

**EVIDENCE ON THE CARCINOGENICITY OF
4-AMINO-2-NITROPHENOL**

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

Draft

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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).

4-Amino-2-nitrophenol was assigned a final priority of ‘high’ carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on June 12, 1998. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced in the *California Regulatory Notice Register* on June 12, 1998.

This draft document *Evidence on the Carcinogenicity of 4-Amino-2-nitrophenol* was developed to provide the Committee with relevant information for use in its deliberations. It reviews the available scientific evidence on the carcinogenic potential of 4-amino-2-nitrophenol. A public meeting of the Committee to discuss this evidence is scheduled for December 1998. The exact meeting date will be published in the *California Regulatory Notice Register*. Written public comment on the document should be submitted to OEHHA by November 24, 1998, in order to be considered by the Committee in advance of the meeting. During the December meeting, the public will have an opportunity to present verbal comments to the Committee.

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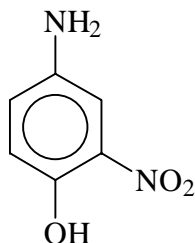
1 EXECUTIVE SUMMARY

4-Amino-2-nitrophenol ($C_6H_6N_2O_3$) has been used as an oxidation base in formulations of hair dyes. Technical grade 4-amino-2-nitrophenol induced tumors of the urinary bladder in male rats, and there is some evidence for a similar effect among female rats. Tumors of the urinary bladder are extremely rare among untreated rats. Preparations of 4-amino-2-nitrophenol have also demonstrated genotoxic potential in a number of short-term tests in bacterial and mammalian cells, *in vitro* and *in vivo*. There is some evidence that a purified preparation of 4-amino-2-nitrophenol loses its genotoxic potential, although this has only been investigated in a single study. In support of the concern for the carcinogenicity of 4-amino-2-nitrophenol itself, the purity of the test substance administered in the studies showing the development of bladder tumors was of relatively high purity, i.e., $99.6 \pm 0.3\%$. Furthermore, aromatic amines with structural homology to 4-amino-2-nitrophenol have shown evidence of carcinogenicity, some with the urinary bladder as the site of tumor development.

There is evidence for the carcinogenicity of *4-amino-2-nitrophenol*, with development of transitional cell carcinomas of the urinary bladder, a rare tumor, observed in male mice. The weight of evidence is supported by observations of a small number of the same rare tumor type in female mice, by mutagenicity in several short-term tests, and by chemical structural analogies with known carcinogens, including several bladder carcinogens.

2 INTRODUCTION

2.1 Identity of 4-Amino-2-nitrophenol



Molecular Formula: C₆H₆N₂O₃

Molecular Weight: 154.13

CAS Registry No.: 119-34-6

Synonyms: *o*-nitro-*p*-aminophenol; *p*-aminonitrophenol; 4-hydroxyl-3-nitroaniline; fourrine 57; fourrine brown; oxidation base 25; C.I. 76555

The International Agency for Research on Cancer described 4-amino-2-nitrophenol as dark red plates or needles with a melting point of 131°C (IARC, 1978).

2.2 Occurrence and Use

The primary use of 4-amino-2-nitrophenol is as a component of permanent or semi-permanent hair dye formulations, producing a wide range of colors depending on the concentrations applied (IARC, 1978). Its role in the development of permanent hair color is as an intermediate (sometimes also called a 'primary') undergoing oxidation (usually by hydrogen peroxide) to form a benzoquinone imine which then reacts with a 'coupler' in the preparation to form a colored compound within the hair (Iyer *et al.*, 1985). Aromatic amines such as 4-amino-2-nitrophenol have also been used in semi-permanent hair coloring preparations, in which case the colored compound itself permeates the hair and leaches out slowly over time because of its relatively small molecular size compared with that when covalently coupled (Cordle and Thompson, 1981). In addition to an intended use on human hair, 4-amino-2-nitrophenol has been used in fur-dyeing processes (NCI, 1978). 4-Amino-2-nitrophenol has also been used as an intermediate in the synthesis of other dyes and in the etching of copper printing plates (HSDB, 1997).

