males ($p = 0.015$, Cochran-Armitage trend test). Incidences relative to

controls were significant ($p < 0.01$, Fisher Exact test) for liver tumors in high-dose males and in females at both high and low doses.

The incidence of lung tumors in high-dose females was also statistically significant ($p < 0.01$, Fisher Exact test). Lung tumors were also elevated in treated males ($p < 0.01$, Cochran-Armitage trend test). In addition, some bile-duct carcinomas were observed, in the high-dose male rats only, but the incidence was not statistically significant ($p = 0.26$, Fisher Exact test).

Table 1: Tumors in Fischer 344 rats receiving 2,4,5-trimethylaniline, 200 ppm or 800 ppm, in the diet for 101 weeks (NCI, 1979)

Tumor Site and Type		Dose, ppm[a]		
		0	200	800
Males				
Liver	Neoplastic Nodules	1/19[b,d]	3/50	11/50[h]
	Hepatocellular Carcinomas	0/19[d]	3/50	11/50[h]
	Bile-duct carcinoma	0/19[f]	0/50	4/50
	All liver tumors	1/19[c]	6/50	20/50[g]
Lung	Adenomas	0/20	0/49	5/50
	Carcinomas	1/20	0/49	2/50
	All lung tumors	1/20[e]	0/49	7/50
Females				
Liver	Neoplastic Nodules	0/20[c]	12/49[g]	20/50[g]
	Carcinomas	0/20[c]	0/49	9/50[h]
	Bile-duct carcinoma	0/19	0/49	1/50
	All liver tumors	0/20[c]	12/49[g]	27/50[g]
Lung	Adenomas	0/20	1/43	9/50[h]
	Carcinomas	0/20	2/43	2/50
	All lung tumors	0/20[d]	3/43	11/50[g]

[a] Concentration of 2,4,5-trimethylaniline in feed

[b] Number of lesion-bearing animals/total examined at 101 weeks.

[c, d, e, f] Dose-related trend was significant by the Cochran-Armitage trend test ([c] $p < 0.001$; [d] $p < 0.005$; [e] $p < 0.01$; [h] $p = 0.015$)

[g, h] Incidence relative to control group was significant by the Fisher Exact test ([f] $p < 0.01$; [g] $p < 0.05$)

Rat (males only) Dietary Exposure: Weisburger et al., 1978

Groups of 25 male CD rats (from Charles River, Sprague-Dawley derived) received 1000 ppm or 2000 ppm 2,4,5-trimethylaniline (as the hydrochloride) in the diet for 78 weeks, with a further observation period of 26 weeks on plain diet. A matched control group, containing 22 animals, received plain diet; pooled (111 animals) control data were also reviewed. A complete gross necropsy was performed an all animals dying after six months on the diet, or at the termination of the experiment.

Incidences of subcutaneous fibromas and fibrosarcomas, and of liver tumors, were elevated in the low-dose group, but these results were statistically significant only relative to a pooled control group. Tumor incidences in the matched controls, pooled controls, and exposed dose groups are reported in Table 2. Only results of significance or interest to Weisburger *et al.* (1978) were included in the journal publication of this study

Table 2: Tumors in male CD rats receiving 2,4,5-trimethylaniline hydrochloride, 1000 ppm or 2000 ppm, in the diet for 78 weeks (Weisburger *et al.*, 1978)

Tumor Site and Type	Dose, ppm[a]			
	0 (matched)	0 (pooled)	1000	2000
Liver tumors	2/22[b]	2/111	3/17[c]	2/25
Subcutaneous fibromas and fibrosarcomas	4/22	18/111	6/17[c]	1/25

[a] Concentration of 2,4,5-trimethylaniline in feed

[b] Number of lesion-bearing animals/total examined at 101 weeks.

[c] Incidence relative to pooled control group was significant ($p < 0.025$, Fisher Exact test).

Mouse Dietary Exposure: NCI, 1979

Groups of 50 male and 50 female $B6C3F_1$ mice received 50 ppm or 100 ppm 2,4,5-trimethylaniline (as free amine) in the diet for 101 weeks. Matched control groups for each sex, containing 20 animals, received plain diet. Data from concurrent untreated groups of 20 animals of each sex, and historical and pooled control data were also reviewed.

