National Toxicology Program
Toxicity Report Series
Number 37

NTP Technical Report
on Toxicity Studies of

Sodium Cyanide

(CAS No. 143-33-9)

Administered in Drinking Water
to F344/N Rats and B6C3F$_1$ Mice

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Post Office Box 12233
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NIH Publication 94-3386
November 1993

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund (Superfund) by an interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

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Note to the Reader

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- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

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The draft report on the toxicity studies of sodium cyanide was evaluated in June 1993 by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the peer reviewers were reviewed by the NTP staff and were addressed in this toxicity study report.

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ABSTRACT

Sodium Cyanide

NaCN

CAS Number	143-33-9
Molecular Weight	49.02
Synonym	Cyanogran

Cyanide and its salts are used extensively in industry and manufacturing and are found in water and food consumed by humans. Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans. Data in the literature indicate that long-term exposure to near-lethal concentrations of cyanide may produce lesions in rodents similar to those linked to chronic cyanide exposure in humans. However, few data are available on the effects of subchronic exposure to cyanide concentrations that are not acutely toxic. To address this lack of data, 13-week toxicity studies on cyanide were conducted with male and female F344/N rats and B6C3F$_1$ mice administered low doses of sodium cyanide in drinking water. Animals were evaluated for histopathology, clinical chemistry, hematology, urine chemistry, and reproductive toxicity. In addition, the mutagenicity of sodium cyanide was assessed in ***Salmonella typhimurium***.

Groups of 10 rats and 10 mice per sex were administered sodium cyanide in drinking water at concentrations of 0, 3, 10, 30, 100, and 300ppm for 13weeks. No deaths attributed to sodium cyanide administration occurred in either species. In animals exposed to 300ppm, male rats had slightly lower final mean body weights and mean body weight gains and female mice had slightly lower final mean body weights than the respective controls. Water consumption by rats and mice in the 100 and 300ppm groups was 10% to 30% lower than that by the controls; however, no clinical signs attributable to sodium cyanide administration or to dehydration were observed. No gross or microscopic changes specifically related to cyanide toxicity occurred at any site in males or females of either species. In particular, no lesions were found in the brain or thyroid gland. Differences between absolute and relative organ weights of exposed and control animals were minor and sporadic and were not exposure concentration dependent; these differences were not considered to be related to sodium cyanide administration.

Hematologic, clinical chemistry, and urinalysis evaluations of rats and mice revealed minimal changes that were not considered biologically significant, although the

decreased urine volume and increased urine specific gravity observed in male rats in the 300ppm group of the supplemental clinical pathology study were consistent with the observed decreases in water consumption. The concentration of urinary thiocyanate (the primary metabolite of cyanide) increased with increasing exposure concentration at all time points.

Sperm motility and vaginal cytology examinations were performed on rats and mice in the 0, 30, 100, and 300 ppm groups. Sodium cyanide caused a slight reduction in cauda epididymal weight in all groups of exposed male rats and in male mice exposed to 300-ppm. In male rats, the number of spermatid heads per testis in the 300ppm group was less than the number in the controls, and sperm motility in all exposed groups was marginally lower than in the controls. Sodium cyanide produced no adverse effects on estrous cyclicity in female mice, but at higher concentrations (100 and 300 ppm), sodium cyanide caused a significant increase in the amount of time spent by female rats in proestrus and diestrus relative to estrus and metestrus.

Sodium cyanide was not mutagenic in ***Salmonella typhimurium*** strain TA100, TA1535, TA97, or TA98 with or without exogenous metabolic activation.

In summary, administration of low concentrations of sodium cyanide in drinking water to rats and mice for 13 weeks resulted in no clinically significant body weight, organ weight, histopathologic, or clinical pathology changes. The absorption of administered cyanide was confirmed by increases in urinary thiocyanate excretion. Concentrations of 100 ppm and greater resulted in reduced water consumption. Thus, higher concentrations of sodium cyanide could not be administered by the drinking water route of administration. Alterations in reproductive parameters indicate that subchronic exposure to low concentrations of sodium cyanide may produce mild but significant adverse effects on rat reproductive systems. These changes are probably insufficient to decrease fertility in rats; however, humans are considered to be relatively more sensitive to such changes than rats, and the potential for reproductive toxicity in humans from low concentrations of cyanide warrants further investigation.

NTP TECHNICAL REPORTS ON TOXICITY STUDIES PRINTED AS OF NOVEMBER 1993

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	***n***-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	***o***-Cresol ***m***-Cresol ***p***-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	***p***-Chloro-_,_,_-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	***t***-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	***o***-Nitrotoluene ***m***-Nitrotoluene ***p***-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
PRINTED AS OF NOVEMBER 1993 (continued)

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385

INTRODUCTION

Physical Properties, Production, Use and Exposure

Sodium cyanide and other cyanide salts are used extensively in a multitude of industrial processes, including the production of dyes, pigments, nylon, and chelating agents; the cleaning, electroplating, and case-hardening of metals; the extraction of gold and silver from ore; the manufacture of adiponitrile; coal gasification; and the fumigation of ships, buildings, and soil (Klaassen, 1980; Hartung, 1982). The estimated production capacity for cyanide in the United States was approximately 202million kilograms in 1964 and rose to approximately 616million kilograms in 1987 (Towill ***etal.***, 1978; ATSDR, 1988). An estimated 454 million kilograms of sodium cyanide was produced in the U.S. in 1987, and approximately 7.3million kilograms of cyanide salts were imported during 1982 and 1984.

Sodium cyanide is prepared by the direct reaction of hydrogen cyanide with alkali, by melting sodium chloride with calcium cyanamide, or by heating sodium amide with carbon (Hartung, 1982). Under dry conditions, the compound is a white, odorless, crystalline powder that can absorb moisture from the atmosphere and, when moist, gives off a faint odor of almonds. Sodium cyanide has a molecular weight of 49.02, a melting point of 564°C (1,027°F), and a boiling point of 1,496°C (2,645°F). The compound is readily soluble in aqueous solutions and slightly soluble in alcohol. The vapor pressure of sodium cyanide is 1mm Hg at 817°C and 10mm Hg at 983°C; these temperatures are frequently attained in metal-treatment processes using cyanide (Hartung, 1982). Upon contact with water, steam, or acid, sodium cyanide gradually releases the highly toxic and flammable gas hydrogen cyanide.

Exposure to cyanide can take place by a variety of routes, including oral, dermal, and inhalation. In industries using cyanide, occupational exposures occur primarily by the dermal and inhalation routes. Nonindustrial sources of cyanide in air include malfunctioning catalytic converters on automobiles and residential and commercial fires involving the burning of plastics (Way, 1982). Tobacco smoke contains significant levels of cyanide, which has been associated with a nervous disorder known as tobacco amblyopia (Chisholm ***et al.***, 1967). Oral exposure to cyanides in the environment can take place through consumption of food bearing residual cyanide from insecticides or drinking water contaminated by industrial effluents or insecticides. Other potential sources of cyanide exposure include pharmaceutical preparations such as laetrile, a controversial cancer medication which can be degraded to cyanide; nitroprusside, a pharmaceutical agent used to treat hypertension and to control bleeding during surgery; and succinonitrile, a commonly used antidepressant (Solomonson, 1982). Additionally, a

number of vegetables contain significant levels of cyanogenic compounds and can contribute to cyanide-like poisoning. These vegetables include lima beans, sorghum, sweet potatoes, maize, millet, bamboo shoots, and cassava, a staple foodstuff in many regions of Africa (Solomonson, 1982).

Cyanide is produced as a waste component in a number of industries, including mining, the steel industry, and paint manufacturing. Due to the well-known acute toxicity of cyanide, waste management procedures in industry are usually quite rigorous, and a relatively small fraction of the cyanide produced is believed to be discharged into the environment. Nevertheless, it is estimated that, nationwide, paint manufacturing accounts for approximately 355,000 pounds of cyanide released annually (Towill ***et al.***, 1978). High levels of cyanide have been found in surface water at numerous sites, including Denver, Colorado, where a number of ponds downstream from a landfill were contaminated with high levels of cyanide; Oak Ridge, Tennessee, where cyanide in effluent from a sewage facility was responsible for a large fish kill; and Byron, Illinois, where leakage from stored drums containing cyanides resulted in cyanide concentrations of up to 365ppm in surface water, causing environmental damage and loss of livestock (USEPA, 1974, 1975; Towill ***etal.***, 1978). Data from the Nationwide Urban Runoff Program revealed that 16% of urban runoff samples collected from cities across the U.S. contained cyanide concentrations of 2 to 33 ppb (Cole ***etal.***, 1984). The threshold limit value recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) and the standard recommended by the National Institute for Occupational Safety and Health (NIOSH) for cyanide in air are 5 mg/m^3, and the ACGIH adds a skin notation to indicate the potential for dermal absorption of airborne cyanide (ACGIH, 1991; Sittig, 1991). The U.S. Environmental Protection Agency (USEPA) has recommended a maximum permissible concentration of 0.2mg/L for cyanide in water for the protection of human health, and the World Health Organization has set a drinking water standard of 0.05mg/L (Sitting, 1991).

Biochemistry

The cyanide ion (CN^-) forms stable but reversible complexes with biologically active metal ions. Such complexation inhibits the activity of the enzymes containing the metal atom(s). Cyanide inhibits the activity of numerous enzymes, including cytochrome oxidase, catalase, peroxidase, nitrogenase, and nitrite and nitrate reductase (Hartung, 1982; Solomonson, 1982). In addition, cyanide can interact with and inhibit nonmetalloenzymes such as ribulose diphosphate carboxylase. Such inhibition is thought to involve a reaction between cyanide and a Schiff-base intermediate to form an inhibitory compound. Isom ***etal.*** (1975) reported that potassium cyanide altered the glucose metabolism of mice injected with sublethal doses, causing a change from aerobic

(Embden-Meyerhoff and tricarboxylic acid) to anaerobic (pentose phosphate shunt) pathways.

Absorption, Disposition, Metabolism, and Excretion

Absorption of cyanide occurs rapidly through the gastrointestinal tract, lungs, and skin. While data on actual absorption rates are not available, the extremely rapid onset of symptoms after exposure to cyanide occurs makes it clear that the compound is readily taken up and distributed throughout the body. In some cases of human ingestion, victims have become unconscious within seconds and died within minutes of exposure. The rate of absorption of cyanide from the gastrointestinal tract depends on the chemical form of the cyanide and the presence of food in the tract. Food in the stomach significantly delays the absorption of cyanide, and experimental results have demonstrated that dogs and cattle can be protected from the lethal effects of cyanide by the presence of carbohydrates in the stomach (Couch, 1934; Liebowitz and Schwartz, 1948). Cyanide can be released in lethal concentrations from cyanogenic glycosides in foodstuffs; however, the uptake of cyanide in such cases is usually slow, and the onset of symptoms is often delayed (Towill ***etal.***, 1978). Cyanide inhaled as either vapor or dust is rapidly absorbed and distributed within the body; in humans, inhalation of 220 to 270-ppm hydrogen cyanide has resulted in immediate death (Towill ***et al.***, 1978; Way, 1982). The absorption of cyanide after inhalation of low concentrations is demonstrated by the increased levels of thiocyanate in the blood of smokers. However, because of the rapid metabolism of cyanide to thiocyanate, plasma cyanide levels in such cases are usually not elevated. Cyanide is also absorbed percutaneously by humans, and such absorption can result in poisoning (Drinker, 1932; Potter, 1950).

Although the distribution of cyanide to the various tissues in the body is fairly uniform, the highest levels are typically found in the liver, lungs, blood, and brain. Gettler and Baine (1938) found that in dogs exposed to cyanide by inhalation or by stomach intubation, the highest concentrations of cyanide were found in the lungs and blood with either route of exposure, even though tissue distribution varied somewhat with the route of administration. In several cases of human poisoning after ingestion of cyanide, the compound was uniformly distributed throughout the body (Gettler and Baine, 1938). Yamamoto ***et al.*** (1982) found that in rats dosed with cyanide by gavage, the highest concentrations of cyanide were in the liver, followed by the lungs and blood. After inhalation exposure, the highest concentrations of cyanide in rats were found in the lungs, followed by the blood and the liver.

Some of the cyanide in blood can bind reversibly to the Fe^{3+} of methemoglobin and become sequestered, and experimental results have shown that human erythrocytes

can concentrate cyanide from the surrounding fluid (McMillan and Svoboda, 1982). After cessation of exposure, plasma cyanide in humans returns to baseline levels within 4 to 8hours ($t_{½}$ = 20 minutes to 1 hour). Plasma thiocyanate is therefore a better marker of cyanide exposure than cyanide itself.

Although cyanide can interact with substances such as methemoglobin in the bloodstream, the majority of cyanide metabolism occurs within the tissues. Cyanide is metabolized in mammalian systems by one major route and several minor routes. By far the most important mechanism for cyanide metabolism is the conversion of the cyanide ion to thiocyanate by the enzyme rhodanese. This enzyme catalyzes the transfer of the sulfane sulfur of thiosulfate to the cyanide ion to form thiocyanate, which is then excreted in the urine (Westley ***et al.***, 1983; Rutkowski ***et al.***, 1986). The highest concentrations of rhodanese are found in the liver, kidney, brain, and muscle. In addition to rhodanese, a number of other sulfurtransferases can metabolize cyanide, and albumin, which carries elemental sulfur in the body in the sulfane form, may aid in the catalysis of cyanide to thiocyanate as well (Westley ***et al.***, 1983). The carbon of cyanide and thiocyanate can also be metabolized via several minor routes, including conversion of hydroxocobalamin to cyanocobalamin (Boxer and Rickards, 1952), conversion of cystine to cysteine and β-thiocyanoalanine (Wood and Cooley, 1956), and conversion to carbon dioxide.

Absorbed cyanide is mainly excreted as thiocyanate in the urine; however, traces may also be excreted unchanged or as a variety of metabolic products (***e.g.***, carbon dioxide, β-thiocyanoalanine) in expired air, saliva, and sweat (Friedberg and Schwarzkopf, 1969; Hartung, 1982).

Toxicity

The toxic effects of cyanide in humans and animals are generally similar. These effects, whether acute or chronic, are thought to result from inhibition of cellular respiration and consequent histotoxic anoxia. However, the syndromes resulting from acute and chronic cyanide poisoning are distinctly different.

Acute Toxicity

Cyanide is an extremely potent and fast-acting poison. In humans, inhalation of 110 to 135ppm cyanide results in death within a few hours, and inhalation of 220 to 270ppm is immediately fatal (Towill ***etal.***, 1978; Way, 1982). While the lethal dose of cyanide depends on many factors, including the form of cyanide used, the dosing regimen, the route of administration, and the presence of food in the stomach, the LD_{LO} and the LD_{50} in mammals generally range from 1.3 to 10mg/kg body weight (Table1). The

minimum absorbed lethal dose in humans is estimated to be 1.4 to 3.0 mg/kg (Gettler and Baine, 1938; Way, 1982). Typical signs of acute cyanide poisoning include tachypnea, headache, vertigo, lack of motor coordination, weak pulse, cardiac arrhythmias, vomiting, convulsion, coma, and death. No specific gross or histopathologic lesions are seen following acute cyanide poisoning, and there are no autopsy characteristics that are considered pathognomonic for death from cyanide poisoning. Pathologic findings may include tracheal congestion with hemorrhage; cerebral and pulmonary edema; gastric erosions; and petechiae of the brain, meninges, and pericardium (Way, 1982; Ballantyne, 1983).