As of 1979, 4-amino-2-nitrophenol was reportedly not produced commercially in the U.S. (HSDB, 1997) and is not known to occur naturally (IARC, 1978). 4-Amino-2-nitrophenol has been listed as an ingredient in products in FDA's voluntary cosmetic

registration files as late as 1983 (Bronaugh and Congdon, 1984). [As of March 30, 1998, the FDA has suspended the Cosmetics Voluntary Registration Program in its entirety and cannot respond to technical inquiries regarding ingredient usage or product formulation.]

3 DATA ON 4-AMINO-2-NITROPHENOL CARCINOGENICITY

Two series of carcinogenicity studies of technical grade 4-amino-2-nitrophenol have been reported in which the compound was administered in the diet to rats or mice. Several additional studies of technical grade 4-amino-2-nitrophenol have been conducted in which the compound was administered by repeated dermal application to three different strains of mice and to rabbits. In a series of short-term experiments, technical grade 4-amino-2-nitrophenol was administered by repeated intraperitoneal injections to Strain A mice in two separate laboratories. Technical grade 4-amino-2-nitrophenol has also been tested in several *in vitro* and *in vivo* assays in bacterial and mammalian cells.

3.1 Epidemiological Studies of Carcinogenicity in Humans

No data on long-term effects of human exposure to 4-amino-2-nitrophenol were found in an earlier search by IARC (1978) or more recently by OEHHA.

3.2 Carcinogenicity Studies in Animals

In a series of animal carcinogenicity studies, both rats and mice received 4-amino-2-nitrophenol in the diet (NCI, 1978). A clear increase in transitional cell carcinomas of the urinary bladder was observed in treated male rats, and a small increase in the same tumor type was observed in female rats.

The NCI (1978) found as follows: "...under the conditions of the bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in three dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol. The test chemical was not carcinogenic for male or female B6C3F₁ mice at the doses tested."

No treatment related carcinogenic effect of technical grade 4-amino-2-nitrophenol was observed in repeated dermal exposure studies in female Swiss mice and rabbits (Stenbäck *et al.*, 1977) or in male and female DBAf and Strain A mice (Venitt and Searle, 1976; Searle, 1977; Searle and Jones, 1977). These studies were limited by size, inadequate dosing, or less-than-lifetime duration and thus may be of limited value for the determination of a carcinogenic effect. The studies in DBAf and Strain A mice were confounded by the presence of other compounds, since the test material applied was a hair dye mixture.

A series of Strain A mouse intraperitoneal injection studies with 4-amino-2-nitrophenol were also negative (Maronpot *et al.*, 1986). The study's authors noted that the Strain A assay was limited in its sensitivity to detect carcinogenic effects for certain types of carcinogens, particularly aromatic amines. This assay has also been considered relatively insensitive to bladder carcinogens (Stoner, 1991).

3.2.1 Oral Exposure Studies

Rat Dietary Exposure: NCI, 1978

Fischer rats (50/sex/dose) were fed diet containing 1250 or 2500 ppm 4-amino-2-nitrophenol for 103 weeks followed by a two week observation period during which the animals were maintained on control diet. Control groups of rats (20/sex) received untreated feed. The test chemical was obtained from Aldrich Chemical Company (Milwaukee, WI, Lot No. 100737) and was reported to be $99.6 \pm 0.3\%$ pure by ultraviolet, infrared, and nuclear magnetic resonance spectra. Reanalysis after the bioassay showed a purity of $98.7 \pm 0.2\%$. One impurity was identified by vapor-phase and thin-layer chromatography, but it was not characterized; water content was reported to be less than 0.2%. No compound-related effect on body weight, clinical signs, or survival was observed.