The design of this study, one of the early series conducted by NCI, is not consistent with the standard protocol used by National Toxicology Program. The matched control groups had 20 rather than 50 animals, and the test animals were housed in the same room as animals exposed to several other potentially carcinogenic test materials. Thus, studies run under the current NTP protocol would have greater power than the older NCI studies.

NCI reported that gas chromatography indicated two components, one of which was > 99.9 %, and provided other identity data including mass spectrometric identification of the major component as 2,4,5-trimethylaniline. IARC (1982) on the other hand asserted that the purity of the test compound had not been established and noted the identification of at least one impurity.

At the end of the study, hepatocellular carcinomas were observed in both male and female mice. The incidences of hyperplastic nodules were also somewhat elevated in the high-dose group in both sexes. Incidences of lung tumors, and hemangiosarcomas (mostly in lymph nodes, but also at various other sites) were also marginally increased ($0.05 < p < 0.12$) in female mice. A few tumors of the biliary system (bile duct, gall bladder) were also observed in male mice. Tumor incidences are reported in detail in Table 3.

In female mice, the dose-related trends for hepatocellular carcinoma and for all liver tumors were significant ($p < 0.001$, Cochran-Armitage trend test), and incidences relative to controls were significant ($p < 0.01$, Fisher Exact test) at both high and low doses. In males incidences, of liver tumors were elevated in both dose groups, but the statistical significance was lower ($p < 0.05$, Fisher Exact; $p = 0.035$, Cochran Armitage trend test). The incidence of liver tumors in males of this strain of mice is high and variable in historical data. Because of this, and the possible applicability of the Bonferroni correction for multiple observations, NCI was unwilling to conclude that the

elevated incidence of liver tumors in the male mice was necessarily related to exposure to 2,4,5-trimethylaniline.

Table 3: Tumors in B6C3F$_1$ mice receiving 2,4,5-trimethylaniline, 50 ppm or 100 ppm, in the diet for 101 weeks (NCI, 1979)

Tumor Site and Type		Dose, ppm[a]		
		0	50	100
Males				
Liver	Hyperplastic nodule	1/20[b]	3/50	7/50
	Hepatocellular carcinoma	5/20[d]	26/50[f]	27/50[f]
	Bile duct carcinoma	0/20	2/50	2/50
	Gall bladder carcinoma	0/20	1/50	0/50
	All liver tumors	5/20[d]	27/50[f]	27/50[f]
Females				
Liver	Hyperplastic nodule	0/20	4/49	13/50
	Hepatocellular carcinoma	0/20[c]	18/49[e]	40/50[e]
	All liver tumors	0/20	18/49[e]	40/50[e]
Lung	Carcinoma	0/19	4/49	6/48[g]
	Adenoma	0/19	1/49	0/48
	All lung tumors	0/19	5/49	6/48[g]
Vascula-ture	Hemangiosarcoma	1/20	11/49[g]	7/50

[a] Concentration of 2,4,5-trimethylaniline in feed

[b] Number of lesion-bearing animals/total examined at 101 weeks

[c, d] Dose-related trend was significant by the Cochran-Armitage trend test ([c] $P < 0.001$; [d] $P < 0.05$)

[e-g] Incidence relative to control group (p values by the Fisher Exact test: [e] < 0.01; [f] < 0.05; [g] $0.05 < p < 0.12$)

Table 4: Tumors in CD-1 mice receiving 2,4,5-trimethylaniline hydrochloride, 1000 ppm or 2000 ppm in the diet, for 78 weeks (Weisburger *et al*., 1978).

Tumor Site and Type	Dose, ppm[a]			
	0 (matched)	0 (pooled)	1000	2000
Males				
Liver tumors	3/18[b]	7/99	9/14[c]	19/21[c]
Lung tumors	5/18	24/99	11/14[c]	10/21[d]
Vascular tumors	0/18	5/99	3/14	3/21
"Multiple tumors"	6/18	14/99	9/14[d]	14/21[d]
Females				
Liver tumors	0/20	1/102	6/15[c]	14/22[c]
Lung tumors	6/20	32/102	11/15[c]	12/22[d]
Vascular tumors	0/20	9/102	3/15	3/22
"Multiple tumors"	6/20	21/102	9/15[d]	12/22[d]

[a] Concentration of 2,4,5-trimethylaniline in feed

[b] Number of lesion-bearing animals/total examined at 91 weeks

[c] Incidence was significant relative to both pooled and concurrent control groups ($p < 0.025$, Fisher Exact test)

[d] Incidence relative to pooled control group was significant ($p < 0.05$, Fisher Exact test)

Mouse Dietary Exposure: Weisburger et al., 1978

Groups of 25 male and 25 female CD-1 mice (from Charles River, random bred and derived from HaM/ICR strain) received 1000 ppm or 2000 ppm 2,4,5-trimethylaniline (as the hydrochloride) in the diet for 78 weeks, with a further observation period of 13 weeks on plain diet. Matched control groups for each sex, containing 20 animals, received plain diet; historical and pooled (100 animals) control data were also reviewed.

