EVIDENCE ON THE CARCINOGENICITY OF

1-CHLORO-4-NITROBENZENE

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

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by

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PREFACE

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

1-Chloro-4-nitrobenzene 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*, also on June 12, 1998. No information was received as a result of this request.

This draft document *Evidence on the Carcinogenicity of 1-Chloro-4-Nitrobenzene* 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 1-chloro-4-nitrobenzene. A public meeting of the Committee to discuss this evidence is scheduled for October 7, 1999. At this meeting it is expected that the Committee will render an opinion on whether 1-chloro-4-nitrobenzene has been clearly shown to cause cancer. Written public comment on the document should be submitted to OEHHA by September 14, 1999, in order to be considered by the Committee in advance of the meeting. During the October 1999 meeting, the public will have an opportunity to present verbal comments to the Committee.

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1-Chloro-4-nitrobenzene is used as an intermediate in the synthesis of certain drugs, dyes, pesticides and other substances in commerce. It is not known to occur naturally. Administration of 1-chloro-4-nitrobenzene in feed to rats did not produce tumors, but administration in feed to mice produced vascular tumors (hemangiomas or hemangiosarcomas) in both males and females. It also produced hepatocellular tumors in male mice at the low dose but not at the high dose. 1-Chloro-4-nitrobenzene produced mutations in some, but not all, tests using the *Salmonella typhimurium* mutagenesis assay. In mammalian cells it produced sister chromatid exchanges and chromosomal aberrations *in vitro* and DNA strand breaks *in vitro* and *in vivo*. One of the metabolites of 1-chloro-4-nitrobenzene in rabbits, rats and humans is the known carcinogen 4-chloroaniline.

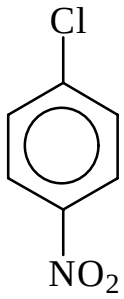
There is evidence for the carcinogenicity of *1-chloro-4-nitrobenzene*, based on observations of vascular tumors in male and female mice. Further evidence of carcinogenic potential is provided by observations of liver tumors at the lower of two doses in a study in male mice, chromosomal effects *in vitro*, DNA strand breaks *in vitro* and *in vivo*, and metabolism to a known carcinogen.

2 INTRODUCTION

2.1 Identity of 1-Chloro-4-Nitrobenzene

1-Chloro-4-nitrobenzene is a crystalline yellow solid at room temperature with a sweet odor (HSDB, 1997) and is slightly soluble in water (243 mg/L at 20°C). Its chemical structure and physical properties are shown below.

Molecular Formula:	$C_6H_4ClNO_2$
Molecular Weight:	157.56
CAS Reg. No.:	100-00-5
Chemical Class:	nitroaromatic
Melting Point:	82.6°C
Boiling Point:	242°C
Vapor Pressure:	0.15 mm Hg (at 30°C)



Synonyms: *para*-chloronitrobenzene, 4-chloro-1-nitrobenzene, 4-nitrochlorobenzene, *para*-nitrochlorobenzene, 1-nitro-4-chlorobenzene, 4-nitro-1-chlorobenzene.

2.2 Occurrence and Use

1-Chloro-4-nitrobenzene is produced and used in the chemical industry and is not known to occur naturally. It is used in the synthesis of industrial chemicals (*e.g.*, *para*-nitrophenol, *para*-nitroaniline, *para*-aminophenol, 4-nitroanisole, and *para*-anisidine), pesticides (*e.g.*, parathion, methyl parathion, ethyl parathion and nitrophen), the analgesic drugs phenacetin and acetaminophen, and the antimicrobial drug dapsone, which is used to treat leprosy among other conditions. 1-Chloro-4-nitrobenzene is also used in the synthesis of 4-nitrodiphenylamine-based antioxidants for rubber (HSDB, 1997).

1-Chloro-4-nitrobenzene is produced at two sites in the U.S. One is located in Deepwater, New Jersey and the other in Sauget, Illinois (HSDB, 1997). Total production in 1994 was estimated to be approximately 145 million pounds (SRI International, 1994). Occupational exposure may occur through the use of 1-chloro-4-nitrobenzene in the manufacture of other chemical products; exposure to the general population may occur through environmental contamination (Travlos *et al*., 1996).