Table 1 Selected Toxicity Data for Sodium Cyanide[1]

Species	Route of Exposure	Dose (mg/kg body weight)
Rat	Intraperitoneal	LD_{50} = 4.3
	Oral	LD_{50} = 15[2]
Mouse	Intraperitoneal	LD_{50} = 4.9 to 5.9
	Subcutaneous	LD_{LO} = 10
Human	Oral	LD_{LO} = 2.9
Dog	Subcutaneous	LD_{50} = 5.4[3]
	Intravenous	LD_{LO} = 1.3
Rabbit	Subcutaneous	LD_{LO} = 2.2
Guinea pig	Subcutaneous	LD_{50} = 5.8

1 Based on Sax, 1984.
2 Smyth *etal.*, 1969.
3 Chen and Rose, 1952.

The toxic effects of cyanide poisoning are thought to result primarily from inhibition of tissue cytochrome oxidase activity, with resulting histotoxic anoxia. Isom and Way (1976) found that cyanide administered with thiosulfate was lethal to mice at doses that caused no inhibition of hepatic cytochrome oxidase; however, brain cytochrome oxidase was inhibited. The brain is the organ that is most sensitive to cyanide toxicity, and death from cyanide poisoning is believed to result from central nervous system depression subsequent to inhibition of brain cytochrome oxidase activity. Although acute doses of cyanide cause cardiovascular, respiratory, and neuroelectric alterations, many studies have shown that cessation of brain activity occurs prior to respiratory or cardiac arrest (Way, 1982). However, Pettersen and Cohen (1985) found a similar degree of inhibition of brain cytochrome oxidase activity in CD-1 mice administered lethal or nonlethal doses of cyanide, suggesting that the inhibition of cytochrome oxidase in the

brain may not be responsible for the lethality of cyanide. Persson ***et al.*** (1985) described rapid and fairly specific changes in the central dopaminergic and γ-aminobutyric acid-ergic systems of rats and mice dosed intraperitoneally with sodium cyanide, and these changes may contribute to central nervous system depression and to the lethality of cyanide.

CHRONIC TOXICITY

While the acute toxicity of cyanide has been thoroughly investigated for many species, relatively few experimental data exist on the effects of subchronic and chronic cyanide exposure. However, the data that are available indicate that the same kinds of effects occur in humans and experimental animals. In experiments with rats (Ibrahim ***et al.***, 1963; Lessell, 1971; Lessell and Kuwabara, 1974; Philbrick ***et al.***, 1979), cats, and monkeys (Ferraro, 1933; Hurst, 1940), selective destruction of white matter in the brain was a striking and consistent feature of poisoning from prolonged exposure to cyanide. In most of these experiments, animals were injected with increasing doses of sodium or potassium cyanide for up to 132days, and the doses used were high enough to cause significant death rates from acute toxicity. However, in the study by Philbrick ***et al.*** (1979), weanling rats exposed to low concentrations of potassium cyanide in feed had a marked decrease in weight gain, but no deaths or clinical signs of toxicity. Early necrosis of gray and white matter was a common occurrence in rats and monkeys, but repeated exposure appeared to selectively favor destruction of white matter. The histopathologic lesions observed in all species consisted of demyelination, especially of the optic nerve tracts and the corpus callosum. Swelling of astrocytes and myelin damage were apparent within 2days in rats injected with sodium cyanide at doses sufficient to keep the rats comatose for 225 to 260 minutes (Lessell and Kuwabara, 1974). Axonal damage, with vacuolation and loss of microtubules, also occurred. Blindness was common in cyanide-treated animals and was considered to be a result of persistent anoxia in the brain.

Neurologic lesions attributed to subchronic cyanide poisoning in humans are similar to those described for experimental animals. In rats, however, the corpus callosum appears to be more sensitive than the optic nerves, whereas in humans, optic nerve damage is frequently the only central nervous system lesion (Way, 1982). Numerous studies have implicated cyanide as the etiologic agent in human neuropathies, including Nigerian nutritional neuropathy, tobacco amblyopia, and Leber's optical atrophy (reviewed in Towill ***etal.***, 1978). The syndrome of tropical ataxic neuropathy includes bilateral optic atrophy, nerve deafness, sensory spinal ataxia, weakness of legs, and numbness of feet (Osuntokun, 1968). This condition is believed to be due to cyanide-induced demyelination in the brain and spinal cord and is attributed primarily to

consumption of the plant cassava, which contains high levels of cyanogenic glycosides (Way, 1982). Elevated plasma and urinary thiocyanate levels and demyelination of peripheral nerves, with decreased conduction velocity, were observed in patients from Nigeria with tropical ataxic neuropathy (Osuntokun, 1968; Osuntokun ***et al.***, 1970). Cyanide poisoning from tobacco smoke has also been implicated in the occurrence of tobacco amblyopia, an optic disorder that is common in people who smoke tobacco. Tobacco smoke is known to contain cyanide, and Wilson (1965) reported that smokers have elevated levels of plasma and urinary thiocyanate. Hydroxocobalamin and cyanocobalamin, which are capable of complexing cyanide in the bloodstream, have been shown to be effective in treating tobacco amblyopia, suggesting that cyanide itself is the etiologic agent in this disorder (Chisholm ***et al.***, 1967). Finally, an inborn error in cyanide metabolism is thought to be the cause of Leber's hereditary optic atrophy, a condition in which bilateral vision failure occurs. Low levels of plasma thiocyanate in smokers with this condition suggests a hereditary deficiency in the ability to metabolize cyanide to thiocyanate (Wilson, 1965). The neurologic lesions seen with all of these neuropathies are thought to be the result of cyanide-induced histotoxic anoxia.

A second clinical feature observed in cases of subchronic cyanide poisoning is disturbance of thyroid function. Significant decrements in thyroid function, reflected as decreases in plasma thyroxine levels and thyroxine secretion and increases in thyroid weight, were observed in weanling rats exposed to 1,500 ppm cyanide or 2,240 ppm thiocyanate in feed for 11.5 months (Philbrick ***et al.***, 1979). No deaths, clinical signs of toxicity, or histopathologic lesions in the eye, thyroid gland, or neural tissues were observed. The effect of cyanide on the thyroid is believed to be indirect, occurring through the action of thiocyanate, and to be exacerbated by nutritional deficiency. Thiocyanate inhibits both the uptake and the utilization of iodine by the thyroid gland (Solomonson, 1982).

Epidemiologic studies have demonstrated an increased incidence of goiter in workers exposed to cyanide in case-hardening and electroplating factories (Hardy ***et al.***, 1950; El-Ghawabi ***et al.***, 1975; Towill ***et al.***, 1978). Thyroid dysfunction in these men was often accompanied by neurologic symptoms including headache, dizziness, confusion, and "psychotic episodes" and was associated with increased urinary thiocyanate levels. This neurologic and thyroid gland damage cannot be unequivocally ascribed to cyanide due to the fact that exposures to other substances may have occurred; however, the similarity of the lesions in humans in these factories to those produced in experimental animals treated with cyanide and the cumulative epidemiologic evidence make a strong case for the role of cyanide in these conditions.

Reproductive and Developmental Toxicity

Relatively few data are available on the reproductive and developmental toxicity of cyanide. Tewe and Maner (1981a) fed pregnant rats diets of cassava containing 500 ppm cyanide throughout gestation and lactation; no effects on the number or body weight of offspring or weight gain of pups during lactation were observed. In similar experiments with pregnant pigs, 250 to 500 ppm cyanide in the diet during gestation had no effect on the number or weight of offspring or subsequent lactational performance (Tewe and Maner, 1981b). At the 500 ppm concentration, pregnant sows had proliferative changes in the kidney glomeruli and increased thyroid weights. However, no teratogenic effects ***per se*** were reported. In contrast, when pregnant hamsters were dosed subcutaneously with 6.125 to 6.517mmol/kg sodium cyanide per hour beginning on Day6 of gestation through delivery (a period covering organogenesis in the hamster), severe fetal malformations were observed at all doses greater than 6.125mmol/kg per hour, and a dose of 6.517 mmol/kg per hour caused 100% fetal mortality and some maternal deaths (Doherty ***et al.***, 1982). Malformations included exencephaly, encephalocele, nonclosure of the neural tube, and microphthalmia. Maternal toxicity in these experiments did not correlate with the incidence of malformations, and thiosulfate protected the dams and fetuses from the toxic effects of cyanide.

Carcinogenicity

Cyanide and cyanogenic glycosides (***e.g.***, amygdalin) have been employed as therapeutic agents in cancer chemotherapy of humans and animals in the past (Stone ***et al.***, 1959; Brown ***et al.***, 1960). Cyanide has been reported to be a selective inhibitor of some types of neoplasms, such as the Ehrlich ascites tumor in mice (Brown ***et al.***, 1960), and several laboratories have reported an anticancer effect of amygdalin (Morrone, 1962; Krebs, 1970). However, reports of anticancer activity of cyanide and cyanogenic glycosides have been difficult to substantiate and have been refuted in a number of investigations (Laster and Schnabel, 1975; Hill ***et al.***, 1976; Lewis, 1977). No thorough long-term investigations into the carcinogenic or anticarcinogenic activity of cyanide were found in the literature.

Genetic Toxicity

Investigations of the genetic toxicity of cyanide have yielded conflicting results, but overall, cyanide does not appear to have significant mutagenic activity. Sodium cyanide was not mutagenic in any of several strains of ***Salmonella typhimurium*** with or without S9 activation (Kleinhofs and Smith, 1976; Rietveld ***et al.***, 1983; Owais ***et al.***, 1985), and it did not induce DNA-strand breaks in cultured mouse lymphoma cells (Garberg ***et al.***, 1988). Potassium cyanide was not mutagenic in five strains of ***S. typhimurium*** with or without S9 at any dose level tested (DeFlora, 1981). Conversely, hydrogen cyanide was reported to induce

mutations in ***S. typhimurium*** strain TA100 in the absence of S9 activation (Kushi ***et al.,*** 1983).

Study Rationale and Design

Cyanide is frequently found in chemical waste sites, and exposure of humans to cyanide can take place through a variety of routes, including consumption of contaminated water and foodstuffs, consumption of food containing cyanogenic glycosides, exposure to pesticide sprays containing cyanide, and occupational exposure. The National Institute of Environmental Health Sciences (NIEHS) selected cyanide for study from a preliminary list of chemicals compiled by the USEPA under the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund) because the toxicologic data available in the literature were considered by the USEPA to be inadequate for the calculation of an acceptable intake level for subchronic exposure. Toxicity testing was undertaken by the NIEHS to provide data for calculation of an acceptable daily intake level with regard to groundwater contamination around dump sites. Sodium cyanide was selected as the form of cyanide to be studied because it is relatively stable in water, the chosen exposure route.

Thirteen-week drinking water studies of sodium cyanide were performed with male and female F344/N rats and B6C3F$_1$ mice. Gross and histologic examinations, sperm motility and vaginal cytology evaluations, and hematology, clinical chemistry, and urinalysis evaluations were performed on both species. The mutagenicity of sodium cyanide was assessed in four strains of ***S. typhimurium*** with and without S9 activation.

MATERIALS AND METHODS

Procurement and Characterization of Sodium Cyanide

Sodium cyanide was obtained in one lot (Lot01410ML) from Aldrich Chemical Company (Milwaukee, WI). Initial identity and purity analyses were performed by Midwest Research Institute (MRI, Kansas City, MO). The chemical, a white powder, was identified as sodium cyanide by infrared and ultraviolet/visible spectroscopy; spectra were consistent with those expected for the structure of sodium cyanide. The results of elemental analyses for carbon, nitrogen, and sodium were slightly lower than the theoretical values; elemental analysis also indicated 0.15% potassium. Spark source mass spectroscopy indicated 140ppm chlorine and 160ppm phosphorus; all other impurities detected by spark source mass spectroscopy were present at a total concentration of less than 132ppm. Karl Fischer analysis indicated 0.4% ± 0.1% water. Functional group titration performed by adding ammonium hydroxide and 10% potassium iodide to samples and then titrating the samples with 0.1N silver nitrate indicated a purity of 99.9% ± 0.9%. The cumulative data indicated an overall purity of approximately 98%.

Because literature references indicate that sodium cyanide is stable when kept dry and protected from exposure to acids (***Merck Index***, 1983), no accelerated stability studies were performed on the bulk chemical. Throughout the 13-week studies, the bulk chemical was stored in the dark at room temperature; reanalyses performed by the study laboratory with functional group titration and infrared or visible spectroscopy indicated no decomposition of the bulk chemical.

Dose Formulations

Drinking water solutions were prepared by mixing sodium cyanide with charcoal-filtered, deionized water. The pH of the premix was adjusted to slightly above 8.5 with hydrochloric acid, and the mixture was then further diluted with deionized water to the desired final volume and stirred. The pH was readjusted, if necessary, to 8.5 with hydrochloric acid.

Stability studies of the drinking water solutions with and without the pH adjusted to 8.5 were performed at MRI with visible light spectroscopy at 582nm. The results indicated that aqueous solutions of 0.03 mg/mL (30ppm) sodium cyanide were stable for at least 3-weeks when stored in the dark at room temperature; 0.03mg/mL solutions with the pH adjusted to 8.5 were also stable for at least 3weeks when stored in the dark at 5°C. Aqueous solutions of 0.3mg/mL (300ppm) sodium cyanide, with or without the pH

adjusted, were stable for 3weeks when stored in the dark at room temperature. All solutions, with or without the pH adjusted, were stable for at least 4days under animal room conditions.

During the 13-week studies, the drinking water formulations were stored in the dark at 5°C for up to 24days (rats) or 17days (mice). The study laboratory periodically analyzed the drinking water formulations and animal room samples by visible light spectroscopy. All formulations used for dosing were within 10% of the theoretical concentrations when analyzed within 2days of preparation. Water bottles were changed at least once every 4days, and the total storage and use time for any preparation did not exceed 24-days (rats) or 17days (mice). For the first, middle, and last mixing periods, samples of drinking water at each dose level were taken from the animal room water bottles on the last day of use and analyzed for sodium cyanide. Five of 15animal room samples for rats and 11 of 15animal room samples for mice were more than 10% lower than the theoretical concentrations. The loss of sodium cyanide from preparations is probably due to volatilization, because samples were not analyzed until 4 to 7days after removal from the animal rooms, and the samples were stored in the water bottles with sipper tubes in place prior to analysis. Results of referee analyses performed by MRI on the drinking water solutions were in agreement with study laboratory results.

Toxicity Study Designs

13-Week Base Studies

Male and female F344/N rats used in these studies were obtained from Taconic Laboratory Animals and Services (Germantown, NY); male and female B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA). Rats and mice were approximately 31days old at receipt and were quarantined 11days; the animals were approximately 6-weeks old when the studies began. Blood samples were collected from five control rats and five sentinel mice per sex at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman ***etal.***, 1986; Rao ***etal.***, 1989a,b); all results were negative. Additional details concerning the study design are provided in Table2.

