

Animal Toxicity

Although toxic effects such as respiratory tract irritation, hemolytic anemia, kidney and liver damage, CNS effects, and tumorigenic effects have been observed in experimental animals given hydrazines, the type and severity of the response induced by each hydrazine may be different, despite the similarity in molecular structures; therefore, each compound will be discussed separately. Relevant literature for each compound will be grouped into three areas: systemic effects, metabolism, and carcinogenicity and effects related to reproduction.

(a) Hydrazine

(1) Systemic Effects

In 1954, Comstock et al [55] described the effects of hydrazine vapor on rats, mice, dogs, and guinea pigs. Several experimental designs were used, from a 6-hour/day, 5-day/week, 6-month exposure to a single exposure of 0.5-4 hours. When rats were exposed at several hundred mg/cu m for 2-4 hours in 20-liter glass jars, 50% or more died and they had pulmonary edema and localized damage of the bronchial mucosa. However, the concentrations of hydrazine at which these rats were exposed were in question; in similar experiments, nominal concentrations of 16,000-27,000 mg/cu m were calculated from mass balance, but titration analysis indicated only 106-831 mg/cu m. The authors found that 74% of the hydrazine in the air was lost in an empty jar, but 96-99% was lost when six rats were placed in the jar. This loss seemed to be largely or entirely caused by surface sorption.

To minimize the adsorption of hydrazine by the walls, the authors then used a 440-liter chamber, and only analytical concentrations were

reported [55]. Of 20 rats and 10 mice exposed at 295 mg/cu m, 6 hours/day, 5 days/week, 16 rats and 8 mice died (80% mortality) in the 1st week of exposure. Fatty degeneration of the liver was found, in addition to pulmonary changes similar to those mentioned above. Of the 16 rats and 10 mice exposed at 70 mg/cu m, 70-87% mortality was reached after 3 weeks of exposure (13 exposures). Of the animals exposed at 26 mg/cu m for 6 weeks, 7 of 10 mice died by the 14th exposure and the rest survived, and 11 of 13 rats died by the end of exposure. For the 6-month study, a 1,000-liter chamber was used. Four dogs, 30 rats, 20 mice, and 10 guinea pigs were exposed at 18 mg/cu m. By the end of the experiment, 2 dogs, 23 rats, 15 mice, and 8 guinea pigs had died. Necropsies on surviving dogs revealed lipid deposition in the spleen and Kupffer cells of the lobular zone of the liver. Two of the dogs also had evidence of anemia. Necropsies on surviving mice showed no abnormalities. No necropsies were performed on the other animals. In addition, 2 dogs and 20 rats were exposed at 6 mg/cu m for 6 months. While two rats died, the dogs survived but had toxic signs such as loss of appetite, loss of body weight, vomiting, irregular breathing, fatigue, and tremors. From these tests, the authors [55] suggested that the maximum allowable concentration for hydrazine should be lower than 6 mg/cu m.

The acutely toxic effects of hydrazine and some derivatives were studied by Jacobson et al [20] in 1955. Rodents exposed to hydrazine were restless, and they had breathing difficulties, convulsions, and exophthalmos. Most of the convulsions were clonic, but some were tonic-clonic. The LC50 values for rats and mice were 570 ppm (750 mg/cu m) and 252 ppm (330 mg/cu m), respectively.

Haun and Kinkead [56], in 1973, reported a 6-month inhalation study of hydrazine. Four experimental groups and a control group were used, each containing 8 male beagle dogs, 4 female Rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR mice. The experimental groups were exposed to anhydrous hydrazine for 6 months at a concentration of 1 or 0.2 ppm continuously, or at 5 or 1 ppm intermittently. Continuous exposure was for 24 hours/day, 7 days/week, and intermittent exposure was for 6 hours/day, 5 days/week. Therefore, the corresponding products of the exposure concentration and the duration of exposure are 168 or 33.6 ppm-hours/week and 150 or 30 ppm-hours/week.

The authors [56] observed that mortality and weight changes were dose-related, regardless of whether exposure was continuous or intermittent. Exposure at 150 or 168 ppm-hours/week caused 35-40% mortality in mice within 2 months, while exposure at 30 or 33.6 ppm-hours/week caused only 2.5-7.5% mortality. No monkeys died and the one rat death was not attributed to hydrazine. Two of the eight dogs exposed at 168 ppm-hours/week died after 16 weeks; there were no other deaths in dogs.