3 DATA ON 1-CHLORO-4-NITROBENZENE CARCINOGENICITY

1-Chloro-4-nitrobenzene has been tested for carcinogenicity in rats and mice of both sexes. It has also been tested for mutagenicity in bacteria and in *Drosophila* and has been tested for clastogenic activity in mammalian cells both *in vitro* and *in vivo*.

3.1 Epidemiological Studies of Carcinogenicity in Humans

No data on long-term effects of human exposure to 1-chloro-4-nitrobenzene were found in an earlier search by the International Agency for Research on Cancer (IARC, 1996) or more recently by OEHHA.

3.2 Carcinogenicity Studies in Animals

1-Chloro-4-nitrobenzene was tested for carcinogenicity in male rats and in male and female mice in bioassays performed under contract with the National Cancer Institute (Weisburger *et al.,* 1978). As noted below, the extent of information published in this report does not meet current standards for carcinogenicity bioassays. Nonetheless, the report documents a statistically significant increase in the incidence of vascular tumors in treated male and female mice. Another series of bioassays conducted in male and female Sprague-Dawley rats (Schroeder and Daly, 1984), which did not find effects, employed much lower doses of 1-chloro-4-nitrobenzene than those used by Weisburger *et al*.

3.2.1. Oral Exposure Studies

Rat Dietary Exposure: Weisburger et al., 1978

Groups of 25 male Charles River CD rats (derived from Sprague-Dawley rats) were administered 1-chloro-4-nitrobenzene mixed with feed for 18 months. For the first three months, the concentration in feed was 2,000 ppm for the low-dose group and 4,000 ppm for the high-dose group. For the next two months these concentrations were reduced to 250 and 500 ppm, respectively, because signs of toxicity were evident in the treated animals. The concentrations in feed during months six through 18 were 500 and 1,000 ppm for the low- and high-dose groups, respectively. Animals were observed without further exposure for an additional six months before they were killed and examined for tumors. The average daily dose of 1-chloro-4-nitrobenzene for the low- and high-dose group was, respectively, approximately 17 and 33 mg per kg body weight. A group of 25 control rats (simultaneous controls) received the standard laboratory diet throughout the experiment. Animals in this control group, together with animals in control groups used for bioassays of other chemicals that were conducted in the same laboratory during an overlapping period, comprised a pooled control group.

Animals that died during the first six months were excluded from the statistical evaluation of the carcinogenicity. Animals that died more than six months after the start were subjected to necropsy and all tumors noted were examined microscopically. Histopathological examination also included lung, liver, spleen, kidney, adrenal, heart, bladder, stomach, intestines, testes and pituitary. No increases in tumor incidence in groups given 1-chloro-4-nitrobenzene were reported.

Rat Gavage Exposure: Schroeder and Daly, 1984

Groups of 60 male and 60 female Sprague-Dawley rats were given 1-chloro-4-nitrobenzene in corn oil (5 ml/kg_{bw}) by gavage five days/week for 24 months. Control,

low-, mid-, and high-dose groups were calculated to receive 0, 0.1, 0.7, and 5.0 mg/kg-d 1-chloro-4-nitrobenzene in corn oil, respectively. All surviving animals were killed after 24 months and examined for tumors and other pathological changes. Methemoglobinemia was noted in mid- and high-dose groups of both sexes. Hemosiderin accumulation in the spleen and anemia were found in high-dose groups of both sexes. No increases in tumor incidence were observed in treated females. In males the incidences of interstitial cell tumors of the testes in the control, low-, mid-, and high-dose groups were, respectively, 1/60, 1/60, 4/59, 5/60, and 6/60. These increases are not statistically significant, but the increase in the high-dose group is nearly statistically significant when compared to the incidence in controls ($p=0.057$ by Fisher's exact test). The trend of increasing incidence with increasing dose is significant ($p<0.01$). The incidences in groups of treated males are within the range of incidences of interstitial cell tumors of the testes observed in historical control groups of Sprague-Dawley rats at the testing laboratory. (Data from 14 bioassays: range = 3.4% - 23.4%, mean = 9.8%). The unusually low incidence of interstitial cell tumors in the control group does not appear to be related to survival, which was similar in all groups of male rats.

It is notable that the dose rates used in the Weisburger *et al*. (1978) rat studies were approximately 3- and 7-fold greater than the highest dose rates used in the Schroeder and Daly (1984) studies.