Transitional-cell carcinomas of the urinary bladder were found in 4-amino-2-nitrophenol treated rats of both sexes (Table 1). Among male rats, the incidence was significantly elevated relative to control animals and the trend was significantly dose-related. The first tumor in males was observed at 90 weeks and at 61 weeks in females (high-dose). In addition to the carcinomas, transitional-cell papillomas were observed in two high-dose male rats and transitional-cell hyperplasia was observed in four high-dose male rats. At the time of reporting of this study, no bladder tumors were observed in the 20 concurrent controls or among 220 male and 220 female rats used as control animals at the laboratory.

Table 1: Tumors in Fischer 344 rats receiving 0, 1250 or 2500 ppm in the diet for 103 weeks (NCI, 1978).

Tumor Site and Type		Dose, ppm ^{a, b}		
		0	1250	2500
<i>Males</i>				
Bladder	Transitional-cell carcinoma	0/15 ^c	0/46	11/39 ^d
<i>Females</i>				
Bladder	Transitional-cell carcinoma	0/15	1/43	2/44

^a Concentration of 4-amino-2-nitrophenol in feed.

^b Number of lesion-bearing animals/total examined microscopically at the termination of the study.

^c Dose-related trend was significant by the Cochran-Armitage trend test ($p < 0.001$).

^d Incidence relative to control group was significantly higher by the Fisher Exact test ($p=0.018$).

Mouse Dietary Exposure: NCI, 1978

B6C3F₁ mice (50/sex/dose) were fed diet containing 1250 or 2500 ppm 4-amino-2-nitrophenol for 103 weeks followed by a two week observation period. The test compound was the same as that used in the rat studies reported above. Control groups (20/sex) received untreated feed. No compound-related effects on survival were observed; mean body weights were slightly decreased among animals of both sexes. Because of little to no change in body weight and survival among the mice, NCI suggested that they may have been able to tolerate a higher dose.

No significant increases in tumor incidence were reported among treated mice of either sex. Among male mice, the incidence of alveolar/bronchiolar carcinomas was significantly decreased in treated animals (3/20 control, 0/49 low-dose, 0/48 high-dose), although when the incidence was combined with adenomas at the same site, the statistical significance disappeared.

3.2.2 Repeated Dermal Exposure Studies

Stenbäck et al., 1977

Female Swiss mice (50/group) and rabbits (5/group) were treated dermally twice weekly with 5 or 10% 4-amino-2-nitrophenol (Aldrich Chemical Co., Milwaukee, WI) in 0.02 ml acetone (Stenbäck et al., 1977). Mice were treated on shaved interscapular skin and rabbits on the inside of the left ear. Mice were treated until moribund or they died spontaneously, and rabbits until week 85. A negative control group (93 mice, 5 rabbits) and a positive control group receiving dimethylbenzanthracene (40 mice, 5 rabbits) were

also included. No 4-amino-2-nitrophenol treatment-related toxic effects were observed and no treatment-related increase in tumor incidence was observed among the animals. The lack of compound-related toxic effects suggests that the animals may have tolerated a higher dose. The size and duration of the rabbit study may have limited the ability to detect a carcinogenic response weaker than that of the dimethylbenzanthracene positive control.

Venitt and Searle, 1976; Searle, 1977; Searle and Jones, 1977

Male and female DBAf and A strain mice were treated dermally twice weekly with a tenfold dilution (with 50% aqueous acetone) of a semi-permanent hair dye solution containing CI Acid Black 107 (an azo-dye metal complex) and 4-amino-2-nitrophenol in a “detergent base containing perfume and other additives” (a Rimmel hair coloring shampoo called “Really Brown”; quantitative information on chemical composition was not presented) (Venitt and Searle, 1976; Searle, 1977; Searle and Jones, 1977). Strain A mice were treated with 0.4 ml per application, while DBAf mice were treated with 0.2 ml per application.

