

Apparent Correlation Between Structure and Carcinogenicity of Phenylenediamines and Related Compounds

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The carcinogenicity of 23 phenylenediamines and related compounds was reviewed. An extensive analysis of the methods used indicated that the bioassays were conducted well. The data suggest that the carcinogenicity of 4-substituted 1,3-phenylenediamines is reduced substantially or eliminated completely by oxidation of one or both amine groups or by *N*-substitution. Oxidation of a methyl substituent on nitroaniline to a carboxyl group eliminated all carcinogenic activity. It required dichlorination to make ring-substituted 1,4-phenylenediamine carcinogenic whereas only one chlorine atom was needed to make 1,2- and 1,3-phenylenediamine carcinogenic. While the available data suggest that as a class, 4-substituted 1,3-phenylenediamines are carcinogenic more often than ring-substituted 1,4-phenylenediamines, the type of added substituent and its position on the benzene ring also are important in exerting carcinogenic activity.

Introduction

Phenylenediamines comprise one of several classes of chemicals which are thought to contribute to the increase of cancer risk observed among workers in the dye manufacturing industry (1). The National Institute for Occupational Safety and Health has estimated that more than 64,000 people are potentially exposed in the workplace to a group of seven phenylenediamines, the production of which is over 50 million pounds. Exposure of the general public is estimated at more than 15 million individuals, since phenylenediamines are used in dyes, either directly as color-yielding compounds which include hair and fabric dyes or as intermediates and photographic development fluids (1).

Phenylenediamines were defined by the Interagency Testing Committee (ITC) as "all nitrogen-unsubstituted phenylenediamines with zero to two substituents on the ring selected from the same or different members of the group of halo, nitro, hydroxy, hydroxy-lower alkoxy, lower alkyl, and lower alkoxy" (2). For this purpose, the term "lower" is defined as a group between one and four carbons. The ITC listed 50 phenylenediamines as occurring in the Toxic Substances Control Act (TSCA) public inventory (2).

Long-term testing for carcinogenicity of all phenylenediamines and related compounds (i.e., those compounds in which $-NH_2$ is replaced by $-NO_2$) would

require a significant economic burden on industry. It would be useful, therefore, to be able to determine, from structural characteristics, which chemicals are most likely to be carcinogenic. It was the aim of this investigation to examine those published carcinogenicity studies for which there is adequate information for analysis and to attempt to identify correlations between chemical structure of phenylenediamines and their carcinogenic activity.

Materials and Methods

Carcinogenicity Studies

Carcinogenicity studies with phenylenediamines and related compounds were conducted for the National Cancer Institute (NCI) in both sexes of Fischer 344 (3-21) or Osborne-Mendel rats (22) and B6C3F1 mice (3-22), and generally followed the procedures outlined in the literature (23). Carcinogenicity studies also were conducted by Weisburger et al. (24) in male Charles River CD rats and in random-bred albino CD-1 mice of both sexes, derived from HaM/ICR. The chemicals were administered in feed. Prechronic studies were conducted to determine the maximum tolerated dose (MTD) to be used for each sex of each species in the chronic study. The MTD was defined by the NCI as the highest dose that causes a noticeable weight loss, but no more than a 10% decrement in weight gain when compared to appropriate controls, and that does not produce any clinical signs of toxicity or pathologic

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lesions (other than those related to neoplastic response) that would be predicted to shorten the animal's lifespan (23). With the exception of 2-chloro-1,4-phenylenediamine sulfate, *N*₂-phenyl-1,4-phenylenediamine, and clonitralid, all chemicals were 99% pure (3-22,24).

Analysis of Methodologies

The methods used for the conduct of the bioassays were analyzed according to the following criteria in order to ensure that meaningful conclusions could be made from the results obtained.

Chemical Purity. A plus (+) was designated when analytical tests indicated a chemical of high (99%) purity. A minus (-) indicated the presence of impurities (Table 1).

Number of Animals. A plus (+) designated that there were 49 to 50 animals in chemical-treated and control groups. A minus (-) indicated that only 20 to 25 control animals and 49 to 50 chemical-treated animals were used, except in the studies by Weisburger et al. (24), in which only 25 chemical-treated animals per group were used (Table 1).

Dose Schedule. A plus (+) indicated no change in dose scheduling throughout the bioassay. A minus (-) indicated that some modifications in the dose schedule occurred during the study (Table 1).

Length of Dosing. The length of dosing was noted in weeks since it affects the total dose administered (Table 1).

Weight. A plus (+) designated a decrease in weight gain relative to control which is not greater than 10% at the high dose. A minus (-) indicated a greater than 10% weight decrement relative to control at the high dose. "NC" indicated no noticeable change in weight gain relative to control (Table 1).

Survival. A plus (+) designated that sufficient numbers of animals in all groups were at risk for the development of late-appearing tumors. A minus (-) designated poor survival, generally less than 20% at 103 weeks (Table 1).

Amount of Dosing. The concentration of test material in the feed was noted in ppm/day since it provides a comparison of amounts of different test substances presented to test animals (Table 1).

Results

Analysis of Test Protocols

Before attempting to correlate carcinogenic activity of phenylenediamines and related compounds and changes in chemical structure, it was important to

examine first the methods used in the bioassays in order to ensure that the conclusions were based on adequate test procedures. These findings can be seen in Table 1.