For the 13-week studies, groups of 10 rats and 10 mice of each sex were administered 0,-3, 10, 30, 100, or 300 ppm sodium cyanide in drinking water. The exposure levels selected for the 13-week studies were based on the results of 2-week drinking water studies conducted by SRI, International (1987a,b). In these studies, male and female rats and mice exposed to sodium cyanide at concentrations greater than 300ppm had significantly depressed weight gains. Animals exposed to 1,000ppm sodium cyanide gained 50% to 86% less weight than the controls; animals receiving 3,000ppm actually

lost weight over the course of the studies. These weight gain depressions were due to decreases in water consumption by animals in the 1,000 and 3,000ppm groups.

During the 13-week studies, rats were housed five per cage by sex and mice were housed individually. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available ***ad libitum***. Animal rooms were maintained at 71° to 74°F (rats) or 69° to 76°F (mice) and 48% to 87% (rats) or 35% to 67% (mice) relative humidity, with 12hours of fluorescent light per day and at least 10room air changes per hour.

Complete necropsies were performed on all base-study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all animals in the 0 and 300ppm groups. Tissues examined microscopically are listed in Table2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman ***etal.*** (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

Clinical pathology evaluations were performed on male rats designated for clinical pathology supplemental studies and on base-study rats and mice of each sex after 13-weeks of exposure. Ten animals per exposure group were evaluated. Blood for hematology and clinical chemistry evaluations was collected from supplemental clinical pathology study rats on Days5, 25, 45, and 92; blood was collected from base-study rats on Days86 (males) and 93 (females). Urinalysis samples were collected from supplemental study rats on Days 8, 22, 43, and 88. For mice, blood for hematology and clinical chemistry evaluations was collected from base-study animals on Days89 (males) and 93 (females). For the hematology and clinical chemistry evaluations, animals were anesthetized with CO_2, and blood samples were drawn from the retroorbital sinus. Samples for hematology analysis were placed in tubes containing EDTA; samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant. The

latter samples were allowed to clot at room temperature; the samples were then centrifuged and serum was removed.

Hematologic determinations were made on an Ortho ELT-8 hematology analyzer (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table2. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy of blood smears stained with a Romanowsky stain. Smears were prepared from mixtures of methylene blue and whole blood (1:1, v:v) and were incubated for at least 20 minutes at room temperature prior to microscopic examination for quantitative determination of reticulocytes.

Clinical chemistry variables were measured on a Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters that were evaluated are listed in Table2. Reagents for assays of sorbitol dehydrogenase were obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the manufacturer.

Urine samples were collected overnight from fasted rats individually housed in metabolism cages (Hoeltge, Inc., Cincinnati, OH). Animals had free access to water during the urine collection period. Urine collection containers were kept immersed in ice water during sampling to minimize evaporation and suppress bacterial growth. After volume, pH, and specific gravity were measured, sorbitol dehydrogenase and ***N***-acetyl-β-D-glucosaminidase were measured on a Roche Cobas Fara chemistry analyzer. Ribonuclease and thiocyanate in frozen urine samples were measured spectrophotometrically.

Sperm Motility and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm motility evaluations were performed on base-study rats and mice (10animals per sex) from the 0, 30, 100, and 300ppm groups at the end of the 13-week studies. The parameters that were evaluated are listed in Table2. Methods were those described by Morrissey ***et al.*** (1988). Briefly, for the 12days prior to sacrifice, the vaginal vaults of 10females of each species per dose group were moistened with saline, if necessary, and samples of vaginal fluid and cells were spread on a glass slide, dried, and stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (***i.e.***, diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy (Dunnick ***etal.***, 1986). The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed

from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Distal cauda were teased and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

TABLE 2 Experimental Design and Materials and Methods in the 13-Week Drinking Water Studies of Sodium Cyanide

EXPERIMENTAL DESIGN

Study Laboratory	Southern Research Institute (Birmingham, AL)
Size of Study Groups	Base Studies: 10 males and 10 females per species per exposure group Clinical Pathology Study: 10 male rats per exposure group
Route of Administration	Drinking water
Doses/Duration of Dosing	Rats and mice: 0, 3, 10, 30, 100, or 300ppm daily for 13weeks
Date of First Dose	Rats: 7 November 1988 Mice: 19 December 1988
Date of Last Dose	Rats: 7-9 February 1989 Mice: 21-23 March 1989
Necropsy Dates	Rats: 7-9 February 1989 Mice: 21-23 March 1989
Type and Frequency of Observation	Animals were observed twice daily and were weighed at the start of the study, weekly thereafter, and at necropsy. Clinical observations were recorded weekly. Water consumption by cage was measured weekly.
Necropsy and Histologic Examinations	Complete necropsies were performed on all animals in the base studies. Histopathologic evaluations were performed on all animals in the control and 300ppm groups. The following tissues were examined: adrenal glands, brain (threesections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (threesections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). The liver (males only), spleen, and urinary bladder of rats, the spleen and mammary gland (females only) of mice, and gross lesions in rats and mice were examined in the lower exposure groups.
Supplemental Evaluations	Clinical Pathology Studies: Blood for hematology and clinical chemistry evaluations was collected on Days5, 25, 45, and 92 from rats in the supplemental clinical pathology study group. Base-study rats were evaluated on Days86 (males) and 93 (females). Urine samples were collected from supplemental rats overnight on Days8, 22, 43, and 88. Blood for hematology and clinical chemistry evaluations was collected from base-study mice on Days89 (males) and 93 (females). Hematology parameters included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, nucleated erythrocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and leukocyte (WBC) count and differential. Clinical chemistry parameters included urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase (CK), sorbitol dehydrogenase (SDH), 5'-nucleotidase, and total bile acids. Urinalysis parameters included thiocyanate, sorbitol dehydrogenase (SDH), *N*-acetyl-β-D-glucosaminidase (NAG), ribonuclease, volume, specific gravity, and pH. Sperm Motility and Vaginal Cytology Evaluations: Sperm motility and vaginal cytology evaluations were performed on base- study animals at the end of the 13-week studies. Animals in the 0, 30, 100, and 300ppm groups were evaluated. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various stages.

TABLE 2 Experimental Design and Materials and Methods in the 13-Week Drinking Water Studies of Sodium Cyanide (continued)

ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N rats B6C3F$_1$ mice
Animal Source	Rats:Taconic Laboratory Animals and Services (Germantown, NY) Mice:Simonsen Laboratories (Gilroy, CA)
Time Held Before Study	11 days
Age When Study Began	6 weeks
Age When Killed	19 weeks
Method of Animal Distribution	Animals were weighed and were randomized with a table of random numbers.
Diet	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and deionized, filtered water (City of Birmingham) containing appropriate doses were available *ad libitum*.
Animal Room Environment	Rats were housed five animals per cage by sex and mice were housed individually. The temperature was maintained at 71° to 74° F (rats) and 69° to 76° F (mice) and relative humidity at 48% to 87% (rats) and 35% to 67% (mice), with at least 10 air changes per hour. Fluorescent light was provided for 12hours per day.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger ***etal.***(1992). Sodium cyanide was sent to the testing laboratory as a coded aliquot and was incubated with the ***S. typhimurium*** tester strains (TA97, TA98, TA100, and TA1535) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with ***l***-histidine and ***d***-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of sodium cyanide.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed with the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry, hematology, spermatid, and epididymal spermatozoal data, which

typically have skewed distributions, were analyzed with the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

Analysis of Vaginal Cytology Data

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

Analysis of Mutagenicity in *Salmonella typhimurium*

A positive response in the ***Salmonella typhimurium*** assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Quality Assurance

The animal studies of sodium cyanide were performed in compliance with U.S. Food and Drug Administration Good Laboratory Practices regulations (21CFR, Part58). The Quality Assurance Unit of Southern Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

13-Week Drinking Water Study in F344/N Rats

All rats survived to the end of the study (Table3). The final mean body weights and mean body weight gains of males in the 10 and 300ppm groups were slightly less than those of the controls (Table3 and Figure1), while the final mean body weights and mean body weight gains of exposed and control female rats were similar. No clinical signs were observed that were considered related to exposure to sodium cyanide. Water consumption by males and females in the 100 and 300ppm groups was more than 10% less than that by the controls (Table3).

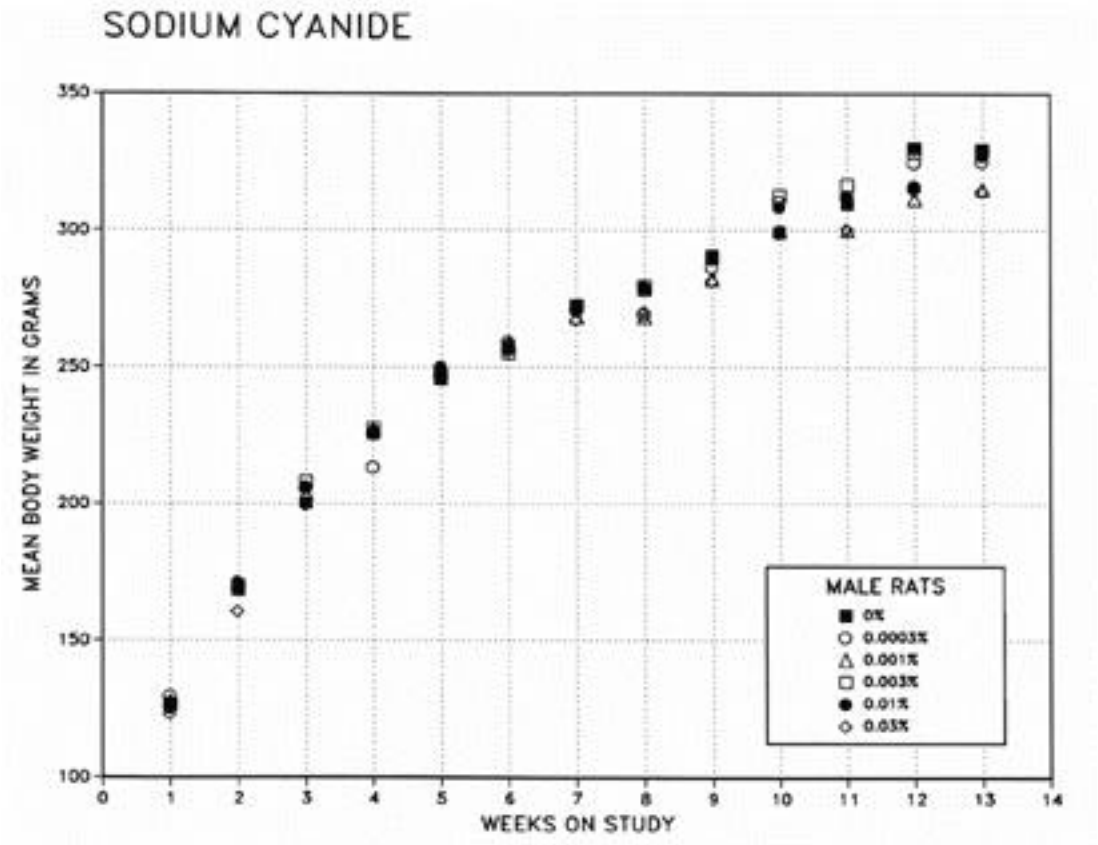
TABLE 3Survival, Body Weight, Water Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide

Dose		Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Water Consumption[3]	Average Dose[3]
(ppm)	Survival[1]	Initial	Final	Change		(g/day)	(mg/kg/day)
MALE							
0	10/10	121	330	209		24.6	
3	10/10	127	325	199	99	23.9	0.3
10	10/10	126	315	189	96	22.6	0.9
30	10/10	123	330	207	100	23.0	2.7
100	10/10	124	327	203	99	22.1	8.5
300	10/10	121	315	193	95	20.1	23.6
FEMALE							
0	10/10	105	197	91		18.4	
3	10/10	105	195	90	99	17.3	0.3
10	10/10	106	196	90	100	17.6	1.0
30	10/10	105	198	93	101	18.3	3.2
100	10/10	101	196	96	100	15.4	9.2
300	10/10	107	198	91	101	13.5	23.5

1 Number surviving at 13 weeks/number of animals per group.

2 (Dose group mean/control group mean) × 100.

3 Average of individual consumption values for Weeks2-13 for all animals in the base study.



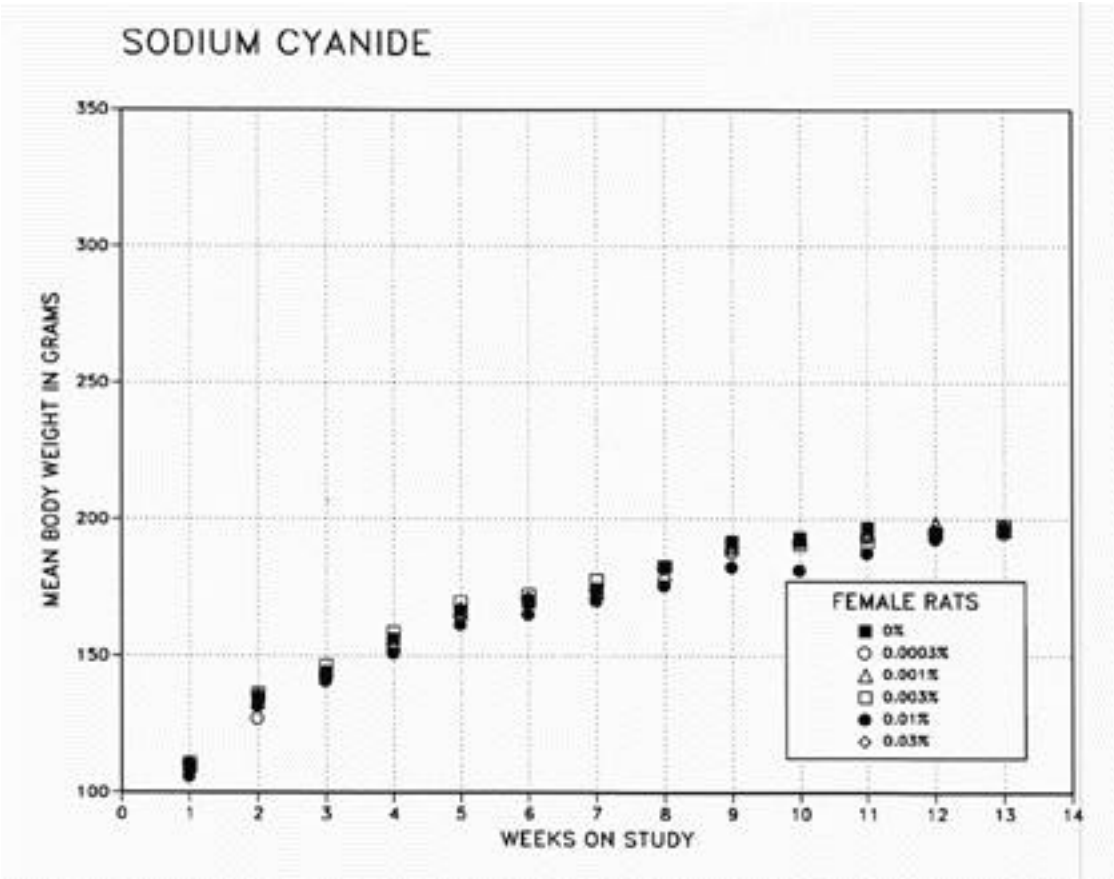


FIGURE 1 Body Weights of F344/N Rats Administered Sodium Cyanide in Drinking Water for 13Weeks

For male and female rats, statistically significant differences in organ weights (TableA1) were minor and sporadic and were not considered to be related to chemical administration.