The reported tumor incidences are shown in detail in Table 4. Liver tumors (hepatomas) and lung tumors were observed in both males and females. Incidences of these tumors in all exposed groups were statistically significant relative to a pooled control group, and also (except in the case of lung tumors in both sexes in the high-dose groups) relative to the corresponding concurrent control group. There was also an increase in the numbers of vascular tumors but this was not statistically significant. The authors also reported an increase in animals with multiple tumors, which was statistically significant relative to pooled controls. This classification apparently included subcutaneous fibromas or fibrosarcomas, but they did not report the actual incidences of these tumors. Survival was poor in both sexes. The IARC (1982) Working Group noted "that the poor survival and the scant detail in the reporting make evaluation of the study difficult."

3.3 Other relevant data

In addition to the reported animal bioassays, additional evidence related to the possible carcinogenicity of 2,4,5-trimethylaniline is available. This includes studies of genetic toxicity, observations of the pharmacokinetics and metabolism, and structure-activity comparisons.

3.3.1 Genetic Toxicology

2,4,5-Trimethylaniline was found to be mutagenic in the *Salmonella* reverse mutation assay with metabolic activation by Aroclor-induced rat liver enzymes (Zimmer *et al.*, 1980; Kugler-Steigmeier *et al.*, 1989). 2,4,5-trimethylaniline did not induce strand breaks in the DNA of Chinese hamster V79 cells *in vitro* (Zimmer *et al.*, 1980). Kugler-Steigmeier *et al.* (1989) observed mutations with this compound in a 6-thioguanine resistance test in primary cultured rat fibroblasts; the toxicity of the compound at higher doses

interfered with the observation of a dose response curve, and maximum response was observed at the second lowest concentration (148 µg/ml medium) studied. The latter authors also observed a positive response with 2,4,5-trimethylaniline in a somatic mutation and recombination test *in vivo* in *Drosophila melanogaster*.

The data now available on genotoxicity of 2,4,5-trimethylaniline are more extensive than those available to NCI and IARC in their earlier evaluations. No genotoxicity studies were reviewed by NCI (1979), and only the study by Zimmer *et al.* (1980) was reviewed by IARC (1982).

3.3.2 Structure-Activity Comparisons

The dye Ponceau 3R, which was made from trimethylaniline mixtures, was identified as a liver carcinogen and its registrations for food and cosmetic uses were withdrawn. Ponceau 3R is cleaved metabolically *in vivo* to 1-amino-2-naphthol-3,6-disulfonic acid and trimethylanilines (including 2,4,5-trimethylaniline). The carcinogenic effect of the dye has been attributed to the trimethylanilines (NCI, 1979; Lindstrom *et al.*, 1969).

The isomeric compound 2,4,6-trimethylaniline was studied in the series of carcinogenicity bioassays by Weisburger *et al.* (1978) and induced tumors in all three test systems (male rat, male and female mouse), although this evidence was considered inadequate by IARC (1982). 2,4,6-trimethylaniline is mutagenic in Salmonella, fibroblasts and *Drosophila* (Kugler-Steigmeier *et al.*, 1989), and induces DNA repair in rat hepatocytes (Yoshimi *et al.*, 1988).

There is a considerable literature documenting carcinogenicity and mutagenicity of aromatic amines in general, and substituted anilines in particular. The structure-activity prediction program Oncologic, which is based on a set of rules generated by US EPA experts from data on known carcinogens, indicated a “High-Moderate” concern for carcinogenicity to 2,4,5-trimethylaniline, which is the highest level found for agents which do not actually appear in the program’s source database of known carcinogens.

3.3.3 Pharmacokinetics and Metabolism

No studies of pharmacokinetics or metabolism of 2,4,5-trimethylaniline were identified in the literature. However, the role of metabolic activation in the genotoxicity and carcinogenicity of other aromatic amines is well established.