Mouse Dietary Exposure: Weisburger et al. 1978

Groups of 25 male and 25 female HaM/ICR CD-1 mice were administered 1-chloro-4-nitrobenzene mixed with feed at concentrations of 0 ppm (simultaneous control groups), 3,000 ppm (low-dose groups) or 6,000 ppm (high-dose groups) for 18 months. Animals were then observed without further exposure for an additional three months before they were killed and examined for tumors. Animals in control groups for 1-chloro-4-nitrobenzene, together with mice in control groups used for bioassays of other chemicals that were conducted in the same laboratory during an overlapping period, comprised a pooled control group.

Animals that died during the first six months were excluded from the statistical evaluation of the carcinogenicity. Animals that died more than six months after the start were subjected to necropsy and tumors noted were examined microscopically. Histopathological examination also included lung, liver, spleen, kidney, adrenal, heart, bladder, stomach, intestines, and reproductive organs. The incidences of vascular tumors (hemangiomas or hemangiosarcomas) at all sites in simultaneous control, low-dose and high-dose groups of males were 0/14, 2/14 and 4/14, respectively, and in these groups of females were 0/15, 2/20 and 7/18 (Table 1). In pooled controls, the incidences of vascular tumors were 5/99 in males and 9/102 in females. The incidence in high-dose males was significantly increased above both the incidence in simultaneous controls ($p=0.049$) and the incidence in pooled controls ($p=0.013$). The incidence in high-dose females was also significantly increased above the incidence in simultaneous controls ($p=0.007$) and the incidence in pooled controls ($p=0.003$). In male mice, the incidence of hepatocellular carcinomas (Table 1) in low-dose males was significantly higher than the incidence in pooled controls ($p=0.03$) but the increase did not reach statistical

significance when compared to simultaneous controls (p=0.16). No hepatocellular carcinomas were observed in the high-dose males.

Table 1: Tumor incidence in HaM/ICR mice administered 1-chloro-4-nitrobenzene at concentrations of 3,000 or 6,000 ppm in feed for 18 months (Weisburger *et al.*, 1978).

Tumor Type	Dose (ppm in feed)			
	0[a]	0[b]	3,000	6,000
Males				
Hepatocellular carcinomas	1/14[c] *(7%)*[d]	7/99 *(7%)*	4/14[e] *(29%)*	0/14 *(0%)*
Vascular tumors	0/14 *(0%)*	5/99 *(5%)*	2/14 *(14%)*	4/14[f] *(29%)*
Females				
Vascular tumors	0/15 *(0%)*	9/102 *(9%)*	3/20 *(15%)*	7/18 [e] *(39%)*

[a] Simultaneous control group
[b] Pooled control group
[c] Number of lesion-bearing animals/total examined.
[d] Percentage of animals examined with lesions.
[e] P=0.16 and p=0.03 calculated for comparison to incidences in simultaneous controls and pooled controls, respectively, using Fisher's exact test.
[f] P=0.049 and p=0.013 calculated for comparison to incidences in simultaneous controls and pooled controls, respectively, using Fisher's exact test.
[g] P=0.007 and p=0.003 calculated for comparison to incidences in simultaneous controls and pooled controls, respectively, using Fisher's exact test.

In reviewing these studies, the IARC Working Group (IARC, 1996) noted that the number of animals in each dose group was small, and the histopathological evaluation and reporting was limited. Nevertheless, the number of animals per group was large enough to observe a statistically significant tumorigenic response in both male and female mice, and the limited histopathological evaluation was able to detect vascular tumors of the lung, liver and spleen, the organs where hemangiomas and hemangiosarcomas most commonly occur in mice.

3.3 Other Relevant Data

Additional information that may be relevant to the possible carcinogenicity of 1-chloro-4-nitrobenzene is reviewed below. This includes studies of genetic toxicity, uptake, metabolism, excretion, and structure-activity comparisons.

3.3.1 Genetic Toxicology

When tested in the *Salmonella typhimurium* mutagenesis assay using strains TA100 and TA1535, 1-chloro-4-nitrobenzene was positive in the presence of metabolic activation. These *Salmonella* strains detect base pair substitution mutations. In the absence of metabolic activation, 1-chloro-4-nitrobenzene did not produce a response in strain TA100 and was weakly positive in strain TA1535 (NTP, 1993). 1-Chloro-4-nitrobenzene did not produce a significant increase in the number of mutations when tested using strains TA1537, TA1538 or TA98, which detect frameshift mutations (Gilbert *et al*., 1980; Haworth *et al*., 1983; NTP, 1993; Shimizu *et al*., 1983; Suzuki *et al*., 1983; 1987), or in the *Drosophila melanogaster* recessive lethal mutation test (Zimmering *et al*., 1985; 1989).