Changes in hematology and clinical chemistry parameters occurred in supplemental and base-study rats in various exposure groups at various time points (TablesB1 and B2). In-general, these changes were minor and sporadic and were not considered to be clinically significant. Decreases in urine volume and increases in urine specific gravity occurred in supplemental rats in the 300 ppm group at all time points and in the 100ppm group on Day 8 (TableB3). These changes were consistent with the observed decreases in water consumption and with subsequent decreases in urine output, suggesting a palatability problem with the dosed water. Increases in urinary thiocyanate occurred in rats at all but the 3 and 10ppm exposure levels on Days 22 and 88 and all but the 3ppm exposure level on Day43. Changes in urine pH, sorbitol dehydrogenase, and ***N***-acetyl-β-D-glucosaminidase were minor and not exposure related; these changes were not considered to be clinically significant.

There were no treatment-related gross or histopathologic lesions in rats of either sex. There were no morphologic differences in the follicle size, colloid staining, or follicular epithelium of the thyroid gland of rats administered sodium cyanide compared to the controls. In histologic sections of the brain, there was no evidence of treatment-related degenerative changes in the corpus callosum.

The left cauda epididymal weights of all groups of exposed males were significantly lower than the control value; left epididymal and testis weights and the number of spermatid heads per testis for males in the 300ppm group were also lower than those of the controls (TableC1). Sperm motility in all groups of exposed males was less than that in the controls, but these motility changes were not considered to be biologically significant. Female rats in the 100 and 300ppm groups spent more time in proestrus and diestrus and less time in estrus and metestrus than control females (TableC2).

13-Week Drinking Water Study in B6C3F$_1$ Mice

One control female mouse died during Week9, and a female in the 30ppm group died during Week13 (Table4); the death of the exposed female was attributed to an ovarian tumor. All males survived to the end of the study. The final mean body weights of males and females in the 3ppm groups and males in the 30ppm group were slightly greater than those of the controls; the final mean body weight of females exposed to 300ppm was less than that of the control females (Table4). No treatment-related clinical signs were noted. Water consumption by males and females in the 100 and 300ppm groups was lower than that by the controls (Table4).

TABLE 4 Survival, Body Weight, Water Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide

Dose		Mean Body Weight (grams)			Final Weight Relative to	Average Water Consumption[3]	Average Dose[3]
(ppm)	Survival[1]	Initial	Final	Change	Controls[2] (%)	(g/day)	(mg/kg/day)
MALE							
0	10/10	22.5	37.1	14.6		5.7	
3	10/10	21.7	39.2	17.5	106	5.8	0.5
10	10/10	22.3	38.3	16.1	103	5.9	1.8
30	10/10	23.0	39.1	16.1	105	5.5	5.1
100	10/10	21.4	38.7	17.3	104	5.2	16.2
300	10/10	23.1	37.6	14.5	101	4.8	45.9
FEMALE							
0	9/10[4]	18.5	31.1	12.2		5.7	
3	10/10	18.5	33.1	14.6	107	5.7	0.6
10	10/10	17.5	32.0	14.4	103	5.6	2.1
30	9/10[5]	17.4	29.8	12.4	96	5.3	6.2
100	10/10	17.1	31.4	14.2	101	5.0	19.1
300	10/10	17.8	29.0	11.2	93	4.4	54.3

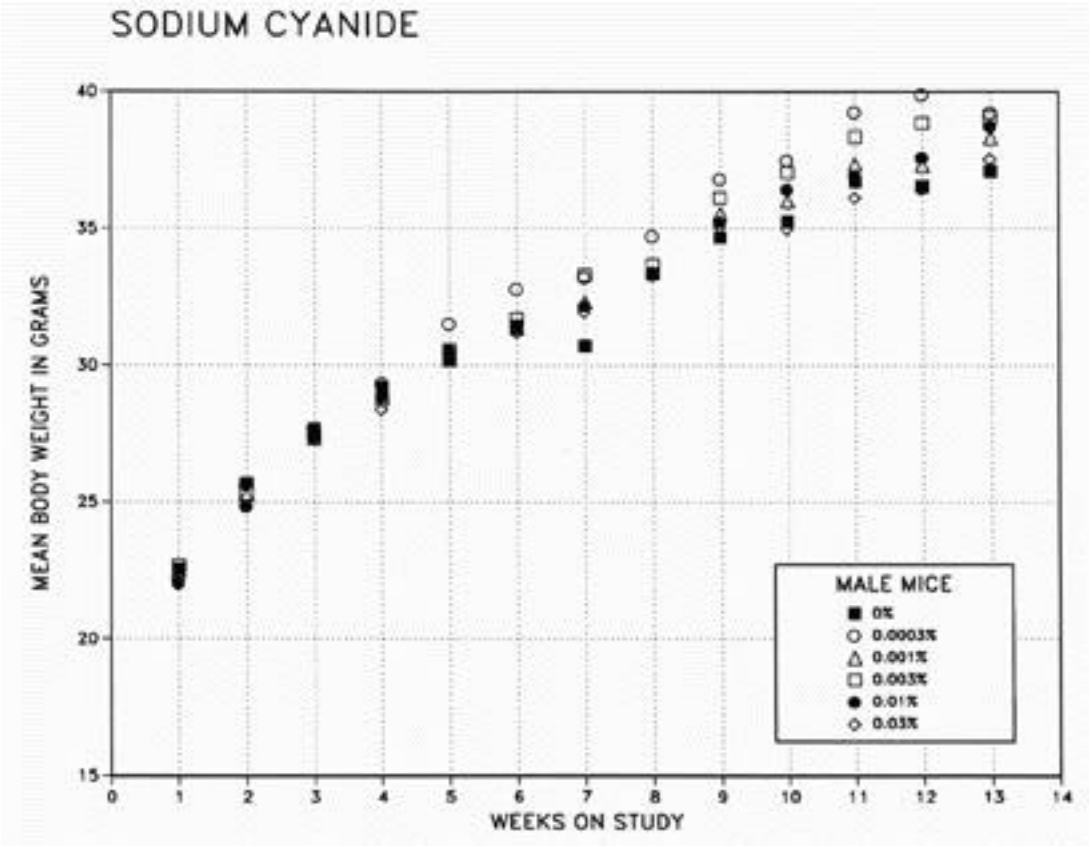
1 Number surviving at 13 weeks/number of animals per group.

2 (Dose group mean/control group mean) × 100.

3 Average of individual consumption values for Weeks2-13 for all animals in the base study.

4 Week of death: 9.

5 Week of death: 13.



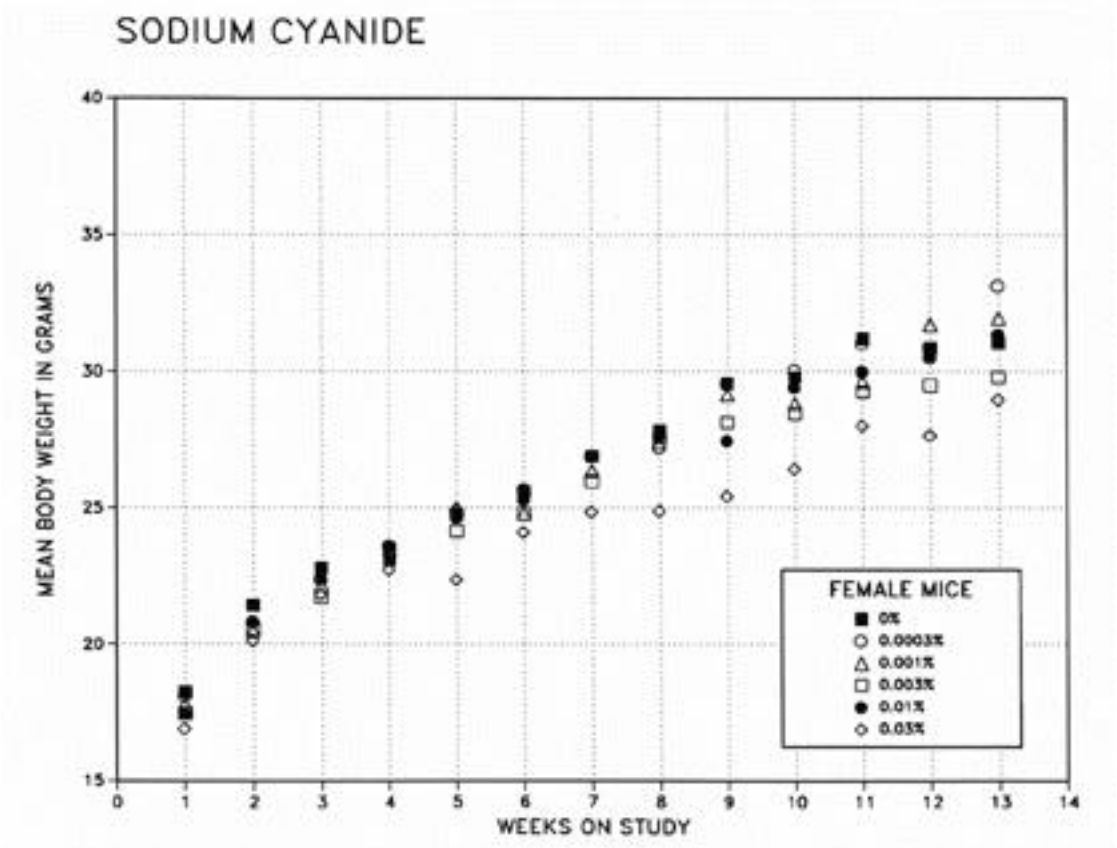


FIGURE 2 **Body Weights of B6C3F$_1$ Mice Administered Sodium Cyanide in Drinking Water for 13Weeks**

Differences in absolute and relative organ weights of male and female mice (TableA1) were sporadic and were not considered related to cyanide toxicity.

Few hematologic or clinical chemistry changes occurred in mice (TablesB4 and B5). These changes were minimal and were not considered to be biologically significant.

There were no treatment-related gross or histopathologic lesions in mice of either sex.

The left epididymal and cauda epididymal weights of males in the 300ppm group were significantly less than those of the controls (TableC3). No changes in sperm motility or spermatid head density such as those seen in male rats exposed to 300ppm sodium cyanide occurred in male mice. No significant changes in estrous cycle length occurred in females (TableC4).

Genetic Toxicity Studies

Sodium cyanide (0.3 to 333µg/plate) was tested for mutagenicity in ***Salmonella typhimurium*** strains TA100, TA1535, TA97, and TA98, with and without Aroclor-induced rat and hamster S9 at concentrations of 10% and 30%; all results were negative (AppendixD).

DISCUSSION

Previous studies with laboratory animals have demonstrated that exposure to acutely toxic doses of cyanide can cause nerve damage and disturbances of thyroid function (Ferraro, 1933; Hurst, 1940; Ibrahim ***etal.***, 1963; Lessell, 1971). In those animal studies, however, the levels of cyanide necessary to produce lesions were near or within the lethal range. The effects of subchronic administration of cyanide are less clear. In a 2-year feed study in which rats were administered feed containing hydrogen cyanide at concentrations up to 300ppm, there were no increases in mortality, decreases in body weight gain, hematologic changes, or gross or histologic lesions in any tissue of any exposure group (Howard and Hanzal, 1955). In rats administered feed containing 1,500-ppm potassium cyanide for 11.5months, Philbrick ***etal.*** (1979) observed decreases in body weight gain, decreases in thyroid function that were not accompanied by discernible histologic lesions, and modest myelin degeneration in spinal cord white matter. Philbrick and coworkers also found evidence of decreased thyroid function and vacuolation of nervous tissue in rats fed a diet containing 2,500ppm potassium thiocyanate for 11.5months. This concentration caused no change in body weight gain. The studies by Philbrick ***etal.*** included only one dose level of each compound, and no data verifying compound levels in the feed were presented; therefore, the significance of the results is difficult to assess. Nevertheless, the literature data do indicate that repeated exposure to doses of cyanide that are marginally toxic is capable of producing thyroid gland and nervous system changes in rodents.

The neurologic and thyroid gland lesions attributed to subchronic poisoning by cyanide and cyanogenic compounds in humans (Hardy ***etal.***, 1950; Wilson, 1965; Osuntokun, 1968; Osuntokun ***etal.***, 1970; El Ghawabi ***etal.***, 1975; Towill ***etal.***, 1978) are similar to those described in experimental animals receiving repeated high doses of cyanide (Ferraro, 1933; Hurst, 1940; Ibrahim ***etal.***, 1963; Lessell, 1971). However, few quantitative exposure data are available in these cases of human poisoning. In studies where disturbances of thyroid function, or goiter, were seen in humans, exposure to cyanide vapors was described as "frequent" or "almost constant," and the thyroid gland effects were accompanied by signs of acute cyanide poisoning, including headache, dizziness, and difficulty in breathing. No studies describing thyroid gland effects in humans exposed to low, nonacute toxic levels of cyanide were found in the literature. Visual and other neurological disturbances attributed to cyanide generally occur in individuals exposed to relatively high levels of cyanide or cyanogenic compounds (***e.g.***, tropical neuropathies in persons consuming cassava as a significant percentage of the diet; tobacco amblyopia in persons who smoke) or individuals with inborn deficiencies in

cyanide detoxification (***e.g.***, optical neuropathy in persons with Leber's hereditary optic atrophy). Thus, while there is strong evidence for neurotoxic and thyrotoxic effects of cyanide in humans, these effects may represent high-dose phenomena, and the risk from low-level chronic exposures may be less. Alternatively, although humans are generally considered to be less sensitive than rodents to the acute effects of cyanide intoxication (McNamara, 1976), it is possible that humans may be more sensitive to the neurologic and thyroid gland effects.

While assessments of thyroid function (***e.g.***, measurement of serum triiodothyronine, thyroxine, and thyroid-stimulating hormone levels) and specific examination of optic nerves were not performed in these NTP studies, the evaluations that were performed (***i.e.***, histopathologic examination of thyroid gland and brain) provided no evidence of thyroid gland or neurologic effects. These results indicate that subchronic administration of sodium cyanide to rats and mice at concentrations that caused no clinical signs of toxicity or reductions in body weight gains was not thyrotoxic or neurotoxic. Furthermore, with the exception of effects on the rat testis and epididymis, there was no evidence of other organ-specific toxicity. These results are in agreement with those of Howard and Hanzal (1955), who used dose levels similar to those employed in the present studies. The concentrations used in the present studies (3 to 300ppm) were chosen because, in earlier short-term studies, concentrations of sodium cyanide greater than 300ppm caused marked decreases in water consumption and significant depressions in body weight gains in both rats and mice, making administration of higher concentrations in the drinking water unfeasible.