Kugler-Steigmeier *et al.* (1989) discussed the interpretation of their test results on the basis of a role for metabolic activation. They described the dependence of the result in *Salmonella* on activation by rat liver enzymes, and possible metabolic influences on the timing of observable effects in the 6-thioguanine resistance test. They also noted that the result seen in the somatic mutation test in *Drosophila*, which consisted of small wing spots only, resulted from a late action in the exposed larva. This was explained as the result of age dependent increases in the levels of activating enzymes.

3.3.4 Pathology

The tumors observed in both studies in rat and mouse liver were considered by the authors (Weisburger *et al.*, 1978; NCI, 1979) to meet standard criteria for hepatocellular adenomas and carcinomas. It is generally considered that these tumor phenotypes are related in origin, and that the adenomas may progress to carcinomas. They are therefore usually aggregated for carcinogen identification and risk assessment purposes. Weisburger *et al.* (1978) illustrated a metastatic hepatocellular carcinoma in the lung of a female mouse given 2,4,5-trimethylaniline. Tumors at other sites, including the mouse lung, were also diagnosed as carcinomas.

3.4 Mechanism

Based on several results in tests *in vivo* and *in vitro*, 2,4,5-trimethylaniline appears to be genotoxic, probably after metabolic activation. A genotoxic mechanism may therefore be responsible for the observed carcinogenic effect. Other aromatic amines which have been more extensively studied than 2,4,5-trimethylaniline are known to cause cancer by this mechanism.

The positive carcinogenicity findings in rat and mouse liver, associated with positive results at other sites and with indications of genotoxic potential, are interpreted as an indication of general, rather than species- or tissue-specific activity (Haseman *et al.*, 1993; Gold *et al.*, 1991; Huff *et al.*, 1991).

4 SUMMARY AND CONCLUSIONS

4.1 Summary of evidence

2,4,5-Trimethylaniline has been demonstrated to induce liver carcinoma in male and female Fischer 344 rats and female $B6C3F_1$ mice in studies by the National Cancer Institute (NCI). In these studies liver carcinomas were also significantly elevated in male mice, but not to the same extent as for the female. Significant increases in lung tumors were also observed in treated rats of both sexes and female mice. The incidence of bile duct carcinoma, which rarely occurs spontaneously, was also observed in male rats, and a few tumors of this type were also observed in male mice; the result statistically significant in the rat but not the mouse.

With regard to study deficiencies, the noted limitations size of control group and animal housing for the NCI studies would limit the study's power to detect effect, rather than result in spurious findings of positive effect. Regarding purity of the NCI test substance, although a second chromatographic peak was reported, the test substance was more than 99.9% pure, and no contaminant was hypothesized to be present at levels of < 0.1% which might otherwise explain the study results.

In a second series of studies using 2,4,5-trimethylaniline hydrochloride (Weisburger *et al.,* 1978), findings of statistically significant increases of liver tumors were also seen in HaM/ICR mice of both sexes. In addition, lung tumors were observed significantly elevated in male and female mice. Tumors were not observed their study in male Charles River rats. Because of limitations in study reporting, the positive findings in mice for this second series are considered to provide supportive but not conclusive evidence of carcinogenicity. This (Weisburger *et al.*) study was undertaken by NCI as part of a large scale initiative to identify industrial carcinogens used as intermediates in manufacturing, and journal publication of study results for 2,4,5-trimethylaniline and a number of other agents was limited to findings of significance or interest. The studies were conducted according to well reviewed protocols. While limited in power to detect effects because of small numbers of animals in control and treatment groups, the study provides information useful in evaluating the overall weight of evidence.

2,4,5-trimethylaniline is mutagenic in the *Salmonella typhimurium* with metabolic activation, cultured rat fibroblasts, and *in vivo* in *Drosophila melanogaster*. These findings of mutagenicity and similar structure-activity relationships to other carcinogenic aromatic amines add to the overall weight of evidence for carcinogenicity.

4.2 Conclusion

Based on the information reviewed in the preparation of this document, there is evidence for the carcinogenicity of *2,4,5-trimethylaniline and its strong acid salts* at multiple sites in two species. Observations of genetic toxicity and chemical structural analogies with known carcinogens contribute to the weight of evidence.

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