1-Chloro-4-nitrobenzene produced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary (CHO) cells (Galloway *et al*., 1987; NTP, 1993). It produced single-strand DNA breaks in cultured rat hepatocytes, and when injected intraperitoneally (*i.p.*) in male Swiss mice it produced single-strand DNA breaks in liver, kidney and brain (Cesarone *et al*., 1983; 1984).

3.3.2 Structure-Activity Comparisons

Studies in both humans and rats show that a major pathway for metabolism of 1-chloro-4-nitrobenzene begins with reduction to 4-chloroaniline (*para*-chloroaniline; see Section 3.3.3), which is on the Proposition 65 list of chemicals known to the state of California to cause cancer. IARC (1993) evaluated 4-chloroaniline for possible carcinogenicity and classified it in group 2B on the basis of sufficient evidence of carcinogenicity in animals and inadequate evidence in humans.

The tumorigenic activity of 4-chloroaniline administered to mice and rats was similar in some respects to that of 1-chloro-4-nitrobenzene. In multiple studies, 4-chloroaniline also produced hemangiosarcomas in male and female mice in different organs. It also produced hepatocellular adenomas and carcinomas in male mice and sarcomas of the spleen and splenic capsule in male rats. Thus, the structurally similar compounds 1-chloro-4-nitrobenzene and 4-chloroaniline produced similar carcinogenic responses in male and female mice but not in male rats.

There are also some similarities in the effects of 4-chloroaniline and 1-chloro-4-nitrobenzene in tests for genotoxicity. The IARC (1993) summary of genetic and related effects of 4-chloroaniline states: "*para*-Chloroaniline preferentially killed the Pol A^- strain in the *Escherichia coli* Pol A^-/Pol A^+ assay, both in the presence and the absence of an exogenous metabolic system. It was not mutagenic to *Salmonella typhimurium*, except for strain TA98, for which conflicting data were obtained. It produced mutations in *Aspergillus nidulans* and in mouse lymphoma L5178Y cells at the *tk* locus, but did not induce mitotic recombination in *Saccharomyces cerevisiae*. *para*-Chlorobenzene transformed primary cultures of Syrian hamster embryo cells, only in the later of two studies in the same laboratory. It induced sister chromatic exchange and chromosomal aberrations in Chinese hamster ovary cells *in vitro*." For those tests performed on both 1-

chloro-4-nitrobenzene and 4-chloroaniline, both chemicals produced a positive response in assays for sister chromatid exchange and chromosomal aberrations in CHO cells. The differing responses in *Salmonella typhimurium* strains suggest that there may be a pathway in this bacterium for metabolism of 1-chloro-4-nitrobenzene that does not produce 4-chloroaniline.

3.3.3 Pharmacokinetics and Metabolism

Dermal uptake and excretion were studied in male Fischer rats using radioactive 1-chloro-4-nitrobenzene. During 72 hours following application, 43-45% of the applied radioactivity was detected in urine and 5-12% in feces (NTP, 1993; Nomeir *et al*., 1992). Thus, dermal absorption was at least 48% in this study.

Following oral administration of radioactive 1-chloro-4-nitrobenzene to male Fischer rats, 73%-78% was absorbed. The fraction of administered radioactive substance remaining in body tissues 24 hours and 72 hours after uptake was 23% and 5% respectively. The highest concentration was in fat followed by red blood cells, kidney, liver and spleen (NTP, 1993). This study shows that 1-chloro-4-nitrobenzene or its metabolites are readily eliminated from rats with a half-life that appears to be less than 24 hours.

Urinary metabolites of 1-chloro-4-nitrobenzene in male Sprague-Dawley rats following *i.p*. injection were investigated using high-performance liquid chromatography (Yoshida *et al*., 1991). The metabolites identified were 4-chloroaniline, 2,4-dichloroaniline, 4-nitrothiophenol, 2-chloro-5-nitrophenol, 2-amino-5-chlorophenol, 4-chloroformanilide, 4-chloro-2-hydroxyacetanilide and 4-chloroacetanilide.