These sodium cyanide studies were initiated by the NIEHS to provide data for safety assessment with regard to groundwater contamination around chemical waste disposal sites. The lack of lesions in the rat and mouse brain in these studies suggests that subchronic exposure to low concentrations of cyanide in drinking water does not present a significant human health hazard. However, the data contained in this report do not address relative species sensitivity to low-dose cyanide exposure, and future research in this area would be helpful in assessing the risk to humans from subchronic cyanide exposure.

Recently published studies suggest that the changes seen in reproductive endpoints in this study are probably not secondary to body weight reductions (Chapin ***etal.***, 1993a,b). Testicular sperm production and testis, epididymal, and cauda epididymal weights decreased with increasing exposure concentration, with the decreases becoming statistically significant at the highest concentration. These data suggest that subchronic exposure to low doses of sodium cyanide may produce mild, but perhaps

significant, adverse effects on the male reproductive system. The observed differences in sperm motility, while statistically significant, are not considered to be biologically significant, as they represent a relatively small percentage difference from the controls and are well within the range of normal values reported by various laboratories. However, the reductions in cauda epididymal weight, cauda sperm count, and testicular spermatid count are all consistent with a small but measurable adverse effect on male rat reproduction. Based on results of previous studies, these collective reproductive changes alone are probably insufficient to decrease fertility in rats (Chapin ***etal.***, 1985; Gray ***etal.***, 1992); however, the interactive effects of fertilization and development were not evaluated. In addition, the relative sensitivity of humans to such changes is considered to be greater than that of rats (Working, 1988). Therefore, a potential for adverse reproductive effects in humans following subchronic exposure to cyanide or cyanogenic compounds exists.

The lack of a dose-response relationship in the differences in female reproductive parameters suggests that these differences are spurious, and the results of this study would need to be replicated before such changes could be unequivocally attributed to sodium cyanide exposure. If the differences in female reproductive parameters were found to be treatment related, it is likely that perturbations in hormonal balance would be involved. Similarly, the male reproductive effects may have a hormonal component, but the present data do not allow elimination of other possible mechanisms.

In summary, administration of sodium cyanide at concentrations up to 300ppm in drinking water to rats and mice for 13 weeks resulted in no significant adverse effects on body weights, organ weights, histopathology, or clinical pathology parameters. No evidence of neurologic or thyroid gland damage was seen. The absorption of administered cyanide was confirmed by increases in urinary thiocyanate excretion. Concentrations of 100 ppm and greater resulted in decreased water consumption by rats and mice, suggesting poor palatability. Alterations in reproductive parameters suggest that subchronic exposure to low concentrations of sodium cyanide may produce mild but significant adverse effects on the male reproductive system. These changes are probably not biologically significant in rats; however, because humans are considered to be relatively more sensitive to such reproductive changes than rats, the potential for reproductive toxicity in humans from low concentrations of cyanide may be underestimated by these studies. Furthermore, the epidemiologic evidence for thyrotoxic and neurotoxic effects of cyanide after prolonged exposure in humans suggests that a difference in species sensitivity to such effects may exist between humans and rodents, and further research in this area is warranted.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	338 ± 5	321 ± 5*	318 ± 4**	335 ± 5	338 ± 4	319 ± 5*
Heart						
Absolute	1.006 ± 0.034	0.948 ± 0.018	0.905 ± 0.016*	0.968 ± 0.021	0.973 ± 0.020	0.923 ± 0.020*
Relative	2.97 ± 0.09	2.96 ± 0.05	2.85 ± 0.03	2.89 ± 0.05	2.88 ± 0.05	2.89 ± 0.05
Right kidney						
Absolute	1.234 ± 0.019	1.129 ± 0.028*	1.148 ± 0.026	1.232 ± 0.035	1.185 ± 0.023	1.196 ± 0.024
Relative	3.65 ± 0.05	3.51 ± 0.06	3.61 ± 0.07	3.68 ± 0.08	3.51 ± 0.04	3.75 ± 0.05
Liver						
Absolute	12.792 ± 0.202	9.525 ± 0.260**	11.783 ± 0.286	13.625 ± 0.528	12.057 ± 0.132	12.821 ± 0.426
Relative	37.84 ± 0.46	29.63 ± 0.43	37.10 ± 0.81	40.63 ± 1.12	35.73 ± 0.41	40.15 ± 1.15
Lungs						
Absolute	1.364 ± 0.038	1.322 ± 0.034	1.306 ± 0.026	1.299 ± 0.039	1.309 ± 0.033	1.227 ± 0.027**
Relative	4.04 ± 0.12	4.12 ± 0.11	4.12 ± 0.09	3.88 ± 0.09	3.87 ± 0.07	3.85 ± 0.09
Right testis						
Absolute	1.469 ± 0.027	1.470 ± 0.035	1.421 ± 0.020	1.441 ± 0.025	1.454 ± 0.020	1.390 ± 0.023
Relative	4.35 ± 0.05	4.58 ± 0.05	4.47 ± 0.04	4.31 ± 0.08	4.31 ± 0.03	4.36 ± 0.08
Thymus						
Absolute	0.288 ± 0.014	0.297 ± 0.026	0.315 ± 0.023	0.280 ± 0.030	0.285 ± 0.013	0.244 ± 0.015
Relative	0.86 ± 0.05	0.92 ± 0.08	0.99 ± 0.07	0.83 ± 0.09	0.84 ± 0.04	0.76 ± 0.04
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	193 ± 4	199 ± 2	203 ± 4	197 ± 3	195 ± 3	198 ± 4
Heart						
Absolute	0.644 ± 0.016	0.641 ± 0.017	0.625 ± 0.015	0.620 ± 0.013	0.618 ± 0.012	0.620 ± 0.017
Relative	3.36 ± 0.10	3.22 ± 0.08	3.09 ± 0.07*	3.16 ± 0.06	3.17 ± 0.04	3.13 ± 0.03
Right kidney						
Absolute	0.709 ± 0.010	0.712 ± 0.011	0.734 ± 0.016	0.729 ± 0.021	0.731 ± 0.018	0.765 ± 0.018*
Relative	3.70 ± 0.09	3.58 ± 0.08	3.63 ± 0.07	3.71 ± 0.10	3.75 ± 0.07	3.87 ± 0.05
Liver						
Absolute	5.820 ± 0.110	6.126 ± 0.110	6.529 ± 0.168	6.115 ± 0.145	5.966 ± 0.160	6.732 ± 0.175**
Relative	30.29 ± 0.57	30.78 ± 0.61	32.25 ± 0.52	31.13 ± 0.68	30.60 ± 0.49	34.05 ± 0.38**
Lungs						
Absolute	0.952 ± 0.028	0.925 ± 0.015	0.916 ± 0.023	0.896 ± 0.029	0.899 ± 0.024	0.932 ± 0.021
Relative	4.95 ± 0.15	4.65 ± 0.10	4.52 ± 0.06*	4.56 ± 0.13*	4.61 ± 0.09	4.72 ± 0.04
Thymus						
Absolute	0.219 ± 0.008	0.246 ± 0.015	0.246 ± 0.015	0.242 ± 0.012	0.242 ± 0.023	0.241 ± 0.017
Relative	1.14 ± 0.05	1.24 ± 0.08	1.21 ± 0.06	1.23 ± 0.06	1.24 ± 0.10	1.21 ± 0.07

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
n	9	10	10	10	10	9
Necropsy body wt	37.3 ± 1.0	40.0 ± 1.0	38.8 ± 0.7	39.2 ± 1.3	38.6 ± 1.1	35.5 ± 1.1
Heart						
Absolute	0.162 ± 0.003	0.179 ± 0.003*	0.177 ± 0.006*	0.166 ± 0.003	0.168 ± 0.003	0.166 ± 0.004
Relative	4.35 ± 0.07	4.50 ± 0.13	4.58 ± 0.19	4.26 ± 0.11	4.38 ± 0.11	4.67 ± 0.07
Right kidney						
Absolute	0.337 ± 0.009	0.368 ± 0.007	0.349 ± 0.011	0.355 ± 0.009	0.339 ± 0.008	0.359 ± 0.010
Relative	9.04 ± 0.22	9.22 ± 0.16	9.00 ± 0.26	9.12 ± 0.30	8.83 ± 0.23	10.12 ± 0.14**
Liver						
Absolute	1.521 ± 0.032	1.696 ± 0.051	1.817 ± 0.047**	1.677 ± 0.058	1.558 ± 0.055	1.630 ± 0.074
Relative	40.85 ± 0.82	42.39 ± 0.77	46.80 ± 0.79**	42.83 ± 0.85	40.37 ± 0.64	45.77 ± 1.28**
Lungs						
Absolute	0.196 ± 0.007	0.194 ± 0.004	0.191 ± 0.006	0.183 ± 0.004	0.183 ± 0.007	0.194 ± 0.009
Relative	5.27 ± 0.25	4.87 ± 0.14	4.94 ± 0.19	4.73 ± 0.24	4.77 ± 0.20	5.48 ± 0.19
Right testis						
Absolute	0.125 ± 0.002	0.128 ± 0.003	0.124 ± 0.002	0.127 ± 0.004	0.123 ± 0.003[2]	0.123 ± 0.003
Relative	3.36 ± 0.10	3.21 ± 0.09	3.20 ± 0.08	3.25 ± 0.12	3.23 ± 0.12[2]	3.49 ± 0.12
Thymus						
Absolute	0.041 ± 0.003	0.045 ± 0.003	0.038 ± 0.002	0.044 ± 0.005	0.039 ± 0.002	0.035 ± 0.003
Relative	1.11 ± 0.09	1.12 ± 0.08	0.97 ± 0.05	1.11 ± 0.12	1.01 ± 0.04	0.99 ± 0.06
FEMALE						
n	8	10	10	8	9	10
Necropsy body wt	31.3 ± 0.9	33.6 ± 1.2	33.0 ± 1.1	30.3 ± 0.7	30.3 ± 1.0	30.0 ± 0.9
Heart						
Absolute	0.125 ± 0.004	0.138 ± 0.002	0.142 ± 0.005**	0.125 ± 0.002	0.130 ± 0.005	0.137 ± 0.002
Relative	4.01 ± 0.12	4.13 ± 0.11	4.35 _±0.20	4.15 ± 0.14	4.29 ± 0.05	4.59 ± 0.12**
Right kidney						
Absolute	0.205 ± 0.005	0.224 ± 0.005	0.237 ± 0.008**	0.218 ± 0.005	0.226 ± 0.006	0.219 ± 0.004
Relative	6.58 ± 0.18	6.71 ± 0.20	7.24 ± 0.30	7.20 ± 0.19	7.48 ± 0.23*	7.33 ± 0.16*
Liver						
Absolute	1.224 ± 0.040	1.394 ± 0.044*	1.464 ± 0.034**	1.313 ± 0.036	1.358 ± 0.054	1.445 ± 0.048**
Relative	39.27 ± 1.15	41.55 ± 0.81	44.61 ± 1.09**	43.37 ± 0.82**	44.83 ± 1.00**	48.19 ± 0.96**
Lungs						
Absolute	0.169 ± 0.005	0.174 ± 0.007	0.178 ± 0.008	0.171 ± 0.007	0.169 ± 0.006	0.170 ± 0.004
Relative	5.41 ± 0.12	5.17 ± 0.08	5.47 ± 0.35	5.67 ± 0.23	5.59 ± 0.18	5.70 ± 0.16
Thymus						
Absolute	0.051 ± 0.005	0.053 ± 0.003	0.050 ± 0.004	0.051 ± 0.004	0.054 ± 0.004	0.054 ± 0.002
Relative	1.64 ± 0.16	1.57 ± 0.09	1.52 ± 0.12	1.69 ± 0.13	1.78 ± 0.11	1.80 ± 0.08