With the exception of 2-chloro-1,4-phenylenediamine sulfate (X) *N*₂-phenyl-1,4-phenylenediamine (XV) and clonitralid (XXIII), all chemicals were 99% pure. Chemical analysis was not performed on 4-ethoxy-*N*₁-acetyl-1,3-phenylenediamine (XIV).

With the exception of mice given *N*₂-phenyl-1,4-phenylenediamine for only 51 weeks because of sudden markedly reduced survival, all test animals were fed the appropriate diet containing the chemical for 78 to 103 weeks.

Only 20 to 25 control or chemical-treated rats and mice were used in some experiments. However, statistical analyses of results from all studies except those with 1,2-phenylenediamine dihydrochloride (I), 1,3-phenylenediamine dihydrochloride (IV) and 1-chloro-2,4-dinitro-benzene (XIX) compared findings in chemical-treated groups with concurrent and with historical controls.

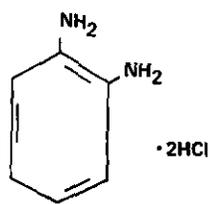
While some dose changes occurred during several chronic studies (Table 1), survival was adequate at terminal sacrifice in all groups except in rats receiving the high dose of 4-methyl-1,3-phenylenediamine (VI), male rats receiving the high dose of 2-methoxy-5-nitroaniline (XXI), and male mice receiving the high dose of clonitralid.

A greater than 10% weight gain depression relative to control was seen in some high dose groups in the various bioassays (Table 1) indicating that the maximal tolerated dose (MTD) may have been exceeded in these groups. This finding, however, was not sufficient to negate the studies since, with the exception of 2-methyl-5-nitroaniline (XVI) and 3-nitro-4-ethoxy-*N*-acetylaniline (XXII), all carcinogenic chemicals in which the high dose caused a greater than 10% depression of mean body weight gain relative to control were also carcinogenic in at least one site of one sex of one species at the lower dose which approximated the MTD (3-22). In the experiment with 3-nitro-4-hydroxyaniline (XX), mice had less than 10% weight gain depression relative to control, indicating that the MTD may not have been obtained.

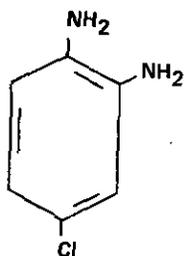
It can be concluded, therefore, that the studies were conducted under conditions in which the MTD was obtained at the high dose or if exceeded, was achieved at the lower dose. Also, good survival was generally maintained in all groups. The period of compound administration (78-103 weeks), although less in some studies than previously recommended (23), nevertheless was adequate. Mice treated with *N*₂-phenyl-1,4-phenylenediamine for 51 weeks may have been able to tolerate a longer dosing schedule at lower doses.

Carcinogenicity of Phenylenediamines and Related Compounds

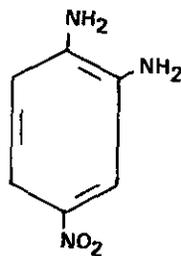
A summary of the carcinogenicity of phenylenediamines and related compounds (1-22,24) is outlined in



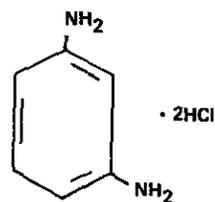
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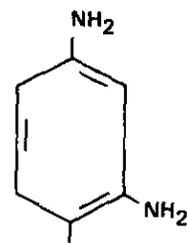
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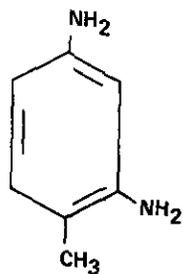
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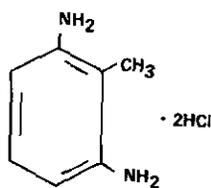
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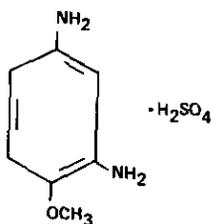
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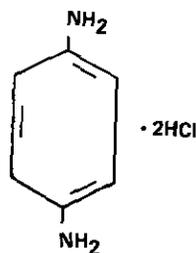
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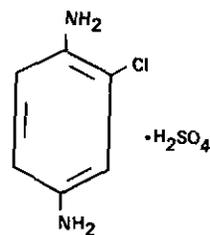
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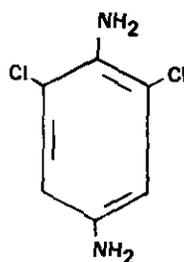
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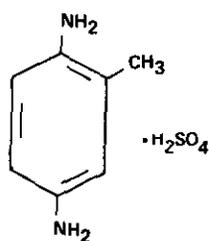
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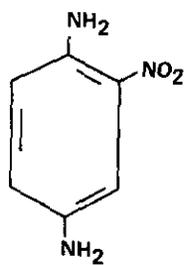
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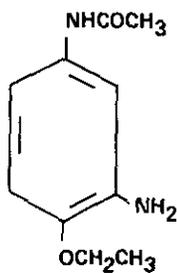
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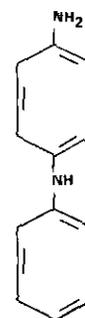
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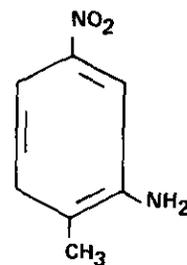
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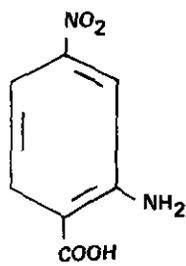
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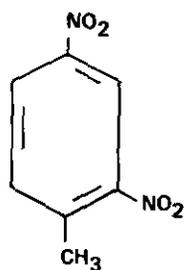
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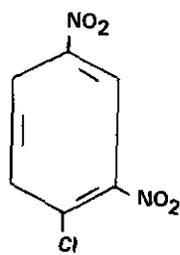
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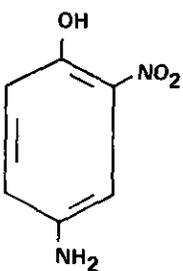
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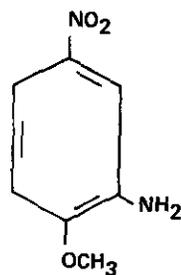
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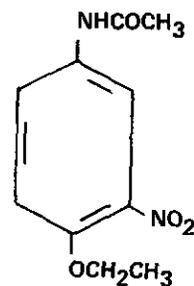
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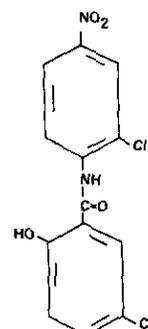
XX