Eight of 32 strain A mice treated with the dye mix developed tumors versus 7 of 32 control mice after 80 weeks of observation. Three of 32 dye-treated DBAf mice developed tumors versus 1 of 32 control mice after 72 or 71 weeks of observation, respectively. While no significant increases in specific tumor types were observed, the time of onset of tumors in dye-treated animals was shorter than in control animals. The studies are of limited value for drawing conclusions regarding carcinogenicity because of poor characterization of the test material and the less-than-lifetime exposure to the test material.

3.2.3 Repeated Injection Exposure Studies

Maronpot et al., 1986

Strain A/St mice (10/sex/group) were injected intraperitoneally three times per week with 0, 31.25, 62.5 or 125 mg/kg_{bw} 4-amino-2-nitrophenol in 0.1 ml tricapylin for 8 weeks, then observed for lung tumors at 24 weeks (Maronpot *et al.*, 1986). Control groups consisted of animals injected with tricapylin, untreated animals and animals injected with 1000 mg urethane/kg_{bw} (positive control). No significant increase in tumors was observed among 4-amino-2-nitrophenol animals when compared to pooled control animals (Table 2).

Table 2: Lung tumors in male and female strain A/St mice treated with 4-amino-2-nitrophenol - University of California, San Diego (Maronpot *et al.*, 1986).

Dose (mg/kg)	Survivors		% with tumors		tumors/mouse	
	male	female	male	female	male	female
31.25	10/10	10/10	20	40*	0.20	0.50*
62.5	7/10	9/10	57*	33	0.57*	0.33
125	10/10	10/10	20	30	0.30	0.30
0 (tricaprylin)	54/60	54/60	13	11	0.167	0.110
0 (untreated)	119/120	79/80	2	8	0.017	0.076
0 (urethane – positive control)	47/50	47/50	96*	96*	11.9*	10.3*

* Significantly different from vehicle control (p < 0.05).

Results of a study conducted in a second laboratory were reported in the same paper. Male strain A/J mice (30/group, except high-dose group of 40) were injected three times per week for 8 weeks with 40, 100, or 200 mg 4-amino-2-nitrophenol/kg_{bw} in 0.1 ml. Control groups consisted of animals injected with corn oil, untreated animals and animals injected with 1000 mg urethane/kg_{bw} (positive control). Survival at 24 weeks, percent of survivors with tumors, and tumors per animal are reported in Table 3. No statistically significant increases in tumor incidence or tumors per animals were found relative to concurrent control animals.

Table 3: Lung tumors in male strain A/J mice treated with 4-amino-2-nitrophenol - Oak Ridge National Laboratory (Maronpot *et al.*, 1986).

Dose (mg/kg)	survivors	% with tumors	tumors/mouse
40	24/30	42	0.54
100	24/30	21	0.21
200	18/40	11	0.16
0 (corn oil)	16/30	31	0.31
0 (untreated)	14/20	21	0.36
0 (urethane -positive control)	17/20	100*	19.41*

* Significantly different from vehicle control (p < 0.05).

These studies of 4-amino-2-nitrophenol were part of a battery of 65 compounds tested which had previously been tested in NCI carcinogenicity tests and included 33 aromatic amines. Based upon the correlation with the NCI bioassay results, the authors suggested that the Strain A assay was “marginal” in its ability to detect carcinogenic aromatic amines. The limited exposure duration (8 weeks) may have also compromised the ability of these studies to detect a carcinogenic effect of 4-amino-2-nitrophenol.

Thus, 4-amino-2-nitrophenol has been shown to induce tumors of the urinary bladder in male mice and a small number of the same rare tumor type in treated female mice. Other studies showing no carcinogenic effect, with the exception of the NTP studies in rats, were compromised by short study duration, inadequate dosing, the application of compound mixtures, or assay limitations for detecting effects of aromatic amines, such as 4-amino-2-nitrophenol.