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

APPENDIX B

Hematology, Clinical Chemistry, and Urinalysis Results

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
Special Clinical Pathology Study						
n						
Day 5	10	10	10	9	10	10
Other	10	10	10	10	10	10
Hematocrit (%)						
Day 5	40.4 ± 0.4	40.0 ± 0.4	39.7 ± 0.4	39.7 ± 0.5	39.6 ± 0.6	41.4 ± 0.4
Day 25	46.2 ± 0.5	45.3 ± 0.4	45.2 ± 0.5	45.2 ± 0.3	44.5 ± 0.4	45.6 ± 0.4
Day 45	49.7 ± 0.4	49.6 ± 0.5	49.6 ± 0.5	50.2 ± 0.3	51.3 ± 0.6	49.9 ± 0.6
Week 13	47.4 ± 0.3	47.1 ± 0.4	47.8 ± 0.5	47.6 ± 0.3	47.5 ± 0.4	46.4 ± 0.3
Hemoglobin (g/dL)						
Day 5	13.7 ± 0.1	13.6 ± 0.1	13.4 ± 0.1	13.4 ± 0.2	13.5 ± 0.2	14.0 ± 0.2
Day 25	16.2 ± 0.2	15.9 ± 0.1	15.7 ± 0.2	15.8 ± 0.1	15.3 ± 0.2**	15.8 ± 0.1
Day 45	16.8 ± 0.1	16.7 ± 0.2	16.6 ± 0.2	16.8 ± 0.1	17.1 ± 0.2	16.8 ± 0.2
Week 13	16.2 ± 0.1	16.1 ± 0.1	16.2 ± 0.2	16.2 ± 0.1	16.1 ± 0.2	16.1 ± 0.3
Erythrocytes (10^6/µL)						
Day 5	7.16 ± 0.08	7.07 ± 0.07	6.96 ± 0.07	7.04 ± 0.09	7.03 ± 0.10	7.37 ± 0.08
Day 25	8.53 ± 0.12	8.42 ± 0.09	8.40 ± 0.09	8.48 ± 0.08	8.32 ± 0.08	8.55 ± 0.06
Day 45	9.26 ± 0.08	9.21 ± 0.11	9.28 ± 0.09	9.43 ± 0.08	9.62 ± 0.10*	9.47 ± 0.11
Week 13	9.56 ± 0.07	9.51 ± 0.06	9.67 ± 0.11	9.69 ± 0.08	9.65 ± 0.08	9.42 ± 0.05
Reticulocytes (10^6/µL)						
Day 5	0.43 ± 0.06	0.44 ± 0.03	0.49 ± 0.03	0.47 ± 0.05	0.46 ± 0.04	0.36 ± 0.03
Day 25	0.13 ± 0.01	0.08 ± 0.01	0.15 ± 0.02	0.12 ± 0.02	0.16 ± 0.02	0.15 ± 0.02
Day 45	0.19 ± 0.02	0.17 ± 0.01	0.20 ± 0.02	0.17 ± 0.02	0.15 ± 0.02	0.17 ± 0.02
Week 13	0.25 ± 0.03	0.24 ± 0.03	0.21 ± 0.02	0.16 ± 0.02*	0.20 ± 0.01	0.24 ± 0.02
Nucleated erythrocytes (10^3/µL)						
Day 5	0.19 ± 0.05	0.17 ± 0.04	0.24 ± 0.05	0.23 ± 0.04	0.24 ± 0.06	0.26 ± 0.05
Day 25	0.05 ± 0.03	0.02 ± 0.01	0.03 ±0.02	0.03 ± 0.02	0.06 ± 0.02	0.04 ± 0.02
Day 45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 13	0.10 ± 0.02	0.11 ± 0.04	0.08 ± 0.03	0.07 ± 0.02	0.10 ± 0.03	0.15 ± 0.05
Mean cell volume (fL)						
Day 5	56.4 ± 0.3	56.5 ± 0.3	57.2 ± 0.3	56.3 ± 0.2	56.4 ± 0.3	56.2 ± 0.3
Day 25	54.2 ± 0.3	54.0 ± 0.3	53.8 ± 0.4	53.2 ± 0.3*	53.6 ± 0.2	53.2 ± 0.2*
Day 45	53.7 ± 0.2	53.8 ± 0.2	53.4 ± 0.3	53.3 ± 0.3	53.6 ± 0.2	52.9 ± 0.2*
Week 13	49.5 ± 0.2	49.4 ± 0.2	49.4 ± 0.2	49.1 ± 0.2	49.3 ± 0.2	49.2 ± 0.1
Mean cell hemoglobin (pg)						
Day 5	19.1 ± 0.1	19.2 ± 0.1	19.3 ± 0.1	19.1 ± 0.1	19.2 ± 0.1	19.0 ± 0.1
Day 25	19.0 ± 0.1	19.0 ± 0.2	18.7 ± 0.1	18.6 ± 0.1*	18.5 ± 0.1**	18.5 ± 0.0**
Day 45	18.1 ± 0.1	18.1 ± 0.1	17.9 ± 0.1*	17.9 ± 0.1*	17.8 ± 0.1*	17.8 ± 0.1**
Week 13	16.9 ± 0.1	16.9 ± 0.0	16.7 ± 0.1*	16.8 ± 0.1*	16.7 ± 0.1*	17.1 ± 0.3
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.9 ± 0.2	34.0 ± 0.1	33.8 ± 0.2	33.8 ± 0.1	34.0 ± 0.2	33.8 ± 0.2
Day 25	35.1 ± 0.2	35.2 ± 0.3	34.8 ± 0.1	34.9 ± 0.3	34.4 ± 0.2*	34.8 ± 0.1
Day 45	33.8 ± 0.1	33.7 ± 0.1	33.4 ± 0.2	33.5 ± 0.1	33.4 ± 0.1	33.7 ± 0.2
Week 13	34.1 ± 0.1	34.1 ± 0.1	33.8 ± 0.1	34.1 ± 0.1	33.9 ± 0.1	34.6 ± 0.6
Platelets (10^3/µL)						
Day 5	920.0 ± 20.1	929.1 ± 11.6	969.6 ± 20.0	948.3 ± 14.3	922.6 ± 21.0	895.4 ± 9.9
Day 25	733.5 ± 11.5	667.4 ± 50.3	807.7 ± 33.0*	767.3 ± 17.9	810.5 ± 15.2**	774.3 ± 18.4*
Day 45	669.4 ± 13.4	656.3 ± 12.6	657.1 ± 16.5	672.9 ± 5.9	677.3 ± 17.5	632.2 ± 7.7**
Week 13	612.8 ± 16.6	609.9 ± 6.6	638.7 ± 14.3	604.5 ± 26.0	606.1 ± 8.7	605.8 ± 7.5

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide (continued)

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE (continued)						
Special Clinical Pathology Study (continued)						
Leukocytes (10^3/µL)						
Day 5	8.65 ± 0.36	8.64 ± 0.43	8.02 ± 0.65	8.21 ± 0.32	8.24 ± 0.40	8.18 ± 0.28
Day 25	7.87 ± 0.59	7.82 ± 0.49	8.80 ± 0.48	7.98 ± 0.73	8.37 ± 0.33	8.55 ± 0.49
Day 45	7.65 ± 0.38	8.29 ± 0.55	9.17 ± 0.43	8.69 ± 0.50	7.24 ± 0.54	8.78 ± 0.35
Week 13	7.64 ± 0.44	7.98 ± 0.24	8.37 ± 0.60	7.49 ± 0.50	7.47 ± 0.35	7.77 ± 0.30
Segmented neutrophils (10^3/µL)						
Day 5	1.08 ± 0.08	0.96 ± 0.11	0.83 ± 0.15*	1.11 ± 0.14	0.89 ± 0.09	0.69 ± 0.09**
Day 25	1.17 ± 0.16	1.14 ± 0.17	1.05 ± 0.11	0.82 ± 0.14	1.30 ± 0.12	0.82 ± 0.14
Day 45	1.05 ± 0.13	1.34 ± 0.16	1.62 ± 0.15	0.91 ± 0.12	0.96 ± 0.12	1.24 ± 0.17
Week 13	1.52 ± 0.16	1.78 ± 0.09	1.83 ± 0.32	1.38 ± 0.17	1.28 ± 0.13	1.15 ± 0.12
Lymphocytes (10^3/µL)						
Day 5	7.18 ± 0.34	7.37 ± 0.46	6.93 ± 0.57	6.91 ± 0.31	6.94 ± 0.38	7.18 ± 0.21
Day 25	6.56 ± 0.45	6.53 ± 0.39	7.59 ± 0.47	6.99 ± 0.59	6.90 ± 0.32	7.52 ± 0.39
Day 45	6.36 ± 0.30	6.50 ± 0.46	7.08 ± 0.33	7.26 ± 0.40	5.97 ± 0.46	6.98 ± 0.38
Week 13	5.80 ± 0.31	5.73 ± 0.17	6.00 ± 0.37	5.74 ± 0.41	5.65 ± 0.22	6.20 ± 0.33
Atypical lymphocytes (10^3/µL)						
Day 5	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.08 ± 0.03	0.00 ± 0.00
Monocytes (10^3/µL)						
Day 5	0.34 ± 0.05	0.22 ± 0.04	0.21 ± 0.04	0.18 ± 0.03	0.34 ± 0.05	0.26 ± 0.05
Day 25	0.08 ± 0.04	0.12 ± 0.04	0.14 ± 0.06	0.14 ± 0.08	0.12 ± 0.06	0.17 ± 0.04
Day 45	0.18 ± 0.08	0.41 ± 0.11	0.40 ± 0.08	0.38 ± 0.10	0.23 ± 0.06	0.50 ± 0.08*
Week 13	0.22 ± 0.04	0.43 ± 0.08	0.44 ± 0.11	0.31 ± 0.07	0.42 ± 0.11	0.35 ± 0.08
Eosinophils (10^3/µL)						
Day 5	0.05 ± 0.02	0.09 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.02
Day 25	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.04 ± 0.02
Day 45	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.03	0.13 ± 0.03	0.07 ± 0.02	0.08 ± 0.03
Week 13	0.15 ± 0.03	0.05 ± 0.02	0.10 ± 0.03	0.07 ± 0.03	0.07 ± 0.02	0.08 ± 0.03
Base Study						
n	9	10	10	10	10	10
Hematocrit (%)	46.6 ± 0.2	46.6 ± 0.2	45.8 ± 0.3*	46.1 ± 0.4	46.0 ± 0.3	45.1 ± 0.5*
Hemoglobin (g/dL)	15.8 ± 0.1	15.8 ± 0.1	15.5 ± 0.1*	15.5 ± 0.2	15.5 ± 0.1*	15.3 ± 0.2**
Erythrocytes (10^6/µL)	9.33 ± 0.05	9.40 ± 0.05	9.32 ± 0.08	9.34 ± 0.09	9.39 ± 0.05	9.18 ± 0.09
Reticulocytes (10^6/µL)	0.18 ± 0.02	0.17 ± 0.02	0.19 ± 0.03	0.16 ± 0.02	0.18 ± 0.03	0.18 ± 0.02
Nucleated erythrocytes (10^3/µL)	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
Mean cell volume (fL)	49.9 ± 0.1	49.6 ± 0.2	49.1 ± 0.2**	49.3 ± 0.2**	49.0 ± 0.2**	49.2 ± 0.1**
Mean cell hemoglobin (pg)	16.9 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.5 ± 0.1**	16.7 ± 0.0
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.1	33.9 ± 0.1	33.9 ± 0.1	33.7 ± 0.2	33.7 ± 0.2	33.9 ± 0.1
Platelets (10^3/µL)	602.4 ± 7.8	589.5 ± 9.1	603.6 ± 5.5	587.5 ± 16.1	611.3 ± 12.0	581.1 ± 19.0
Leukocytes (10^3/µL)	7.44 ± 0.35	8.10 ± 0.35	8.02 ± 0.56	7.04 ± 0.31	8.29 ± 0.32	7.93 ± 0.54
Segmented neutrophils (10^3/µL)	1.33 ± 0.18	1.30 ± 0.12	1.12 ± 0.19	1.08 ± 0.10	1.50 ± 0.15	1.21 ± 0.25
Lymphocytes (10^3/µL)	5.99 ± 0.24	6.74 ± 0.31	6.81 ± 0.45	5.92 ± 0.27	6.74 ± 0.27	6.66 ± 0.41
Monocytes (10^3/µL)	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Eosinophils (10^3/µL)	0.12 ± 0.03	0.04 ± 0.02	0.08 ± 0.03	0.04 ± 0.02	0.07 ± 0.03	0.05 ± 0.02

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide (continued)

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
FEMALE						
n	9	10	9	10	10	10
Hematocrit (%)	45.8 ± 0.4	46.3± 0.3	45.7 ± 0.3	45.7 ± 0.3	44.7 ± 0.7	44.0 ± 0.3**
Hemoglobin (g/dL)	15.6 ± 0.1	15.8 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.3 ± 0.2	15.2 ± 0.1*
Erythrocytes (10^6/µL)	8.65 ± 0.09	8.73 ± 0.07	8.61 ± 0.05	8.61 ± 0.07	8.41 ± 0.14	8.24 ± 0.10**
Reticulocytes (10^6/µL)	0.14 ±0.02	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.02	0.15 ± 0.02	0.16 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.02 ± 0.02	0.08 ± 0.03	0.10 ± 0.02	0.14 ± 0.03*	0.06 ± 0.02	0.17 ± 0.03**
Mean cell volume (fL)	53.0 ± 0.0	53.0 ± 0.0	53.0 ± 0.0	53.0 ± 0.0	53.1 ± 0.1	53.4 ± 0.3
Mean cell hemoglobin (pg)	18.1 ± 0.1	18.1 ± 0.0	18.1 ± 0.1	18.0 ± 0.1	18.2 ± 0.1	18.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.1	34.2 ± 0.1	34.1 ± 0.1	34.0 ± 0.1	34.2 ± 0.2	34.5 ± 0.1
Platelets (10^3/µL)	643.3 ± 31.8	631.0 ± 16.5	636.4 ± 16.1	665.2 ± 18.5	673.6 ± 42.0	666.3 ± 14.1*
Leukocytes (10^3/µL)	5.10 ± 0.42	6.07 ± 0.45	6.18 ± 0.33	6.36 ± 0.34	5.56 ± 0.32	5.10 ± 0.42
Segmented neutrophils (10^3/µL)	0.73 ± 0.09	1.16 ± 0.17	1.03 ± 0.18	0.86 ± 0.12	0.88 ± 0.14	0.66 ± 0.09
Lymphocytes (10^3/µL)	4.18 ± 0.35	4.57 ± 0.32	4.81 ± 0.32	5.11 ± 0.29	4.35 ± 0.28	4.18 ± 0.31
Monocytes (10^3/µL)	0.18 ± 0.06	0.31 ± 0.06	0.30 ± 0.07	0.29 ± 0.06	0.25 ± 0.05	0.19 ± 0.06
Eosinophils (10^3/µL)	0.03 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.11 ± 0.03	0.09 ± 0.03	0.06 ± 0.02

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
Special Clinical Pathology Study						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 5	21.3 ± 0.5	22.8 ± 0.6	22.4 ± 0.6	22.7 ± 0.4	23.2 ± 0.8	28.5 ± 1.2**
Day 25	22.5 ± 0.8	23.2 ± 0.9	22.0 ± 0.7	22.8 ± 0.6	23.0 ± 0.4	23.7 ± 0.9
Day 45	23.4 ± 0.6	23.2 ± 0.4	22.5 ± 0.4	23.8 ± 0.3	24.6 ± 0.7	24.5 ± 0.9
Week 13	24.0 ± 0.7	22.0 ± 0.4	21.9 ± 0.5	22.1 ± 0.5	22.5 ± 0.8	24.2 ± 0.4
Creatinine (mg/dL)						
Day 5	0.44 ± 0.02	0.50 ± 0.02*	0.52 ± 0.02**	0.48 ± 0.01*	0.52 ± 0.02**	0.54 ± 0.02**
Day 25	0.58 ± 0.02	0.59 ± 0.02	0.61 ± 0.02	0.61 ± 0.03	0.61 ± 0.01	0.60 ± 0.02
Day 45	0.57 ± 0.02	0.51 ± 0.02	0.56 ± 0.02	0.59 ± 0.02	0.52 ± 0.01	0.57 ± 0.03
Week 13	0.64 ± 0.04	0.64 ± 0.03	0.63 ± 0.02	0.63 ± 0.03	0.65 ± 0.04	0.70 ± 0.03
Total protein (g/dL)						
Day 5	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.0	5.7 ± 0.1	5.8 ± 0.1*
Day 25	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.4 ± 0.1
Day 45	6.4 ± 0.2	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.6 ± 0.2	6.6 ± 0.1
Week 13	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2	6.7 ± 0.1	6.7 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 5	3.3 ± 0.1	3.3 ± 0.1	3.3 ± 0.0	3.3 ± 0.0	3.3 ± 0.0	3.5 ± 0.1*
Day 25	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	4.1 ± 0.1
Day 45	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	4.0 ± 0.1[2]
Week 13	4.0 ± 0.1	3.8 ± 0.1[2]	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.0	4.0 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	49 ± 1	43 ± 1**	48 ± 1	45 ± 2	53 ± 3	42 ± 1**
Day 25	49 ± 5	45 ± 1	44 ± 1	44 ± 2	39 ± 1**	45 ± 2*
Day 45	51 ± 2	47 ± 1	52 ± 2	51 ± 1	48 ± 1	51 ± 1
Week 13	55 ± 3	52 ± 2	52 ± 2	50 ± 3	51 ± 2	51 ± 2
Alkaline phosphatase (IU/L)						
Day 5	629 ± 14	629 ± 16	650 ± 8	626 ± 14	632 ± 19	635 ± 14
Day 25	470 ± 13	482 ± 13	493 ± 11	476 ± 14	503 ± 8	458 ± 12
Day 45	382 ± 10	371 ± 12	369 ± 9	377 ± 11	375 ± 5	368 ± 8
Week 13	294 ± 10[2]	285 ± 10	251 ± 17	268 ± 6	281 ± 8	275 ± 5
Creatine kinase (IU/L)						
Day 5	629 ± 50	611 ± 42	874 ± 130	742 ± 62	672 ± 66	798 ± 97
Day 25	675 ± 75	578 ± 52	732 ± 70	679 ± 68	792 ± 80	826 ± 132
Day 45	579 ± 47	618 ± 50	617 ± 48	511 ± 71	639 ± 59[2]	761 ± 92
Week 13	544 ± 88	482 ± 100	619 ± 75[2]	494 ± 78	499 ± 48	485 ± 62
Sorbitol dehydrogenase (IU/L)						
Day 5	6 ± 0	7 ± 0	7 ± 0*	7 ± 0*	8 ± 1	8 ± 0**
Day 25	8 ± 1	8 ± 0	8 ± 0	8 ± 1	8 ± 0	8 ± 0[2]
Day 45	10 ± 1	9 ± 0	9 ± 1	10 ± 0	10 ± 1	10 ± 1
Week 13	10 ± 1	9 ±1	9 ± 0	9 ± 0	10 ± 0	10 ± 1
5'-Nucleotidase (IU/L)						
Day 5	33.9 ± 1.2	34.2 ± 0.7	36.6 ± 1.3	33.1 ± 1.1	33.8 ± 0.7	31.7 ± 0.7
Day 25	34.5 ± 1.0	31.8 ± 2.2	33.7 ± 0.9	37.9 ± 2.1	36.7 ± 1.0	32.9 ± 1.0[2]
Day 45	32.1 ± 1.2	31.2 ± 0.6	33.6 ± 1.1	29.8 ± 1.1	30.4 ± 0.5	28.3 ± 0.6**
Week 13	36.6 ± 0.7	37.1 ± 1.4	38.9 ± 1.2	38.4 ± 0.9	38.6 ± 1.7	38.6 ± 1.1
Bile acids (µmol/L)						
Day 5	14.20 ± 1.43	13.00 ± 1.05	13.00 ± 1.70	13.20 ± 1.50	13.60 ± 1.38	10.90 ± 0.72
Day 25	14.80 ± 2.47	12.50 ± 1.35	9.60 ± 0.75*	13.00 ± 1.65	11.80 ± 1.20	15.40 ± 3.29
Day 45	17.60 ± 2.89	15.00 ± 2.01	11.30 ± 1.10	10.60 ± 0.62	13.70 ± 1.94	14.80 ± 2.44
Week 13	14.00 ± 2.03	15.30 ± 1.67	11.60 ± 1.34	16.60 ± 2.33	20.50 ± 3.83	12.50 ± 1.59