XXI



XXII



XXIII

Table 1. Evaluation of test methodologies.

Chemical (CAS No.)	Structure	Sex and species ^a	Dose (time-weighted average, mg/kg diet) ^b		Chem. purity ^c	No. of animals ^d	Dose schedule changes ^e	Length of dosing ^f	Criteria used in evaluation		Reference
			High	Low					Weight ^g	Survival ^h	
1,2-Phenylenediamines 1,2-Phenylenediamine dihydrochloride (615-28-1)	I	MR	4,000	2,000	+	-	+	78	Not reported	Not reported	(24)
		MM	13,743	6,872	+	-	-	78			
		FM	13,743	6,872	+	-	-	78			
4-Chloro-1,2-phenyl- enediamine (95-83-0)	II	MR	10,000	5,000	+	+	+	78	-	+	(3)
		FR	10,000	5,000	+	+	-	78	-	+	
		MM	14,000	7,000	+	+	-	78	-	+	
4-Nitro-1,2-phenyl- enediamine (99-56-0)	III	FM	14,000	7,000	+	+	-	78	-	+	
		MR	750	375	+	-	+	103	+	+	(4)
		FR	750	375	+	-	+	103	+	+	
1,3-Phenylenediamines 1,3-Phenylenediamine dihydrochloride (541-69-5)	IV	MM	7,500	3,750	+	-	+	102	+	+	
		MM	7,500	3,750	+	-	+	102	+	+	
		FM	7,500	3,750	+	-	+	102	+	+	
4-Chloro-1,3-phenyl- enediamine (5131-60-2)	V	MR	4,000	2,000	+	+	+	78	+	+	(5)
		FR	4,000	2,000	+	+	+	78	+	+	
		MM	14,000	7,000	+	+	+	78	+	+	
4-Methyl-1,3-phenyl- enediamine [2,4-Diaminotoluene] (95-80-7)	VI	FM	14,000	7,000	+	+	-	78	-	+	(6)
		MR	176	79	+	-	-	79-103	-	+	
		FR	171	79	+	-	-	84-103	-	+	
2-Methyl-1,3-phenylene- diamine dihydro- chloride [2,6-Toluenediamine dihydrochloride] (823-40-5)	VII	MM	200	100	+	-	+	101	+	+	
		MM	200	100	+	-	+	101	+	+	
		FM	200	100	+	-	+	101	+	+	
4-Methoxy-1,3-phenyl- enediamine sulfate [2,4-Diaminoanisole sulfate] (39156-41-7)	VIII	MR	500	250	+	+	+	103	+	+	(7)
		FR	500	250	+	+	+	103	+	+	
		MM	100	50	+	+	+	103	+	+	
1,4-Phenylenediamines 1,4-Phenylenediamine dihydrochloride (624-18-0)	IX	FM	100	50	+	+	+	103	+	+	
		MR	5,000	1,200	+	+	+	78	+	+	(8)
		FR	5,000	1,200	+	+	+	78	+	+	
2-Chloro-1,4-phenylene- diamine sulfate (61702-44-1)	X	MM	2,400	1,200	+	+	+	78	+	+	
		MM	2,400	1,200	+	+	+	78	+	+	
		FM	2,400	1,200	+	+	+	78	+	+	
1,4-Phenylenediamines 1,4-Phenylenediamine dihydrochloride (624-18-0)	IX	MR	1,250	625	+	-	+	103	+	+	(9)
		FR	1,250	625	+	-	+	103	+	+	
		MM	1,250	625	+	-	+	103	+	+	
2-Chloro-1,4-phenylene- diamine sulfate (61702-44-1)	X	MM	1,250	625	+	-	+	103	+	+	
		MR	3,000	1,500	-	-	+	105-107	+	+	(10)
		FR	3,000	1,500	-	-	+	(High dose male and female mice were 87 weeks)	+	+	
2-Chloro-1,4-phenylene- diamine sulfate (61702-44-1)	X	FR	6,000	3,000	-	-	+	105-107	+	+	
		MM	6,000	3,000	-	-	+	(High dose male and female mice were 87 weeks)	+	+	
		FM	6,000	3,000	-	-	+	(High dose male and female mice were 87 weeks)	+	+	