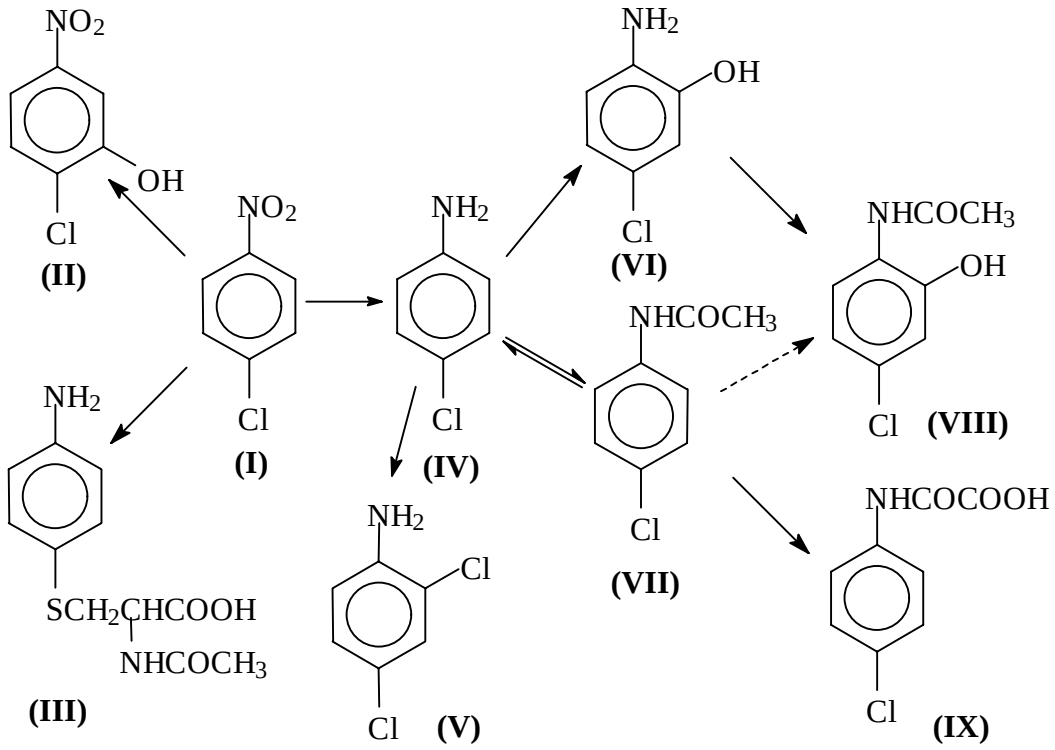
Bray *et al*. (1956) reported that nearly all 1-chloro-4-nitrobenzene administered to female rabbits (route and dose unspecified) was excreted as metabolites in urine. Approximately 40% of the administered dose was excreted as sulfate- or glucuronide-conjugated phenol metabolites and approximately 10% as 4-chloroaniline.

Rickert and Held (1990) reported that during incubation of hepatocytes from male Fischer rats with 4-chloro-1-[^{14}C]nitrobenzene, 15.4% of the amount initially present was metabolized to 4-chloroaniline, 16.3% to 4-chloroacetaniline and 10.4% to S-(4-nitrophenyl)glutathione. Incubation of hepatocyte microsomes with 4-chloro-1-[^{14}C]nitrobenzene resulted in the formation of 4-chloroaniline. However, in the presence of the cytochrome P-450 inhibitors SKF 525-A, metyrapone or carbon monoxide, production of 4-chloroaniline was inhibited, implicating a role for cytochrome P-450 in the reduction of the nitro group (Rickert and Held, 1990).