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide (continued)

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE (continued)						
Base Study						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	25.5 ± 0.5	24.1 ± 0.7	25.0 ± 0.6	27.0 ± 0.5	26.3 ± 0.9	28.3 ± 1.3
Creatinine (mg/dL)	0.60 ± 0.04	0.55 ± 0.02	0.59 ± 0.02	0.59 ± 0.02	0.58 ± 0.01	0.62 ± 0.01
Total protein (g/dL)	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.6 ± 0.4
Albumin (g/dL)	3.8 ± 0.0	3.8 ± 0.1	3.7 ± 0.0	3.8 ± 0.1	3.8 ± 0.0	3.9 ± 0.0*
Alanine aminotransferase (IU/L)	61 ± 2	58 ± 3	55 ± 1	63 ± 2	56 ± 2	65 ± 3
Alkaline phosphatase (IU/L)	281 ± 4	290 ± 6	273 ± 6	266 ± 4	286 ± 6	261 ± 10
Creatine kinase (IU/L)	518 ± 40	414 ± 27	440 ± 30	528 ± 49	485 ± 38	542 ± 43
Sorbitol dehydrogenase (IU/L)	10 ± 1	9 ± 0[2]	9 ± 0	11 ± 1	9 ± 0	13 ± 1*
5'-Nucleotidase (IU/L)	30.5 ± 0.9	30.4 ± 0.7	28.4 ± 0.5	28.3 ± 0.6	29.1 ± 0.7	29.6 ± 0.7
Bile acids (µmol/L)	12.90 ± 2.47	12.11 ± 0.95[2]	9.00 ± 0.33	13.70 ± 1.89	11.10 ± 1.05	14.10 ± 3.02
FEMALE						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	24.7 ± 1.0	25.3 ± 0.7	27.3 ± 1.3	26.8 ± 1.3	25.1 ± 0.9	27.6 ± 1.0
Creatinine (mg/dL)	0.64 ± 0.02	0.66 ± 0.02	0.67 ± 0.02	0.64 ± 0.02	0.65 ± 0.03	0.65 ± 0.02
Total protein (g/dL)	6.7 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
Albumin (g/dL)	4.2 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Alanine aminotransferase (IU/L)	46 ± 2	48 ± 3	50 ± 2	54 ± 3	48 ± 3	49 ± 2
Alkaline phosphatase (IU/L)	250 ± 9	257 ± 9	242 ±8	243 ± 5	246 ± 10	255 ± 8
Creatine kinase (IU/L)	266 ± 33	291 ± 38	268 ± 18	252 ± 22	300 ± 65	201 ± 37
Sorbitol dehydrogenase (IU/L)	10 ± 1	10 ± 1	11 ± 1	11 ± 1	11 ± 1	12 ± 1
5_-Nucleotidase (IU/L)	29.3 ± 0.6	29.7 ± 0.6	29.5 ± 0.6	29.7 ± 0.9	28.6 ± 0.9	28.6 ± 0.5
Bile acids (µmol/L)	25.00 ± 4.78	22.20 ± 4.36	22.50 ± 5.31	31.00 ± 6.01	29.30 ± 3.20	20.60 ± 3.78

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE B3 Urinalysis Data for Male F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
n						
Day 8	10	10	9	10	5	10
Day 22	10	5	9	10	9	10
Day 43	10	10	10	10	10	10
Day 88	6	10	9	10	10	9
Thiocyanate (µg/mL)						
Day 8	5.1 ± 2.0[2]	6.1 ± 1.9[3]	9.7 ± 1.0*[3]	28.3 ± 2.2**[4]	–[5]	202.0 ± 10.8**[6]
Day 22	12.2 ± 3.1[2]	15.7 ± 4.9[7]	21.0 ± 3.3	39.7 ± 6.9**	100.7 ± 9.9**[8]	155.1 ± 39.3**[7]
Day 43	7.4 ± 2.7	7.8 ± 1.7	25.5 ± 6.9**[2]	48.4 ± 8.4**	111.8 ± 9.8**	224.7 ± 55.5**[8]
Day 88	7.5 ± 2.5	5.4 ± 1.6	12.2 ± 1.8[3]	40.9 ± 4.0**	112.1 ± 15.5**	242.4 ± 31.7**[9]
Sorbitol dehydrogenase (IU/L)						
Day 8	3.0 ± 0.8	3.4 ± 0.8	2.4 ± 0.5	2.7 ± 0.4	2.5 ± 0.5[6]	3.3 ± 0.6[3]
Day 22	1.7 ± 0.4	1.4 ± 0.2	2.3 ± 0.6	2.2 ± 0.8	1.8 ± 0.4	2.2 ± 0.6
Day 43	0.9 ± 0.2	1.7 ± 0.5	2.1 ± 0.6	1.7 ± 0.2	2.2 ± 0.5*	3.4 ± 0.5**[2]
Day 88	1.2 ± 0.5	1.8 ± 0.3	1.8 ± 0.6	2.2 ± 0.3	2.3 ± 0.7	4.0 ± 0.9*
N-acetyl-β-D-glucosaminidase (IU/L)						
Day 8	6.7 ± 0.8	8.1 ± 0.9	7.8 ± 0.8	9.5 ± 0.7*	21.6 ± 2.1**[6]	14.5 ± 1.0**[3]
Day 22	6.7 ± 0.7	7.6 ± 1.4	7.4 ± 0.8	7.5 ± 0.8	8.1 ± 1.1	10.3 ± 1.1**
Day 43	6.9 ± 0.9	7.5 ± 0.7	6.6 ± 1.1[2]	5.4 ± 1.0	6.3 ± 0.6	7.8 ± 0.9
Day 88	8.1 ± 1.8	9.0 ± 1.1	8.7 ± 1.4	9.7 ± 0.5	9.2 ± 1.6	11.5 ± 1.8
Ribonuclease (U/mL)						
Day 8	0.06 ± 0.01[2]	0.10 ± 0.01[3]	0.10 ± 0.01*[3]	0.09 ± 0.02[4]	–	0.15 ± 0.00**[6]
Day 22	0.07 ± 0.02[2]	0.07 ± 0.03[7]	0.08 ± 0.01	0.07 ± 0.01	0.12 ± 0.02*[8]	0.15 ± 0.04[7]
Day 43	0.13 ± 0.02	0.12 ± 0.02	0.09 ± 0.02[2]	0.11 ± 0.01	0.13 ± 0.01	0.20 ± 0.01**[8]
Day 88	0.11 ± 0.02	0.14 ± 0.02	0.13 ± 0.01[3]	0.17 ± 0.02*	0.16 ± 0.01	0.21 ± 0.03**[9]
Volume (mL/16 hr)						
Day 8	4.8 ± 0.9	3.9 ± 0.7	3.9 ± 0.6	2.8 ± 0.4	0.5 ± 0.2**	1.3 ± 0.3**
Day 22	7.6 ± 1.6	4.7 ± 1.4[8]	5.2 ± 1.2[10]	6.6 ± 1.1	4.8 ± 1.1	2.8 ± 0.8**
Day 43	8.3 ± 1.3	8.4 ± 0.9	8.9 ± 2.8	7.8 ± 1.1	6.9 ± 1.1	2.8 ± 0.4**
Day 88	9.2 ± 2.1	6.8 ± 0.8	7.1 ± 1.6[10]	5.5 ± 0.6	6.4 ± 1.2	3.3 ± 0.6**[10]
Specific gravity						
Day 8	1.019 ± 0.002	1.026 ± 0.003	1.022 ± 0.002	1.026 ± 0.002*	1.046 ± 0.015*[7]	1.044 ± 0.006**
Day 22	1.019 ± 0.002	1.019 ± 0.004[3]	1.019 ± 0.003[10]	1.017 ± 0.001	1.021 ± 0.004	1.028 ± 0.003*
Day 43	1.019 ± 0.002	1.017 ± 0.001	1.032 ± 0.009	1.023 ± 0.005	1.032 ± 0.005	1.063 ± 0.011**
Day 88	1.024 ± 0.008	1.019 ± 0.002	1.031 ± 0.009	1.037 ± 0.005*	1.031 ± 0.005	1.050 ± 0.007**[1]
pH						
Day 8	6.50 ± 0.07	6.40 ± 0.07	6.56 ± 0.06	6.45 ± 0.09	6.30 ± 0.20	6.40 ± 0.16
Day 22	6.75 ± 0.11	6.40 ± 0.10	6.60 ± 0.10[10]	6.60 ± 0.10	6.44 ± 0.10	6.35 ± 0.08**
Day 43	6.70 ± 0.17	6.35 ± 0.08	6.55 ± 0.16	6.60 ± 0.10	6.35 ± 0.11	6.25 ± 0.11*
Day 88	6.92 ± 0.15	6.85 ± 0.11	6.80 ± 0.11[10]	6.50 ± 0.07*	6.75 ± 0.15	6.50 ± 0.12

1 Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
2 n=9.
3 n=8.
4 n=7.
5 Not measured for this exposure group.
6 n=2.
7 n=4.
8 n=6.
9 n=5.
10 n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE B4 Hematology Data for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
n	10	10	9	10	10	10
Hematocrit (%)	48.9 ± 0.7	48.2 ± 0.4	48.9 ± 0.6	48.7 ± 0.2	48.7 ± 0.4	50.2 ± 0.6*
Hemoglobin (g/dL)	15.7 ± 0.3	15.5 ± 0.1	15.6 ± 0.2	15.8 ± 0.1	15.6 ± 0.2	16.1 ± 0.2
Erythrocytes (10^6/µL)	10.31 ± 0.14	10.15 ± 0.09	10.31 ± 0.15	10.29 ± 0.06	10.26 ± 0.09	10.62 ± 0.13*
Reticulocytes (10^6/µL)	0.20 ± 0.03[2]	0.18 ± 0.02	0.25 ± 0.02	0.18 ± 0.01[2]	0.17 ± 0.02	0.17 ± 0.02[2]
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Mean cell volume (fL)	47.5 ± 0.2	47.3 ± 0.2	47.4 ± 0.3	47.4 ± 0.2	47.5 ± 0.2	47.4 ± 0.3
Mean cell hemoglobin (pg)	15.3 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.2 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.3	32.1 ± 0.3	32.0 ± 0.2	32.4 ± 0.2	32.1 ± 0.2	32.0 ± 0.2
Platelets (10^3/µL)	918.4 ± 56.9	910.1 ± 53.6	907.1 ± 25.2	979.5 ± 13.6	972.9 ± 45.2	928.3 ± 20.1
Leukocytes (10^3/µL)	5.55 ± 0.48	5.88 ± 0.27	5.43 ± 0.15	6.23 ± 0.39	5.95 ± 0.34	6.35 ± 0.47
Segmented neutrophils (10^3/µL)	0.71 ± 0.11	0.74 ± 0.11	0.74 ± 0.07	0.75 ± 0.10	0.75 ± 0.04	0.98 ± 0.10*
Lymphocytes (10^3/µL)	4.71 ± 0.42	4.98 ± 0.27	4.57 ± 0.13	5.35 ± 0.42	5.08 ± 0.33	5.19 ± 0.38
Monocytes (10^3/µL)	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.02
Eosinophils (10^3/µL)	0.12 ± 0.03	0.12 ± 0.03	0.10 ± 0.02	0.13 ± 0.04	0.13 ± 0.02	0.14 ± 0.04
FEMALE						
n	9	10	10	8	10	10
Hematocrit (%)	49.6 ± 1.3	48.5 ± 0.5	48.9 ± 0.4	48.6 ± 0.4	49.0 ± 0.5	48.6 ± 0.5
Hemoglobin (g/dL)	15.9 ± 0.3	15.6 ± 0.2	15.8 ± 0.1	15.6 ± 0.2	15.8 ± 0.1	15.8 ± 0.1
Erythrocytes (10^6/µL)	10.38 ± 0.25	10.07 ± 0.11	10.08 ± 0.06	10.09 ± 0.11	10.28 ± 0.11	10.11 ± 0.11
Reticulocytes (10^6/µL)	0.31 ± 0.05	0.31 ± 0.04	0.32 ± 0.03	0.33 ± 0.05	0.34 ± 0.04	0.36 ± 0.06
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01*
Mean cell volume (fL)	47.8 ± 0.4	48.1 ± 0.2	48.5 ± 0.2	48.1 ± 0.2	47.7 ± 0.2	48.2 ± 0.1
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.5 ± 0.1	15.7 ± 0.1**	15.5 ± 0.1	15.4 ± 0.1	15.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.2	32.3 ± 0.1	32.4 ± 0.1	32.1 ± 0.2	32.3 ± 0.1	32.4 ± 0.1
Platelets (10^3/µL)	883.8 ± 27.4	949.5 ± 33.3	899.1 ± 42.6	841.8 ± 36.0	919.1 ± 13.4	902.1 ± 18.1
Leukocytes (10^3/µL)	5.00 ± 0.33	4.97 ± 0.44	5.11 ± 0.33	5.38 ± 0.52	5.44 ± 0.38	5.59 ± 0.51
Segmented neutrophils (10^3/µL)	0.69 ± 0.07	0.62 ± 0.07	0.48 ± 0.04	0.59 ± 0.08	0.75 ± 0.09	0.71 ± 0.12
Lymphocytes (10^3/µL)	4.21 ± 0.33	4.19 ± 0.41	4.47 ± 0.29	4.65 ± 0.48	4.58 ± 0.38	4.72 ± 0.44
Monocytes (10^3/µL)	0.06 ± 0.02	0.03 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.04 ± 0.03	0.06 ± 0.02
Eosinophils (10^3/µL)	0.06 ± 0.02	0.14 ± 0.04	0.11 ± 0.02	0.11 ± 0.04	0.08 ± 0.02	0.09 ± 0.03

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test.