2,6-Dichloro-1,4-phenyl-enediamine (609-20-1)	MR	2,000	1,000	+	+	+	103	+	+	(11)
	FR	6,000	2,000	+	+	+	103	+	+	
	MM	3,000	1,000	+	+	+	103	+	+	
	FM	3,000	1,000	+	+	+	103	+	+	
2-Methyl-1,4-phenyl-enediamine sulfate [2,5-Toluenediamine sulfate] (6369-59-1)	MR	2,000	600	+	+	+	78	+	+	(12)
	FR	2,000	600	+	+	+	78	+	+	
	MM	1,000	600	+	+	+	78	+	+	
	FM	1,000	600	+	+	+	78	+	+	
2-Nitro-1,4-phenyl-enediamine (5307-14-2)	MR	1,100	550	+	-	+	78	+	+	(13)
	FR	2,200	1,100	+	-	+	78	+	+	
	MM	4,400	2,200	+	-	+	78	+	+	
	FM	4,400	2,200	+	-	+	78	+	+	
N-Substituted 1,3-phenyl-enediamines 4-Ethoxy-N ₁ -acetyl-1,3-phenylenediamine [3-Amino-4-ethoxy-acetanilide] (17026-81-2)	MR	15,000	4,000	(Analysis not performed)	+	+	78	+	+	(14)
	FR	15,000	4,000	+	+	+	78	+	+	
	MM	8,000	4,000	+	+	+	78	+	+	
	FM	8,000	4,000	+	+	+	78	+	+	
N-Substituted 1,4-phenyl-enediamines N ₂ -Phenyl-1,4-phenyl-enediamine (624-18-0)	MR	1,200	600	-	-	+	78	+	+	(15)
	FR	1,200	600	-	-	+	78	+	+	
	MM	4,114	2,057	-	-	+	51	+	+	
	FM	8,170	3,672	-	-	+	51	+	+	
Compounds related to 1,3-phenylenediamine 2-Methyl-5-nitroaniline [5-Nitro- <i>o</i> -toluidine] (99-55-8)	MR	100	50	+	+	+	78	+	+	(16)
	FR	100	50	+	+	+	78	+	+	
	MM	2,300	1,200	+	+	-	78	+	+	
	FM	2,300	1,200	+	+	-	78	+	+	
2-Carboxyl-5-nitroaniline [4-Nitroanthranilic acid] (619-17-0)	MR	15,000	4,600	+	-	+	78	+	+	(17)
	FR	15,000	4,600	+	-	+	78	+	+	
	MM	10,000	4,600	+	+	+	78	+	+	
	FM	10,000	4,600	+	+	+	78	+	+	
2,4-Dinitrotoluene [1-Methyl-2,4-dinitrobenzene] (121-14-2)	MR	200	80	+	-	+	78	+	+	(18)
	FR	200	80	+	-	+	78	+	+	
	MM	400	80	+	+	-	78	+	+	
	FM	400	80	+	+	-	78	+	+	
1-Chloro-2,4-dinitrobenzene	MR	1,024	512	+	-	+	78	+	+	(24)
	MM	3,270	1,635	+	-	+	78	+	+	
	FM	2,663	1,332	+	-	+	78	+	+	
	Not reported							Not reported		
3-Nitro-4-hydroxyaniline [4-amino-2-nitrophenol] (119-34-6)	MR	2,500	1,250	+	-	+	103	+	+	(19)
	FR	2,500	1,250	+	-	+	103	+	+	
	MM	2,500	1,250	+	-	+	103	+	+	
	FM	2,500	1,250	+	-	+	103	+	+	
2-Methoxy-5-nitroaniline [5-Nitro-1,2-anisidine] (99-59-2)	MR	8,000	4,000	+	+	+	78	-	-	(20)
	FR	8,000	4,000	+	+	+	78	-	-	
	MM	8,000	6,000	+	+	+	78	+	+	
	FM	8,000	6,000	+	+	+	78	+	+	

Table 1. Evaluation of test methodologies. (continued)

Chemical (CAS No.)	Structure	Sex and species ^a	Dose (time-weighted average, mg/kg diet) ^b		Chem. purity ^c	No. of animals ^d	Dose schedule changes ^e	Length of dosing ^f	Weight ^g	Survival ^h	Reference
			High	Low							
3-Nitro-4-ethoxy-N- acetylaniline [3-nitro- <i>p</i> -aceto- phenetide] (1770-84-0)	XXII	MR	3,600	1,800	+	+	-	78	-	+	(21)
		FR	3,600	1,800	+	+	-	78	-	+	
		MM	14,600	7,300	+	+	-	78	-	+	
		FM	14,600	7,300	+	+	-	78	-	+	
Compounds related to 1,4-phenylenediamine Clonitralid (1420-04-8)	XXIII	MR	28,433	14,216	-	-	-	78	+	+	(22)
		FR	28,433	14,216	-	-	-	78	+	+	
		MM	549	274	-	-	-	78	+	-	
		FM	549	274	-	-	-	78	+	+	

^a MR = male rats; FR = female rats; MM = male mice; FM = female mice. Fischer 344 rats were used for all studies (3-21) except Osborne-Mendel rats were used in the study with clonitralid (22), and Charles River CD rats were used for the EPA studies (2). B6C3F1 mice were used for all NCI studies in (3-22) and random-bred albino CD-1 mice derived from HaM/ICR mice were used for studies by Weisberger (24).