3.3 Other Relevant Data

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

3.3.1 Genetic Toxicology

Bacterial Assays

4-Amino-2-nitrophenol has been tested in multiple experiments for its ability to induce reverse mutations in *Salmonella typhimurium* using different samples or sources of the test compound. An overview of the results suggests that purity is a concern for the genotoxicity of the compound. In the tests described below, the information regarding the purity of the test compound has been included when available. Test material obtained from the NCI Chemical Repository was considered to be the same as that used in NCI's 1978 bioassay in rats and mice (obtained from Aldrich Chemical Company). Positive results in *S. typhimurium* strains TA1535 and TA100 are considered indicative of base-pair substitutions, while strains TA1537, TA1538, and TA98 indicate frameshift mutation.

4-Amino-2-nitrophenol was reported to be mutagenic only upon metabolic activation in strain TA1538 (Venitt and Searle, 1976). A test in strain TA1535 was negative. Similar results were obtained in a test of a hair dye product containing 4-amino-2-nitrophenol plus a metal-azo-dye complex.

In a second study with 4-amino-2-nitrophenol was found to be weakly mutagenic of *S. typhimurium* strain TA1538 only with the addition of S9 fraction for metabolic activation (Garner and Nutman, 1977). The compound was obtained from Aldrich Chemical Company.

4-Amino-2-nitrophenol was found to be mutagenic with a dose-response in *S. typhimurium* strains TA98, TA100, TA1537 and TA1538 using the same batch of the chemical used in the 1978 NCI bioassays (Dunkel and Simmon, 1980). In the presence of uninduced rat liver S-9 mix the mutagenic response was similar in each of the *S. typhimurium* strains; however, with the addition of induced rat liver S-9 mix, no mutagenic response was observed in TA98, TA100, and TA1537 and a 'greatly reduced' response was observed in TA1538.

In another study, Shahin *et al.* compared the mutagenicity in *S. typhimurium* of technical grade 4-amino-2-nitrophenol (obtained from Aldrich-Europe, a division of Janssen Pharmaceutica, Beerse, Belgium; Lot No. 032097) with that of a sample synthesized in their laboratory assay (Shahin *et al.*, 1982). It was found that the laboratory synthesized and purified sample was non-mutagenic in strains TA98, TA100, TA1535, TA1537, and TA1538 with either an Aroclor 1254-induced or an uninduced metabolic activation system, or in the absence of metabolic activation. In the same battery of strains, technical grade 4-amino-2-nitrophenol was found to be mutagenic in TA98 and TA1538 both with and without metabolic activation by an Aroclor 1254-induced system. A weak response was observed in strains TA100 and TA1537. Removal of toluene-insoluble contaminants from the technical grade sample reduced the mutagenicity. Further purification with silica gel column chromatography eliminated mutagenic activity in all strains. The toluene-insoluble contaminants were found to be mutagenic. The technical grade and laboratory-prepared samples were distinguishable by high-performance liquid and thin-layer chromatography, but not by nuclear magnetic resonance, ultraviolet, or infrared spectra. The impurities in the sample were not characterized.

Using 99% pure 4-amino-2-nitrophenol obtained from Aldrich Chemical Company, mutagenicity was induced by approximately three-fold over background in *S. typhimurium* strain TA98 (Zeiger *et al.*, 1987). The addition of Aroclor 1254-induced hamster liver homogenate resulted in a weak mutagenic effect while the addition of a similar homogenate from rat liver showed no mutagenic activity. Tests in strains TA100, TA1535, and TA97 were negative, both with and without the addition of homogenates from either rat or hamster.

4-Amino-2-nitrophenol was tested in a modified *Salmonella* mutagenesis assay in strain TA98 (Mersch-Sundermann and Krämer, 1993). The compound was obtained from Aldrich (Lot No. 12,721-4). The change in protocol involved a change in incubation time from 16 hours to 12 hours followed by a 2-hour short-term subculture calibrated to 10^8 colony forming units per plate. This modification resulted in an increase from 0.008 to 0.017 revertants per nanomole (113% increase).