Rats showed a dose-related growth rate depression [56]. At the end of exposure, the largest weight difference, 35 g, was found between the 150 ppm-hours/week group and the controls. Weight loss in dogs occurred only in the groups exposed at 150 and 168 ppm-hours/week, and the four dogs retained after exposure recovered their lost weight in 2 weeks.

Results of clinical chemistry tests and blood cell counts in monkeys and rats were reported to be normal [56]. After 8 weeks of exposure, reductions in the hematocrit value, hemoglobin concentration, and erythrocyte count of 11, 16-22, and 10-12% respectively, were observed in

the dogs exposed at 150 and 168 ppm-hours/week. Although, there was a tendency toward recovery, no value reached normal by the end of exposure. All these hematologic values had returned to normal 2 weeks after exposure ended. Reticulocytosis occurred in the dogs exposed at 168 ppm-hours/week 20 weeks after exposure had begun. These dogs also had increased erythropoietic activity, which was evident in the decreased myeloid-erythroid ratios in marrow samples. Blood counts from dogs exposed to hydrazine at 30 or 33.6 ppm-hours/week were within normal limits. Clinical chemistry, Heinz body counts, and methemoglobin concentrations of all exposed dogs were within normal limits. Dogs exposed at 150 or 168 ppm-hours/week began to show increased resistance to osmotic hemolysis in the erythrocytes at 8 weeks. Similar effects were observed in dogs exposed at 30 or 33.6 ppm-hours/week beginning at week 12; these effects continued for all groups throughout the rest of the exposure period.

Gross and microscopic examination of the tissues of the mice from all exposure levels showed moderate to severe fatty liver changes, which the authors considered to have been the cause of death in those mice dying during exposure [56]. Exposed monkeys had slight to moderate fat accumulation in the liver, but the controls also had some degree of fatty changes. In dogs, only those exposed at 150 or 168 ppm-hours/week had fatty degeneration of the liver. There were no significant changes in rats. Organ weights of the exposed rats, monkeys, and dogs did not differ significantly from those of the controls.

Haun and Kinkead [56] concluded that, if humans are not less sensitive than the mice, a Threshold Limit Value (TLV) of 1 ppm would not be safe.

As part of a series of experiments with hydrazine, Thienes and coworkers [57], in 1948, described the effects of contact application to the skin and eyes of animals. Anhydrous hydrazine applied in a petrolatum well to the shaved bellies of a rat (2 drops) and a guinea pig (3 drops) caused death in 2 hours. Three rabbits had 3 ml of hydrazine applied through a cloth onto a shaved area of the belly for 1 minute. In two rabbits, one of which was anesthetized, the bellies were washed with water after the cloth had been removed; in the third, no effort was made to remove the hydrazine. Two rabbits died at 60 and 90 minutes after application. The anesthetized rabbit survived and within 2 hours, the affected skin first reddened, then turned blue, eventually turning brown with a dry, burned appearance. The site of application in this rabbit became dry, scaly, crusted, and inflamed before healing. Other rabbits had gauze containing 3 cc of 5-25% hydrazine applied to their bellies. When the gauze was left in place for 1 hour, only the 25% solution caused slight skin irritation; this 25% solution applied for 4 hours was lethal to two of three rabbits. When 0.2 cc of anhydrous hydrazine was applied in a cloth band over the shaved belly of two rats, they died even though the band was removed after 1 minute and the area washed.

One drop of anhydrous hydrazine permanently damaged the eyes of rats and rabbits when no effort was made to remove it [57]. When 1 drop of diluted hydrazine was placed on the eyes of rabbits six times at 10-minute intervals, permanent damage resulted with solutions of 25% or greater. At 1% or lower, there was no visible reaction.

Rothberg and Cope [58] measured the acute toxicity of several hydrazines by the intravenous (iv) and percutaneous routes. Rabbits were

injected iv and observed for 24 hours. Each hydrazine compound was applied to a 100-sq cm clipped area on the backs of guinea pigs and rabbits. The animals were protected from inhalation of the compound, but the site of application was not covered. Hydrazine (3 μ l) was also placed in the left eyes of two rabbits, and the eyes were examined for damage for 10 days. The LD50 by the iv route was 26 μ l/kg (26 mg/kg). By percutaneous absorption, it was 93 mg/kg in rabbits and 190 mg/kg in guinea pigs. Following application of hydrazine, the skin at the site turned bluish-black. After 24 hours, the discoloration penetrated deeply into the dermis, and there was acute local inflammation and moderate edema. By 72 hours, this area had eroded into the subcutaneous tissue and the surrounding area was mildly erythematous and moderately edematous. Hydrazine in the eyes caused corneal damage. Conjunctivitis and erythema of the eyelids occurred at 48 hours and were followed by a slight corneal opacity that persisted throughout the 7-day observation period.