^b Time-weighted average dose = $[\sigma(\text{dosage} \times \text{weeks treated})]/[\sigma(\text{weeks of treatment})]$.

^c "+" means acceptable chemical purity; "-" means the presence of impurities.

^d "+" means 50 animals in all groups; "-" means 25-50 animals in all chemical-treated groups, and 20-25 animals in control groups.

^e "+" means there was no dose change; "-" means there was a dose change. Chemicals were administered in feed.

^f Numbers given refer to both rats and mice in weeks.

^g "+" means that one or both chemical-treated groups showed a notable weight loss when compared to control but not more than a 10% difference; "-" means that weight changes were greater than 10% compared to control; "NC" means that no weight change relative to control was seen.

^h "+" means that survival in all groups was adequate; "-" means that survival was less than 20% at the end of the study.

Table 2. Of the 23 compounds tested, 11 were judged not to be carcinogenic in rats or mice. Of the 12 carcinogenic chemicals, nine were carcinogenic for the rat and/or mouse liver, two [4-chloro-1,2-phenylenediamine (II) and 3-nitro-4-hydroxyaniline (XX)] for the bladder of rats, and two [4-methoxy-1,3-phenylenediamine sulfate (VIII) and 4-ethoxy-*N*₂-acetyl-1,3-phenylenediamine (XIV)] were carcinogenic for rat or mouse thyroid. Other sites affected by some of these agents were rat adrenals [by 4-chloro-1,3-phenylenediamine (V)], rat zymbal gland [by 4-methoxy-1,3-phenylenediamine sulfate (VIII)], female rat clitoral gland (by 2-methoxy-5-nitroaniline (XXI)), and rat skin (by 4-methyl-1,3-phenylenediamine and 2-methoxy-5-nitroaniline).

Of the nine chemicals that affected the liver of the rodent (Table 2), eight were carcinogenic for mouse liver, whereas only two significantly increased the incidence of liver tumors in the male rat [1,2-phenylenediamine dihydrochloride (I) and 4-methyl-1,3-phenylenediamine (VI)]. 4-Methyl-1,3-phenylenediamine was also carcinogenic for the skin of both sexes of the rat and for the mammary gland of female Fischer 344 rats. Of the mouse hepatocarcinogens, only 4-methoxy-1,3-phenylenediamine was also carcinogenic for other sites (i.e., hematopoietic system). While the high incidence of liver tumors (22%) in untreated male B6C3F1 mice might suggest that this site should not be considered in the evaluation of the carcinogenicity of these chemicals, it should be noted that, with the exception of 3-nitro-4-ethoxy-*N*-acetylaniline, all chemicals which were carcinogenic for the liver of male mice were also carcinogenic for the liver of female B6C3F1 mice where the historical control incidence of these neoplasms is only 4.0%. These results indicate a major difference in the tissue sensitivity of mice and rats to this group of chemical carcinogens.

In general, ring-substituted 1,4-phenylenediamines and related compounds were not carcinogenic (4 of 6 of the 1,4-phenylenediamines and related compounds were not carcinogenic), ring-substituted 1,3-phenylenediamines and related compounds were carcinogenic (8 of 12 were carcinogenic), and not enough information was available to conclude on the carcinogenic potential of ring-substituted 1,2-phenylenediamines (1 of 2 were carcinogenic).

Discussion

False Positives and False Negatives

Before making structure-activity correlations, the possibility of false positives and false negatives must be considered. False positives in carcinogenicity studies may be caused by the use of impure chemicals, the placement of several chemicals in the room, the presence of abnormally low tumor rates in control animals, etc., as well as random variation in results. As can be seen from Table 1, several test criteria were not met by

a number of the agents tested so that the possibility of a false positive in any test does exist.

The possibility of false negatives is likely if effects are small enough to escape detection in 50 animals, if the MTD was not reached in the studies, if impure chemicals were used, if nonsusceptible species or strains were employed, the use of low numbers of animals, poor survival, etc. With some chemicals, several tissue sites did show a positive dose-response trend, suggesting that if the doses were slightly higher then the chemicals might have been found to be carcinogenic at those sites. For example, 2-chloro-1,4-phenylenediamine sulfate was concluded not to be carcinogenic. However, there was a significant ($p < 0.038$) positive association between dosage and the combined incidence of hepatocellular carcinoma or hepatocellular adenoma in male mice. This finding suggests that 2-chloro-1,4-phenylenediamine sulfate may be found to be a carcinogen if retested under more vigorous conditions.

Similar findings were seen with 2-carboxyl-5-nitroaniline (XVII) [positive trend ($p < 0.035$) in circulatory system neoplasms in the male mouse], and with clonitralid [positive trend in thyroid ($p < 0.040$) and uterus ($p < 0.036$) neoplasms in female rats].

Other limitations of this evaluation which should be noted include (1) limited data set; (2) choice of species; (3) choice of doses; (4) variations in the incidence of tumors in control groups; (5) biological variations including interspecies differences in pharmacokinetics; (6) effect of housing (i.e., cage rotation, etc.) on results; and (7) appropriateness of statistical methods employed.

Spontaneous Tumor Incidence

The ability to detect chemicals which are carcinogenic for specific sites of the rodent is directly dependent on the normal, background incidence of tumors at those sites in unexposed animals. For example, the high incidence (22%) of hepatocellular neoplasms in untreated male B6C3F1 mice substantially reduces the sensitivity of the assay method for detecting hepatocarcinogens in this species. Likewise, comparing results from carcinogenicity studies conducted in different strains of rats having differing spontaneous tumor incidences may be difficult.