Mammalian Cell Assays (in vitro)

4-Amino-2-nitrophenol was evaluated for a mutagenic response in two laboratories in the mouse lymphoma cell line L5178Y TK^{+/−} forward mutation assay both with and without the addition of a metabolic activation system (Mitchell *et al.*, 1988). All tested

concentrations (6, 15, and 16 µg/ml) of 4-amino-2-nitrophenol were mutagenic. The addition of Aroclor-induced liver homogenate (S9) increased the activity several fold. Another testing demonstrated the mutagenicity of 4-amino-2-nitrophenol at concentrations greater than 10-15 µg/ml with an approximately 12-fold induction of activity (Myhr and Caspary, 1988). In this testing, however, the addition of S9 homogenate reduced the mutagenic activity with the peak activity rising to only 4-fold over controls.

In one of two laboratories conducting a transformation assay in the mouse embryo cell line C3H/10T1/2 (clone 8), 4-amino-2-nitrophenol (obtained from the NCI Chemical Repository) was found to induce a weak morphological transformation response at doses (0.08-2.0 µg/ml) which were not cytotoxic (Dunkel *et al.*, 1988). Although the laboratory reporting the negative result did not test the compound up to doses which resulted in 10-20% cytotoxicity, no positive findings were found at doses near those which produced a positive result in the other testing laboratory (0.1-1.0 µg/ml). Cells were exposed to the test compound for 24 hours, followed by staining and examination 8-10 days later.

A survival assay was performed evaluating the conferral of anchorage-independent growth of Rauscher leukemia virus-infected rat embryo cells following treatment with 4-amino-2-nitrophenol (Traul *et al.*, 1981). The addition of doses of 11 or 17 µg 4-amino-2-nitrophenol per 5.2×10^4 cells resulted in a >250% increase in anchorage-independent growth (considered by the authors to be a positive result). The test compound was obtained from the NCI Chemical Repository.

In a DNA repair assay in primary cultures of Fischer rat hepatocytes, the same preparation of 4-amino-2-nitrophenol used in the 1978 NCI bioassay failed to elicit a significant response as measured by increased DNA synthesis (repair) (Williams *et al.*, 1982; Williams *et al.*, 1989).

Mammalian Cell Assays (in vivo)

Male Swiss mice (10/group) were intraperitoneally administered 0, 50, 100, 200, 250, 500, or 1000 mg/kg_{bw} 4-amino-2-nitrophenol in water twice over a span of 24 hours (Misra, 1992). The test compound was reported to be 87% pure and was obtained from local sources. The highest dose tested was found to be lethal to the mice and all doses tested showed some degree of cytotoxicity as measured by deviation from the ratio of polychromatic to normochromatic erythrocytes. Percent of normochromatic and polychromatic erythrocytes with micronuclei was significantly elevated among the treated animals and showed a clear dose-response.

Twenty male Charles River CD rats were administered 20 mg/kg 4-amino-2-nitrophenol ('commercially available') by intraperitoneal injection 3 times weekly for 8 weeks (Burnett *et al.*, 1977). Forty control animals received injections of saline solution. The rats were then mated with female CD rats for 5 days. After 17 days, the female rats were examined for dominant lethal effects. No significant differences were observed in total

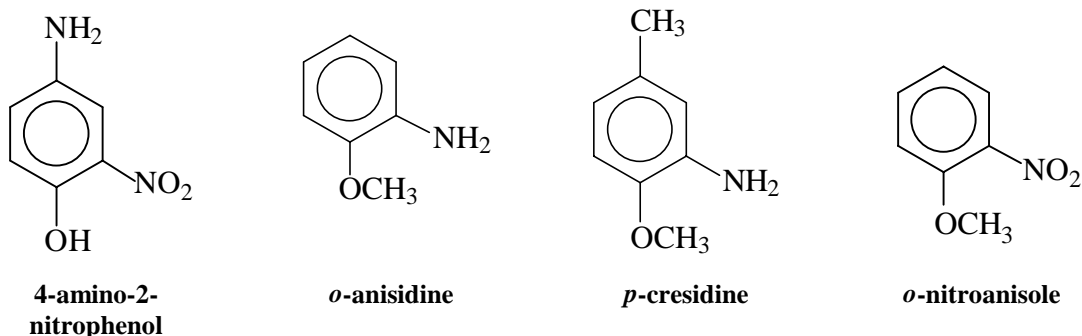
live fetuses, percent of litters with resorptions, mean resorptions per pregnancy, or percent resorptions per litter.