Following accidental poisoning of eight workers exposed to 1-chloro-4-nitrobenzene primarily by inhalation, Yoshida *et al.* (1993) were unable to detect 1-chloro-4-nitrobenzene in the workers' urine but were able to detect several other urinary metabolites of this compound (see Fig. 1 below). The mercapturic acid N-acetyl-S-(4-nitrophenyl)-L-cysteine (III) was the major metabolite (48% of urinary metabolites), indicating that a major route of metabolism in humans involves conjugation with

glutathione. Another 29.9% of the urinary metabolites were comprised of 4-chloroacetanilide (VII) and 4-chloro-oxanilic acid (IX), indicating that reduction of 1-chloro-4-nitrobenzene to 4-chloroaniline, followed by acetylation, is a second major pathway for formation of urinary metabolites of 1-chloro-4-nitrobenzene. An additional 8.7% of urinary metabolites was comprised of 4-chloro-2-hydroxyacetanilide (VIII), suggesting that some 4-chloroacetanilide may be further metabolized by hydroxylation, although it was not clear whether this compound originated from 4-chloroacetanilide or 2-amino-5-chlorophenol (VI) (as noted in IARC, 1996). A small amount of 2,4-dichloroaniline (V) (1.2% of total metabolites) was detected; indicating that some 4-chloroaniline is further metabolized by chlorination. Finally, 12.2% of urinary metabolites was comprised of 2-chloro-5-nitrophenol (II), indicating that hydroxylation at the 3 position of the benzene ring is a third pathway for metabolism of 1-chloro-4-nitrobenzene.

Figure 1. Metabolic pathway of 1-chloro-4-nitrobenzene (adapted from IARC, 1996).



(I) 1-chloro-4-nitrobenzene; **(II)** 2-chloro-5-nitrophenol; **(III)** N-acetyl-S-(4-nitrophenyl)-L-cysteine; **(IV)** 4-chloroaniline; **(V)** 2,4-dichloroaniline; **(VI)** 2-amino-5-chlorophenol; **(VII)** 4-chloroacetanilide; **(VIII)** 4-chloro-2-hydroxyacetanilide; **(IX)** 4-chloro-oxanilic acid.

From the above studies of metabolites identified in urine, it appears that in both rats and humans the major metabolic pathways for 1-chloro-4-nitrobenzene involve conjugation with glutathione, reduction of the nitro group, or hydroxylation of the benzene ring at the 3 position. From these limited data, it appears that there are quantitative differences in the relative activities of these pathways, with conjugation being the main pathway in

humans and reduction of the nitro group being the main pathway in rats. Nevertheless, the data of Yoshida *et al.* (1993) indicate that reduction of the nitro group is a significant metabolic pathway in humans.

3.3.4 Pathology

The vascular tumors found in male and female mice were considered by the authors (Weisburger *et al.*, 1978) to be hemangiomas or hemangiosarcomas (personal communication, J. Weisburger, 1998). Tumors of the vascular endothelium frequently exhibit a range of malignancy without clear distinctions between benign and malignant neoplasms. These tumors can be highly invasive and can produce metastases.

In short-term exposure studies, Travlos *et al.* (1996) found pathological changes in several organs in Fischer 344/N rats and $B6C3F_1$ mice exposed to 1-chloro-4-nitrobenzene by inhalation. Groups of male and female rats and mice (10/dose/sex/species) were exposed six hours per day, five days per week, for 13 weeks at concentrations of 0, 1.5, 3, 6, 12 or 24 ppm 1-chloro-4-nitrobenzene. Hematological and serological analyses, performed only for rats, demonstrated methemoglobinemia that increased in severity with dose in both males and females and was associated with numerous tissue changes secondary to oxidative erythrocyte injury, including a macrocytic, hyperchromic responsive anemia, Heinz bodies, polychromasia, poikilocytosis, and increased bile acid concentration in serum. Hemosiderin was noted in hepatic Kupffer's cells of both mice and rats exposed to 1-chloro-4-nitrobenzene. Hepatocytomegaly with slight focal necrosis was seen in exposed mice and hepatocyte basophilia in exposed rats. Hematopoietic cell proliferation, hemosiderin accumulation and capsular fibrosis were noted in the spleen of dosed rats and mice. Other findings included hyaline droplet nephropathy, degeneration of the testis and inflammation of the Harderian gland in exposed rats, and hyperplasia of the forestomach epithelium in exposed mice.

In other short-term studies, dose-related increases in methemoglobin levels were observed among both male and female Sprague-Dawley rats treated for four weeks by inhalation (6 hours/day, 5 days/week) with concentrations of 0, 0.8, 2.3, or 6.8 ppm 1-chloro-4-nitrobenzene in ethylene glycol monoethyl ether (Nair *et al.*, 1986). Increased severity of extramedullary hematopoiesis and hemosiderosis was observed in the spleens of treated animals.