In 1972, Smith and Clark [59] described the effect of hydrazine absorbed through canine skin. Hydrazine at concentrations of 3-15 millimoles/kg (96-480 mg/kg) was applied to a 15- x 20-cm shaved area on the chest of 25 anesthetized mongrel dogs. The appearance of the skin and signs of toxicity were noted for 6 hours. Hydrazine and glucose concentrations in the blood and urine and reduced glutathione and glutathione peroxidase activity in the erythrocytes were measured at specific intervals. Preexposure measurements were made, and the animals served as their own controls.

When hydrazine was applied to the skin, a chemical burn developed [59]. Ten of the 25 dogs died; the time of death, but not the percentage

dying, was dose-related. Hydrazine was detectable in the blood within 30 seconds after application; the concentration increased to a plateau at 20-60 minutes. The concentration of hydrazine in the urine was variable and did not correlate with blood levels. Blood glucose concentrations were elevated initially, but they then declined to subnormal values. There was no effect on reduced glutathione or glutathione peroxidase activity.

The authors [59] noted the rapidity with which hydrazine was absorbed through the skin. They also commented that about one out of three dogs was a "hyperresponder," exhibiting plasma hydrazine concentrations 2-4 times those of the others.

In 1965, Patrick and Back [60] reported the toxic effects of repeated injections of hydrazine on Sprague-Dawley rats and Rhesus monkeys. Groups of 25 male rats, weighing 308-386 g each, received 10 or 20 mg/kg of practical grade hydrazine (64% hydrazine) intraperitoneally (ip) daily, 5 days/week, for 5 weeks. Five control rats received no injections, and 10 others received distilled water ip. Up to five animals from each dose regimen were killed each week to evaluate progressive tissue changes. Ten rats that received 20 mg/kg daily died between the 8th and 21st injections; all others survived. When blood samples were examined, the major finding was an elevated SGOT activity in both groups of experimental rats. In the 25 rats given 20-mg/kg, gross examination showed severe pulmonary congestion and edema in four rats. Microscopically, slight hepatic cell vacuolization was observed in seven animals. The 10-mg/kg group was normal.

Six Rhesus monkeys received hydrazine ip at 5 mg/kg/day for 5 days/week for 4 weeks; two of these monkeys subsequently received 10

mg/kg/day for 8 additional days and then 20 mg/kg for 4-5 more days [60]. Six other monkeys received 20 mg/kg/day of hydrazine ip for 4-5 days. There were no clinical signs of toxicity in the four monkeys that received hydrazine at 5 mg/kg/day for 20 doses. All monkeys, however, lost between 0.4 and 0.9 kg, and they did not regain their initial weights by the end of the experiment. Seven of eight monkeys that received hydrazine doses of 10 mg/kg or more vomited and showed signs of lethargy and weakness; one animal developed tremors. At the 5-mg/kg dosage level, only slight decreases in hematocrit value and hemoglobin concentration were observed. In the monkeys that received 20 mg/kg of hydrazine for 4 or 5 days, the terminal SGOT activity increased 3- to 200-fold, but plasma glucose levels were insignificantly increased. Grossly, the liver was uniformly pale and slightly enlarged in all animals receiving hydrazine at 20 mg/kg/day. Microscopically, the kidneys, heart, skeletal muscles, and liver showed pronounced fatty changes. One animal that received twenty 5-mg/kg doses showed moderate amounts of lipid deposition in the liver and kidneys, and three had lipid deposition in the myocardium. Monkeys that received doses of hydrazine from 5 to 20 mg/kg had normal kidneys and hearts and showed less accumulation of lipids in the liver than did animals that received four to five doses at 20 mg/kg/day. The authors concluded that the major toxic effect of injected hydrazine was lipid accumulation in the liver. It was noted that monkeys were more susceptible to liver and kidney damage than were rats, as reflected by more lipid accumulation and higher SGOT activities in the former.