Overall, data on the genetic toxicity of technical grade preparations of 4-amino-2-nitrophenol suggest that it has the potential to cause mutational changes in bacterial cells, transformational changes in mammalian cells *in vitro*, and micronuclei in mice *in vivo*.

3.3.2 Structure-Activity Comparisons

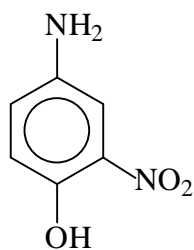
A number of other substituted anilines and nitrobenzenes have showed the potential to cause tumors in experimental animals.

NTP has reported carcinogenic effects on the urinary bladder from exposure of experimental animals to several aromatic amines structurally related to 4-amino-2-nitrophenol, including *o*-nitroanisole*, *o*-anisidine*, and *p*-cresidine* (see structures below). Other aromatic amines showing carcinogenic effects on the urinary bladder of experimental animals in NTP bioassays are 4-chloro-*o*-phenylenediamine*, *m*-cresidine, and *o*-toluidine*.

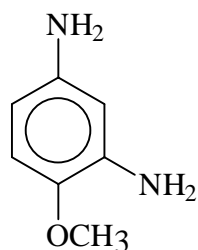


Hair dye components in the substitute aniline and nitrobenzene chemical classes which have shown carcinogenic potential or activity in experimental animals include *o*-phenylenediamine* (2-aminoaniline), 2,4-diaminotoluene* (3-amino-4-methylaniline), 4-chloro-*m*-phenylenediamine, 2,4-diaminoanisole* (4-methoxy-*m*-phenylenediamine), *o*-anisidine*, and 2-nitro-4-aminoaniline (reviewed in Van Duuren, 1980). Other compounds in these chemical classes with carcinogenic potential in experimental animals include *o*-toluidine*, 4-chloro-*o*-toluidine*, 5-nitro-*o*-anisidine*, 2,4,5-trimethylaniline*, 2,4,6-trimethylaniline, and 4-chloro-*o*-phenylenediamine*. Several of the compounds more closely related to 4-amino-2-nitrophenol with *meta*-oriented amine and/or amine generating (nitro) groups are shown below (also see metabolism section).

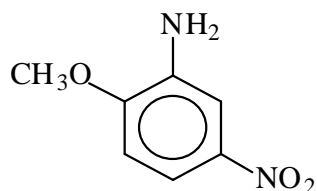
* On Proposition 65 list of chemicals known to the State to cause cancer (as of May 15, 1998).



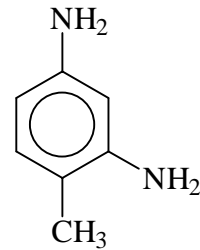
4-amino-2-nitrophenol



2,4-diaminoanisole



5-nitro-*o*-anisidine



2,4-diaminotoluene

A computerized analysis of structure-activity relationships based on a set of rules generated by US EPA experts (OncoLogic[®], 1994) predicts that “an aromatic compound containing one benzene ring, two amino groups [a metabolic derivative of the nitro group] and one hydroxyl group has a carcinogenicity concern of MODERATE.”

3.3.3 Pharmacokinetics and Metabolism

Using three human subjects, the percutaneous penetration of ¹⁴C-labelled 4-amino-2-nitrophenol spiked hair dye (containing 0.433% 4-amino-2-nitrophenol) was examined under normal use conditions as a non-oxidation (semipermanent) product (Wolfram and Maibach, 1985). Among the subjects, the total dose excretion was 0.235% with a half time for urinary excretion of 10 hours.