3.4 Mechanism

The carcinogenic effects of 1-chloro-4-nitrobenzene may be in part mediated by its metabolite, 4-chloroaniline. A role for this known carcinogen is suggested by studies showing that this compound is a significant metabolite of 1-chloro-4-nitrobenzene in rats, rabbits and humans. This role is also supported by observations that 4-chloroaniline and 1-chloro-4-nitrobenzene both produce vascular tumors in male and female mice. While the mechanism of carcinogenesis is not known for either compound, observations of genotoxicity in mammalian cells (single strand DNA breaks (1-chloro-4-nitrobenzene

only), sister chromatid exchanges, and chromosomal aberrations) suggest that DNA damage is involved.

Observations of oxidative erythrocyte injury and accumulation of hemosiderin in the liver and spleen of rats and mice exposed to 1-chloro-4-nitrobenzene (Travlos *et al*., 1996) suggest that oxidative cell injury may play a role in development of vascular tumors in these organs. This possibility is further supported by the observation of capsular fibrosis of the spleen in exposed rats and mice and hepatocytomegaly with slight focal necrosis in the liver of exposed mice. Possible consequences of oxidative cell injury, which might lead to tumor formation, include oxidative DNA damage and regenerative cell proliferation.

4 OTHER REVIEWS

The International Agency for Research on Cancer (IARC) reviewed data relevant to the possible carcinogenicity of 1-chloro-4-nitrobenzene and classified it in Group 3 (not classifiable as to its carcinogenicity) based on the evaluation of the evidence in humans as inadequate and the evidence in laboratory animals as inadequate (IARC, 1996). The animal evidence reviewed by IARC included the studies by Weisburger *et al.* (1978), but not those by Schroeder and Daly (1984). In evaluating the animal evidence, the IARC reviewers expressed concerns for "the small number of animals, the short duration of dosing and the limited histopathological evaluation and reporting." The U.S. Environmental Protection Agency (U.S. EPA) classified 1-chloro-4-nitrobenzene as a probable human carcinogen (Group B2), based on sufficient evidence in animals, as published in the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1995).

5 SUMMARY AND CONCLUSIONS

5.1 Summary of Evidence

1-Chloro-4-nitrobenzene administered in feed to mice produced vascular tumors in both males and females. In male mice, a statistically significant increase in the incidence of hepatocellular carcinomas was seen in the low- but not the high-dose group. When administered in feed to male rats, no statistically significant increases in tumor incidence were found (Weisburger *et al*., 1978). In these studies, the number of animals in each dose group was relatively small and the histopathological examination of tissues was limited. When administered by gavage to male and female rats, no treatment related increases in tumor incidence were observed, with the exception of an equivocal increase in interstitial testicular tumors in treated males (Schroeder and Daly, 1984).

When tested in *Salmonella typhimurium*, 1-chloro-4-nitrobenzene produced a significant increase in the number of mutations in one strain (TA1535) but not in other strains

(TA98, TA1537 and TA1538) or in *Drosophila melanogaster*. It did produce DNA strand breaks, chromosomal aberrations and sister-chromatid exchanges in cultured mammalian cells, and it produced DNA strand breaks in mammalian cells *in vivo*.

In rats and rabbits, a major metabolic pathway for 1-chloro-4-nitrobenzene produces 4-chloroaniline and its metabolites, which are excreted in urine. In humans accidentally exposed to 1-chloro-4-nitrobenzene, approximately 30% of 1-chloro-4-nitrobenzene metabolites in urine were identified as 4-chloroaniline or metabolites of 4-chloroaniline.

4-Chloroaniline is listed as a chemical known to the state of California to cause cancer. 4-Chloroaniline produced vascular tumors in male and female mice and induced sister chromatid exchanges and chromosomal aberrations *in vivo* as did 1-chloro-4-nitrobenzene. 4-Chloroaniline induced sarcomas of the spleen in male rats, but 1-chloro-4-nitrobenzene did not induce these tumors.

5.2 Conclusion

Based on the information reviewed in the preparation of this document, there is evidence for the carcinogenicity of 1-chloro-4-nitrobenzene in male and female mice. Further evidence includes observations of genetic toxicity and metabolism to 4-chloroaniline, a substance with known carcinogenic activity.

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