Most of the studies reported herein were conducted in both sexes of B6C3F1 mice and Fischer 344 rats. Osborne-Mendel rats were used only in the study of clonitralid (XXIII) and that chemical was not found to be carcinogenic.

The spontaneous incidence of neoplasms in untreated Fischer 344 rats is nearly comparable to that of Osborne-Mendel rats, with a few exceptions (25). For example, the incidence of mammary gland tumors in untreated female Osborne-Mendel rats is nearly 37%, whereas in female Fischer 344 rats it is only 18%. Also, kidney tumors appear in greater numbers in unexposed Osborne-Mendel rats of both sexes (approximately

Table 2. Summary of bioassay results on carcinogenicity studies with phenylenediamines and related compounds.^a

Compound	Target organ and result			
	Rats		Mice	
	Male	Female	Male	Female
1,2-Phenylenediamines				
1,2-Phenylenediamine	Liver (1) hepatocellular carcinoma (81%)	Not applicable	None	None
4-Chloro-1,2-phenylenediamine	Urinary bladder (1) transitional-cell carcinoma (37%) (2) papilloma NOS, transitional-cell papilloma, or transitional-cell carcinoma (51%)	Urinary bladder (1) papillary carcinoma or transitional-cell carcinoma (49%) (2) papillary carcinoma, papilloma, papillomatosis, or transitional-cell papilloma or transitional-cell carcinoma (71%)	Liver (1) hepatocellular carcinoma (55%) (2) hepatocellular adenoma or hepatocellular carcinoma (72%)	Liver (1) hepatocellular carcinoma (13%) (2) hepatocellular adenoma or hepatocellular carcinoma (21%)
4-Nitro-1,2-phenylenediamine	None	None	None	None
1,3-Phenylenediamines				
1,3-Phenylenediamine	None	Not applicable	None	None
4-Chloro-1,3-phenylenediamine	Adrenals (1) pheochromocytoma (29%)	None	None	Liver (1) hepatocellular carcinoma (11%) (2) hepatocellular adenoma or hepatocellular carcinoma (18%)
4-Methyl-1,3-phenylenediamine	Integumentary system (1) fibroma (38%) Liver (1) hepatocellular carcinoma or neoplastic nodule (20%)	Integumentary system (1) fibroma (20%) Mammary gland (1) all adenoma (76%) (2) adenoma or carcinoma (82%)	None	Liver (1) hepatocellular carcinoma (39%)
2-Methyl-1,3-phenylenediamine dihydrochloride	None	None	None	None
4-Methoxy-1,3-phenylenediamine sulfate	Thyroid (1) C-cell adenoma or C-cell carcinoma (20%) (2) adenocarcinoma NOS (29%) Preputial gland (1) adenoma NOS, papillary adenoma, or cystadenoma (16%) Zymbal's gland or ear canal (1) squamous-cell carcinoma, or sebaceous adenocarcinoma (16%)	Thyroid (1) adenocarcinoma, follicular-cell carcinoma, or papillary cystadenocarcinoma (20%) Zymbal's gland (1) sebaceous adenocarcinoma (14%)	Thyroid (1) follicular-cell adenoma (24%)	Hematopoietic system (1) malignant lymphoma (18%) Thyroid (1) follicular-cell adenoma (13%) (2) follicular-cell adenoma or follicular cell carcinoma (18%)

Chemical Name	Organ	Findings
Skin (1) squamous-cell carcinoma, or sebaceous adenocarcinoma (14%)	None	None
	None	None
	None	None
1,4-Phenylenediamines	None	None
1,4-Phenylenediamine dithydrochloride	None	None
2-Chloro-1,4-phenylenediamine sulfate	None	None
2,6-Dichloro-1,4-phenylenediamine	None	None
2-Methyl-1,4-phenylenediamine sulfate	None	None
2-Nitro-1,4-phenylenediamine	None	None
N-Substituted 1,3-phenylenediamines	None	None
4-Ethoxy-N ₁ -acetyl-1,3-phenylenediamine	None	None
N-Substituted 1,4-phenylenediamines	None	None
N ₂ -Phenyl-1,4-phenylenediamine	None	None
Compounds related to 1,3-phenylenediamine	None	None
2-Methyl-5-nitroaniline	None	None
2-Carboxyl-5-nitroaniline	None	None
2,4-Dinitrotoluene	None	None
1-Chloro-2,4-dinitrobenzene	None	None
3-Nitro-4-hydroxyaniline	Urinary bladder (1) transitional-cell carcinoma (28%)	None
Skin (1) squamous-cell carcinoma, or sebaceous adenocarcinoma (14%)	None	None
	None	None
	None	None
Liver (1) hepatocellular adenoma or hepatocellular carcinoma (32%)	None	None
	None	None
	None	None
Liver (1) hepatocellular adenoma or hepatocellular carcinoma (32%)	None	None
	None	None
	None	None
Liver (1) hepatocellular adenoma or hepatocellular carcinoma (35%)	None	None
	None	None
	None	None
Thyroid (1) follicular-cell carcinoma (16%) (2) follicular-cell carcinoma or adenocarcinoma NOS (16%) (3) follicular-cell adenoma, follicular-cell carcinoma, adenocarcinoma NOS, or adenoma NOS (27%)	None	None
	None	None
	None	None
Liver (1) hepatocellular carcinoma (64%)	None	None
	None	None
	None	None
Urinary bladder (1) transitional-cell carcinoma (28%)	None	None
	None	None
	None	None