The percutaneous absorption of hair dyes was also examined using excised human skin (Bronaugh and Congdon, 1984). Using human abdominal skin, the stratum corneum:water partition coefficient was determined to be 13.0 with a permeability constant of 0.0028 cm/hr in water (a lower value of 8.6×10^{-5} cm/hr was obtained when the compound was ionized in pH 9.7 borate buffer; pKa = 7.8).

Little information is available regarding the metabolism of 4-amino-2-nitrophenol specifically. The compound contains three functional groups (amino-, nitro-, and hydroxy-groups) on the aromatic ring, which would determine which metabolic pathway the compound is likely to follow. Nitro- groups may undergo reduction to amino-groups via nitro reductase. The amino-group already present or the one reduced from the nitro-group may undergo N-acetylation or N-hydroxylation, which may then, in turn, produce electrophilic intermediates by bioactivation. Such intermediates have the potential to interact with cellular nucleophilic components (e.g. DNA) leading to mutagenesis.

As a part of its coupling reaction to form a colored compound in hair, 4-amino-2-nitrophenol is able to form a benzoquinone imine intermediate in the presence of hydrogen peroxide. It is also possible that such a reactive benzoquinone imine compound may be formed *in vivo* by metabolic oxidation processes.

The functional groups on 4-amino-2-nitrophenol are potential sites of acetyl-, glucuronide- or sulfate- conjugation, which could result in an excretable metabolite. 4-

Amino-2-nitrophenol has been identified as a plausible minor secondary metabolite of 1,3-dinitrobenzene (Rickert, 1987).

3.3.4 Pathology

The tumors observed in the studies in the rat bladders were considered by the authors (NCI, 1978) to meet standard criteria for transitional-cell carcinomas of the urinary bladder. The tumors were described as varying “from papillary structures packed with hyperchromatic epithelial cells, pleomorphic nuclei, and mitotic figures to subepithelial, dome-shaped solid masses of similar tumor cells. The masses often protruded into the bladder lumen. There was invasion of the bladder wall, and in one case metastases appeared in the lungs.” A NCI pathologist speaking at the bioassay review meeting (3/26/78) stated that bladder calculi were not noted by the testing laboratories’ pathologists and that bladder parasites generally had not been found in rats used in bioassays.

3.4 Mechanism

Based on several results in tests *in vivo* and *in vitro*, technical grade 4-amino-2-nitrophenol appears to be genotoxic, probably after metabolic activation. A genotoxic mechanism may therefore be responsible for the observed carcinogenic effect. There were no indications that a chronic inflammation effect in the urinary tract of treated animals may have contributed to the development of bladder tumors.

4 SUMMARY AND CONCLUSIONS

4.1 Summary of Evidence

Technical grade 4-amino-2-nitrophenol clearly induced tumors of the urinary bladder in male mice. A small number of the same rare tumor type was also observed in treated female mice, and is suggestive of a compound-related effect. Other studies showing no carcinogenic effect, with the exception of the NTP studies in rats, were compromised by short study duration, inadequate dosing, the application of compound mixtures, or assay limitations for detecting effects of aromatic amines, such as 4-amino-2-nitrophenol. Data on the genetic toxicity of technical grade preparations of 4-amino-2-nitrophenol suggest that it has the potential to cause mutational changes in bacterial cells, transformational changes in mammalian cells *in vitro*, and micronuclei in mice *in vivo*. 4-Amino-2-nitrophenol also shows structural homology to other aromatic amines which are not only carcinogenic, but which have led to the induction of bladder tumors in experimental animals.

4.2 Conclusion

There is evidence for the carcinogenicity of 4-amino-2-nitrophenol, with development of transitional cell carcinomas of the urinary bladder, a rare tumor, observed in male mice. The weight of evidence is supported by observations of a small number of the same rare tumor type in female mice in the same bioassay series, by mutagenicity in several short-term tests, and by chemical structural analogies with known carcinogens, including several bladder carcinogens.

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