TABLE B5 Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide[1]

	0 ppm	3 ppm	10 ppm	30 ppm	100 ppm	300 ppm
MALE						
n	8	10	10	8	10	10
Urea nitrogen (mg/dL)	29.8 ± 1.4[2]	30.3 ± 0.7	31.7 ± 1.1[3]	29.1 ± 0.8	30.7 ± 0.8	27.2 ± 1.0
Creatinine (mg/dL)	0.40 ± 0.02[4]	0.36 ± 0.02[4]	0.34 ± 0.01[5]	0.40 ± 0.02[4]	0.38 ± 0.02[4]	0.35 ± 0.03[6]
Total protein (g/dL)	6.0 ± 0.2	5.7 ± 0.1[6]	5.9 ± 0.1[4]	5.8 ± 0.1[7]	5.9 ± 0.1	5.9 ± 0.1
Albumin (g/dL)	3.3 ± 0.1	3.2 ± 0.1[7]	3.2 ± 0.1[8]	3.2 ± 0.1[9]	3.2 ± 0.1	3.3 ± 0.1[6]
Alanine aminotransferase (IU/L)	61 ± 15	33 ± 3	48 ± 9[6]	44 ± 6	34 ± 2[6]	54 ± 12
Alkaline phosphatase (IU/L)	71 ± 2[2]	69 ± 2	70 ± 2	68 ± 2[3]	72 ± 2	73 ± 2
Creatine kinase (IU/L)	321 ± 70[2]	221 ± 39	640 ± 112	340 ± 72[2]	304 ± 40	496 ± 142
Sorbitol dehydrogenase (IU/L)	38 ± 2	40 ± 2[3]	37 ± 2[4]	39 ± 2	37 ± 1[3]	43 ± 2
5_-Nucleotidase (IU/L)	39.7 ± 8.2[5]	35.2 ± 2.2[9]	39.0[10]	37.0 ± 2.3[9]	33.0 ± 2.1[9]	40.4 ± 5.0[9]
FEMALE						
n	8	10	10	8	9	10
Urea nitrogen (mg/dL)	28.5 ± 1.4	28.9 ± 1.8	29.2 ± 1.2	27.5 ± 1.6	26.6 ± 1.1	27.6 ± 1.8
Creatinine (mg/dL)	0.34 ± 0.03[8]	0.34 ± 0.02[4]	0.33 ± 0.02[9]	0.35 ± 0.03[5]	0.32 ± 0.03[9]	0.36 ± 0.02[9]
Total protein (g/dL)	5.6 ± 0.1[7]	5.5 ± 0.1	5.6 ± 0.1[6]	5.5 ± 0.1[7]	5.7 ± 0.1[4]	5.7 ± 0.1[3]
Albumin (g/dL)	3.4 ± 0.1[4]	3.5 ± 0.1[6]	3.4 ± 0.0[3]	3.5 ± 0.1[4]	3.6 ± 0.0*	3.6 ± 0.1**[6]
Alanine aminotransferase (IU/L)	29 ± 2[7]	34 ± 5	36 ± 3	28 ± 1	37 ± 4[7]	29 ± 2[7]
Alkaline phosphatase (IU/L)	102 ± 2	107 ± 4	101 ± 5	105 ± 2	106 ± 4	106 ± 4

1 Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
2 n=10.
3 n=9.
4 n=6.
5 n=3.
6 n=8.
7 n=7.
8 n=4.
9 n=5.
10 n=1.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	338 ± 5	335 ± 5	338 ± 4	319 ± 5*
Left epididymis	0.448 ± 0.006	0.437 ± 0.005	0.425 ± 0.007	0.417 ± 0.005**
Left cauda epididymis	0.162 ± 0.003	0.150 ± 0.004*	0.148 ± 0.004*	0.141 ± 0.003**
Left testis	1.58 ± 0.03	1.56 ± 0.02	1.52 ± 0.02	1.46 ± 0.02**
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.35 ± 0.38	10.88 ± 0.53	10.92 ± 0.37	10.57 ± 0.33
Spermatid heads (10^7/testis)	17.86 ± 0.61	16.94 ± 0.81	16.58 ± 0.63	15.42 ± 0.44*
Spermatid count ($mean/10^{-4}$ mL suspension)	89.28 ± 3.05	84.68 ± 4.03	82.90 ± 3.16	77.10 ± 2.20*
Epididymal spermatozoal measurements				
Motility (%)	94.24 ± 0.58	90.67 ± 1.25*	92.09 ± 0.85*	90.66 ± 1.46*
Concentration (10^6/g cauda epididymal tissue)	615 ± 42	684 ± 40	699 ± 33	709 ± 45

1 Data are presented as mean ± standard error. Differences from the control group for spermatid heads/g testis and spermatozoal concentration are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Dunnett's (necropsy body weight only) or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Ratsin the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	10	10	10	10
Necropsy body weight (g)	193 ± 4	197± 3	195 ± 3	198 ± 4
Estrous cycle length (days)	4.95 ± 0.12	5.10 ± 0.16	4.75 ± 0.11	5.25 ± 0.11
Estrous stages[2] (% of cycle)				
Diestrus	33.3	41.7	38.3	41.7
Proestrus	12.5	7.5	14.2	20.0
Estrus	35.0	35.0	33.3	24.2
Metestrus	18.3	15.8	12.5	14.2
Uncertain diagnoses	0.8	0.0	1.7	0.0

1 Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for estrous cycle length are not significant by Dunn's test.
2 Evidence suggests that females in the 100 and 300ppm groups differ significantly (P=0.03, Wilk's Criterion) from the control females in the relative length of time spent in estrous stages. Females in these two groups spent more time in proestrus and diestrus and less time in estrus and metestrus than control females.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	9	10	10	9
Weights (g)				
Necropsy body weight	37.3 ± 1.0	39.2 ± 1.3	38.6 ± 1.1	35.5 ± 1.1
Left epididymis	0.049 ± 0.001	0.047 ± 0.002	0.047 ± 0.001	0.044 ± 0.001*
Left cauda epididymis	0.017 ± 0.001	0.016 ± 0.000	0.015 ± 0.001	0.014 ± 0.001*
Left testis	0.121 ± 0.002	0.113 ± 0.008	0.117 ± 0.002	0.118 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.47 ± 1.13	21.48 ± 2.34	17.42 ± 1.34	18.17 ± 1.62
Spermatid heads (10^7/testis)	2.24 ± 0.14	2.26 ± 0.14	2.03 ± 0.15	2.11 ± 0.16
Spermatid count (mean/10^{-4} mL suspension)	69.94 ± 4.34	70.80 ± 4.25	63.28 ± 4.53	66.06 ± 4.87
Epididymal spermatozoal measurements				
Motility (%)	92.38 ± 0.81	90.63 ± 1.34	91.43 ± 0.55	89.52 ± 0.96
Concentration (10^6/g cauda epididymal tissue)	1,235 ± 82	1,393 ± 70	1,386 ± 70	1,462 ± 101

1 Data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test; differences from the control group for testis weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's or Shirley's test.

* Significantly different (P≤0.05) from the control group by Shirley's test.

TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Micein the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	9	10	10	10
Necropsy body weight (g)	31.3 ± 0.9[2]	30.3 ± 0.7[2]	30.3 ± 1.0[3]	30.0 ± 0.9
Estrous cycle length (days)	4.06 ± 0.06	4.20 ± 0.20	4.25 ± 0.11	4.15 ± 0.08
Estrous stages (% of cycle)				
Diestrus	36.1	37.5	33.3	35.0
Proestrus	21.3	17.5	21.7	19.2
Estrus	30.6	31.7	30.0	30.8
Metestrus	12.0	13.3	15.0	14.2
Uncertain diagnoses	0.0	0.0	0.0	0.8

1 Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Williams' test; differences from the control group for estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in cycle length or in the relative length of time spent in the estrous stages.

2 n=8.

3 n=9.

APPENDIX D

Genetic Toxicology

TABLE D Mutagenicity of Sodium Cyanide in *Salmonella typhimurium*[1]

Strain	Dose (μg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA100	0.0	103 ± 1.5	89 ± 6.2	92 ± 3.5	107 ± 9.3	108 ± 1.2	86 ± 6.9	116 ± 3.5
	1.0	89 ± 9.8		104 ± 4.5		108 ± 5.5		121 ± 4.5
	3.3	108 ± 1.2	83 ± 9.4	89 ± 4.6	104 ± 7.0	111 ± 3.8	94 ± 1.7	120 ± 6.0
	10.0	101 ± 1.5	82 ± 9.0	108 ± 3.7	114 ± 6.1	117 ± 10.0	116 ± 4.6	133 ± 10.3
	33.0	103 ± 6.6	91 ± 8.6	85 ± 1.2	99 ± 11.0	108 ± 4.3	107 ± 12.6	121 ± 6.0
	100.0	101 ± 7.8	3 ± 2.5	96 ± 2.9	79 ± 9.5	115 ± 6.0	80 ± 6.6	122 ± 12.4
	333.0		0 ± 0.0		1 ± 0.7		12 ± 4.4	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[3]		415 ± 31.5	348 ± 15.5	644 ± 59.0	719 ± 25.7	448 ± 41.5	1,125 ± 21.5	706 ± 45.9
TA1535	0.0	14 ± 2.3	10 ± 1.5		13 ± 0.3	16 ± 2.1	11 ± 0.7	13 ± 2.8
	1.0	14 ± 0.3				21 ± 2.7		14 ± 2.5
	3.3	14 ± 0.6	9 ± 0.7		9 ± 2.1	16 ± 3.0	10 ± 2.1	14 ± 1.2
	10.0	9 ± 1.2	7 ± 1.8		13 ± 2.6	13 ± 1.8	8 ± 1.5	14 ± 2.4
	33.0	12 ± 0.9	4 ± 0.7		12 ± 2.7	19 ± 3.8	7 ± 2.6	16 ± 1.0
	100.0	12 ± 2.3	3 ± 0.7		11 ± 2.8	21 ± 1.2	8 ± 1.2	18 ± 1.2
	333.0		0 ± 0.0		4 ± 1.7		3 ± 1.5	
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		516 ± 8.2	200 ± 6.5		80 ± 0.9	92 ± 6.4	141 ± 6.1	178 ± 6.6
TA97	0.0	143 ± 3.2	115 ± 2.3		142 ± 4.7	191 ± 11.9	134 ± 8.5	206 ± 9.2
	1.0	119 ± 4.5				167 ± 12.7		226 ± 9.5
	3.3	133 ± 3.5	123 ± 5.9		154 ± 6.0	167 ± 9.6	144 ± 4.4	217 ± 8.0
	10.0	119 ± 6.2	100 ± 4.5		149 ± 9.4	179 ± 9.4	134 ± 4.3	192 ± 3.8
	33.0	130 ± 4.6	106 ± 5.9		156 ± 7.5	173 ± 9.1	143 ± 9.1	219 ± 3.9
	100.0	163 ± 3.0	109 ± 4.9		163 ± 6.6	179 ± 5.0	128 ± 8.9	229 ± 7.8
	333.0		76 ± 15.6		130 ± 21.5		116 ± 7.7	
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		721 ± 103.8	355 ± 32.1		1,222 ± 40.0	1,900 ± 122.0	1,533 ± 62.5	1,097 ± 43.9

TABLE D Mutagenicity of Sodium Cyanide in *Salmonella typhimurium* (continued)

Strain	Dose	Revertants/plate -S9			+hamster S9		
	(µg/plate)	Trial 1	Trial 2	Trial 3	10%	10%	30%
TA98	0.0	19 ± 1.5	10 ± 2.3	15 ± 2.1	18 ± 2.4	23 ± 2.5	18 ± 2.4
	0.3			14 ± 1.5		16 ± 2.2	
	1.0	12 ± 1.5		13 ± 3.6		20 ± 2.0	20 ± 2.0
	3.3	14 ± 1.5	5 ± 2.0	16 ± 3.3	13 ± 2.6	16 ± 3.8	21 ± 3.2
	10.0	14 ± 1.8	0 ± 0.0	13 ± 1.7	14 ± 2.9	22 ± 3.3	24 ± 1.2
	33.0	11 ± 1.0	1 ± 1.0	12 ± 0.7	8 ± 0.6	16 ± 1.5	13 ± 3.2
	100.0	0 ± 0.0	0 ± 0.0		3 ± 0.6		7 ± 2.2
	333.0		0 ± 0.0		0 ± 0.3		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		321 ± 10.5	253 ± 67.0	423 ± 9.7	706 ± 24.1	406 ± 26.5	387 ± 44.5

TA98 (continued)

		+rat S9		
		10%	10%	30%
	0.0	18 ± 3.0	19 ± 2.3	22 ± 0.0
	0.3		19 ± 3.2	
	1.0		23 ± 2.2	24 ± 2.2
	3.3	21 ± 2.6	17 ± 1.7	24 ± 2.6
	10.0	12 ± 2.4	20 ± 1.5	23 ± 1.8
	33.0	9 ± 0.7	17 ± 1.5	22 ± 2.1
	100.0	3 ± 0.0		7 ± 1.5
	333.0	0 ± 0.0		
Trial summary		Negative	Negative	Negative
Positive control		433 ± 24.2	199± 8.0	407 ± 46.6

1 Study was performed at Microbiological Associates, Inc. The detailed protocol is presented in Zeiger *et al.* (1992); 0µg/plate is the solvent control.

2 Revertants are presented as mean ± standard error from three plates.

3 The positive controls in the absence of metabolic activation were 4-nitro-*o*-phenylenediamine (TA98), sodium azide (TA100 and TA1535), and 9-aminoacridine (TA97). The positive control for metabolic activation with all strains was 2-aminoanthracene.

NTP TECHNICAL REPORTS ON TOXICITY STUDIES PRINTED AS OF NOVEMBER 1993

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	***n***-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	***o***-Cresol ***m***-Cresol ***p***-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	***p***-Chloro-_,_,_-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	***t***-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	***o***-Nitrotoluene ***m***-Nitrotoluene ***p***-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
PRINTED AS OF NOVEMBER 1993 (continued)

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385