Table 2. Summary of bioassay results on carcinogenicity studies with phenylenediamines and related compounds.^a (continued)

Compound	Target organ and result			
	Male	Female	Male	Female
2-Methoxy-5-nitroaniline	Male	Rats	Mice	Mice
	Male	Female	Male	Female
	Skin	Clitoral gland	None	Liver
	(1) basal cell carcinoma (63%)	(1) carcinoma NOS (15%)		(1) hepatocellular (19%)
	(2) trichoeptithelioma (19%)	(2) carcinoma NOS or squamous-cell carcinoma (20%)		
(3) squamous cell carcinoma (23%)	(3) adenoma NOS or papillary adenoma or carcinoma NOS or squamous-cell carcinoma (30%)			
(4) sebaceous adenocarcinoma (38%)				
(5) sebaceous adenoma or sebaceous adenocarcinoma (48%)				
3-Nitro-4-ethoxy-N-acetylaniline	Preputial gland	None	Liver	None
	(1) adenoma NOS or carcinoma NOS (10%)		(1) hepatocellular adenoma or hepatocellular carcinoma (53%)	
Compounds related to 1,4-phenylenediamine	None	None	None	None
	Clonitralid	None	None	None

^aPhenylenediamines and related compounds were tested for carcinogenicity as described in "Materials and Methods." Fischer 344 rats were used in all studies except as follows: Clonitralid study used Osborne-Mendel rats; 1,2-phenylenediamine dihydrochloride, 1,3-phenylenediamine dihydrochloride, and 1-chloro-2,4-dinitrobenzene studies used Charles River CD rats. B6C3F1 mice were used in all studies except in studies with 1,2-phenylenediamine dihydrochloride, 1,3-phenylenediamine dihydrochloride, and 1-chloro-2,4-dinitrobenzene where random-bred albino CD-1 mice derived from HaM/ICR were used. Numbers in parentheses represent the observed tumor incidence in high dose groups. NOS denotes "not otherwise specified."

3.5%) compared to Fischer 344 rats (0.4%) as are thyroid tumors in female Osborne-Mendel rats (11% versus 7%, respectively). Tumors of the uterus and pituitary gland, however, are significantly reduced in untreated female Osborne-Mendel rats compared to female Fischer 344 rats (4% versus 16% and 21% versus 30%, respectively).

Differences in spontaneous tumor incidence can also be seen between sexes of the same strain and species. For example, whereas liver and lung neoplasms are more abundant in untreated male B6C3F1 mice than in the corresponding females, the incidence of pituitary gland tumors and leukemia/lymphoma is greater in female B6C3F1 mice than in the males (26). Similarly, the incidence of mammary gland and pituitary gland tumors is increased in female Fischer 344 or Osborne-Mendel rats over the corresponding males, whereas adrenal gland tumors appear at two to three times greater frequency in untreated males than in female rats (25).

Structure-Activity Relationships

1,2-Phenylenediamines. Only three 1,2-phenylenediamines were examined for carcinogenicity in this review, and these gave conflicting findings so that no definitive conclusion could be made on the carcinogenic potential of this class of compounds. Compounding further the interpretation of the results from this class of compounds is the fact that 1,2-phenylenediamine dihydrochloride was tested in male Charles River CD rats and both sexes of random-bred albino CD-1 mice derived from HaM/ICR whereas 4-chloro-1,2-phenylenediamine and 4-nitro-1,2-phenylenediamine (III) were tested in Fischer 344 rats and B6C3F1 mice of both sexes.

Both 1,2-phenylenediamine dihydrochloride and 4-chloro-1,2-phenylenediamine were carcinogenic. However, changing the 4-chloro- to a 4-nitro- group eliminated all carcinogenic activity. Additional studies with varied analogs are needed to analyze more fully the carcinogenic potential of this group of compounds.

1,3-Phenylenediamines. While 1,3-phenylenediamine dihydrochloride was not carcinogenic in rats or mice, several of its ring-substituted analogs were. For example, chlorination, methylation, or hydroxylation at a position *para* to an amine group produced a compound with carcinogenic activity. In comparison, methylation at a position *ortho* to the amine function, i.e., 2-methyl-1,3-phenylenediamine dihydrochloride (VII) yielded a compound which was completely inactive.

As the substituent at the fourth position of 1,3-phenylenediamine was changed from -Cl to -CH₃ to -OCH₃, the compound became carcinogenic for more species, more sexes, and more target sites. However, *N*-substitution (i.e., 4-ethoxy-*N*₁-acetyl-1,3-phenylene-

diamine and 3-nitro-4-ethoxy-*N*-acetylaniline reduced the carcinogenicity to only one site, of one sex, of one species (male mouse thyroid or liver).

Oxidation of one of the -NH₂ groups to -NO₂ function substantially reduced the carcinogenicity of the compounds that fell into this class. For example, 4-methyl-1,3-phenylenediamine was carcinogenic for several sites of male and female rats and male mice, while the oxidation of the -NH₂ function at position -1 to -NO₂ group (i.e., 2-methyl-5-nitro-aniline) limited the carcinogenicity of the chemical to the liver of both sexes of mice only. In a similar manner, 4-methoxy-1,3-phenylenediamine sulfate was carcinogenic for several sites of rats and mice of both sexes whereas 2-methoxy-5-nitro-aniline, the *N*₁-oxidized analog, was carcinogenic only for female rats and female mice.

Oxidation of the 4-methyl group of the *N*₁-oxidized analog of 1,3-phenylenediamine (i.e., 2-methyl-5-nitro-aniline) to a carboxyl group (i.e., 2-carboxyl-5-nitro-aniline) eliminated all carcinogenic activity. Oxidation of both amine functions, i.e., 2,4-dinitrotoluene (XVIII), also produced a compound which was completely inactive.

It can be concluded, therefore, that while it appears that 4-substituted 1,3-phenylenediamines tend to be carcinogenic, the carcinogenicity of chemicals in this small and nonrandom set is substantially reduced or eliminated completely by oxidation of one or both amine groups or by *N*-substitution. Oxidation of the methyl substituent on nitroaniline to a carboxyl group produced a compound which was devoid of carcinogenic activity.

1,4-Phenylenediamines. Only seven 1,4-phenylenediamines and related compounds were examined for carcinogenicity in this review so that conclusions on the carcinogenic potential of this class of compounds must be viewed with caution. Of these agents, only 2,6-dichloro-1,4-phenylenediamine (XI) and 2-nitro-1,4-phenylenediamine (XIII) were carcinogenic. These results suggest, therefore, that as a class, ring-substituted 1,4-phenylenediamines may be carcinogenic less often than 4-substituted 1,3-phenylenediamines.

While the monochloro analog of 1,2- and 1,3-phenylenediamine and the monomethyl analog of 1,3-phenylenediamine were carcinogenic for both species at several sites, the monochloro and monomethyl analog of 1,4-phenylenediamine were completely inactive. It required dichlorination (2,6-dichloro-1,4-phenylenediamine) to make 1,4-phenylenediamine carcinogenic, whereas only one chlorine atom was needed to make 1,2- and 1,3-phenylenediamine carcinogenic. Therefore, while the data are limited, it appears that the position of the chlorine atom in relation to the diamines on the benzene ring may be important for carcinogenic activity. Both 1,2- and 1,3-phenylenediamines were carcinogenic when the chlorine atom was *para* to an amine group. The chlorine atom on 1,4-phenylenediamine, on the

other hand, can only be *ortho* or *meta* to the amines. This may explain the noncarcinogenicity of monochloro-1,4-phenylenediamine.

That the relationship of the chlorine atom to the amine groups on the benzene ring may be important for the carcinogenicity of the benzenediamines is further supported by the following examples. 4-Chloro-1,2-phenylenediamine was carcinogenic for both sexes of mice and rats producing rare bladder tumors in both sexes of rats. On the other hand, 4-chloro-1,3-phenylenediamine was carcinogenic only for male rats and female mice and adrenal tumors rather than bladder tumors were observed in male rats treated with this agent. 2-Chloro-1,4-phenylenediamine was completely inactive in either species tested.

Similar correlations were seen with methylated and nitrated benzenediamines. For example, 4-methyl-1,3-phenylenediamine was carcinogenic for several sites of rats of both sexes and female mice, but 2-methyl-1,4-phenylenediamine sulfate (XII) was inactive. Also, 2-nitro-1,4-phenylenediamine was carcinogenic for the liver of female mice while 4-nitro-1,2-phenylenediamine was devoid of carcinogenic activity.

While 1,4-phenylenediamine dihydrochloride (IX) was inactive in mice and rats, so were the monochloro and monomethyl analogs of the parent compound. Addition of a nitro group (2-nitro-1,4-phenylenediamine), however, elicited a carcinogenic response in the liver of female B6C3F1 mice.

Based on the available data, it can be concluded that ring-substituted 1,4-phenylenediamines tend to be noncarcinogens (four of six of the 1,4-phenylenediamines and related compounds were not carcinogenic). Also, there is one example (2,6-dichloro-1,4-phenylenediamine) in which twice as many electron-withdrawing groups (i.e., dichlorides) were needed on 1,4-phenylenediamine than on 1,2- or 1,3-phenylenediamines in order to obtain nearly comparable carcinogenic activities.

Conclusions

Sontag (27) suggested that "phenylenediamines appeared to be least active when the amine groups were *para* to one another, and gained activity as they became *ortho* to the substituted groups." Our evaluation, although limited by the number of compounds examined, is more extensive than Sontag's in that it includes several compounds not in his review, and it analyzes in detail the methods and results of the bioassays. Moreover, our evaluation partially supports Sontag's conclusion and indicates that 4-substituted 1,3-phenylenediamines are carcinogenic more often than ring-substituted 1,4-phenylenediamines. Therefore, in setting priorities for consideration of phenylenediamines for testing for carcinogenicity, the likelihood that ring-substituted 1,3-phenylenediamines will be carcinogenic in a long-term animal bioassay appears greater than that of ring-substituted 1,4-phenylenediamines. How-

ever, the type of added substituent and its position on the benzene ring also are important in predicting carcinogenic potential. Since sufficient information is not available presently to draw conclusions related to 1,2-phenylenediamines, these chemicals should be considered on a case by case basis.

A qualitative assessment of the potential for carcinogenicity based on structural changes should provide the basis of additional definitive quantitative structure-activity relationship studies based on relevant molecular descriptors and reactivity indicators. Such studies, however, were beyond the scope of this review.

This report has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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