In 1966, Wong [61] described changes in renal function in anesthetized mongrels for up to 4 hours after the iv injection of

hydrazine. Eight fasted females weighing 12.5-21.0 kg and six control dogs were given creatinine and glucose by sustained infusion and urine was collected by catheterization. In controls, creatinine clearance was measured every 20 minutes for 5 hours. In the experimental group, three baseline values were obtained, then 20 mg/kg of hydrazine was given iv and 20-minute urine samples were collected for the next 4 hours.

The average creatinine clearance and glucose resorption rates in the controls remained relatively constant throughout the test period, ranging from 52 to 57 ml/minute and from 150-170 mg/minute, respectively [61]. In the experimental group, both creatinine clearance and glucose resorption rates were lower than controls and declined throughout the test period. For example, creatinine clearance rates were 48 ml/minute at 20 minutes, 38 at 120 minutes, and 30 at 240 minutes. Glucose resorption rates were 150 mg/minute at 20 minutes, 120 at 120 minutes, and 90 at 240 minutes. These results, indicative of impaired proximal tubular function, suggested a nephrotoxic effect to the author.

Effects of hydrazine and several derivatives on renal function were also observed by Van Stee [62]. Anesthetized dogs were injected iv with 0.50 millimole/kg (16 mg/kg) of hydrazine, and inulin and para-aminohippuric acid (PAH) clearance rates and renal plasma flowrate were measured. All these indicators of renal function were significantly decreased during the first 4 hours after injection. Hydropic degeneration of the tubular epithelium of the kidneys was also seen. The author concluded that the decreased glomerular filtration rate was caused by the decreased renal plasma flow and attributed the decreased PAH clearance rate to the decreased glomerular filtration rate and interference with active

transport by the proximal renal tubular epithelium.

In 1966, Fortney [63] reported the effect of hydrazine on liver glycogen, arterial glucose, lactate, pyruvate, and acid-base balance. Blood and liver biopsies were taken from anesthetized male mongrels that had been fasted 12-48 hours and then given hydrazine iv at doses of 25-100 mg/kg. Control dogs received saline or ammonium hydroxide. Serial blood sampling and liver biopsy continued for 6 hours, and then all surviving animals were killed.

There was an immediate rise in blood lactate and pyruvate concentrations at all doses [63]. The pH rose initially and then stabilized in 30 minutes; this transient alkalosis was followed by a slowly developing acidosis beginning 3 hours after injection. In the first 1.5-2 hours, the rise in pyruvate paralleled that of lactate, but by 3 hours the ratio was altered and lactic acidosis developed. After 15 minutes, the rise in lactate and pyruvate was dose-dependent from 25 to 75 mg/kg, but this effect leveled off above 75 mg/kg. All animals with an initial liver glycogen of less than 590 mg/100 g developed hypoglycemia; those with higher glycogen levels developed hyperglycemia, with hypoglycemia following in 3-5 hours as liver glycogen was depleted. Convulsions appeared 4-5 hours after hydrazine injection at 25 mg/kg. At 50-100 mg/kg, convulsions appeared within 1.5-2 hours. None of these effects was observed in any control animal.

Fortney [63] believed that the hyperglycemia, glycogen depletion and hypoglycemia indicated a profound change in normal carbohydrate metabolism. Similar effects on blood glucose and liver glycogen were also observed by Taylor [64].

Aleyassine and Lee [65], in 1971, described the effects of hydrazine on insulin release. Four groups of six rats each were fasted overnight and then given two 1-millimole/kg ip injections of sodium sulfate or hydrazine sulfate 45 minutes apart, with or without simultaneous dextrose injection. Blood samples were collected 15 minutes after the last injection. Serum insulin decreased by 65-74%, both with or without simultaneous injections of dextrose, but serum glucose decreased by 35% only in animals not given dextrose. Thus, hypoinsulinemia occurred even when glucose levels in the blood were artificially elevated.

The authors [65] also conducted in vitro experiments and found that the stimulatory effect of glucose on insulin release in the rat pancreas was inhibited by hydrazine and that this inhibitory effect was reversible. They concluded that hydrazine directly affected the ability of the pancreas to secrete insulin. However, the mechanism of the hydrazine-induced hypoinsulinemia was obscure.

(2) Metabolism

In 1955, McKennis et al [66] studied the excretion of hydrazine and its metabolites. Six male mongrels, weighing 9.5-20 kg, were anesthetized with pentobarbital and then given hydrazine sulfate iv at a dose of 50 mg/kg. Hourly urine samples were collected for up to 8 hours or until the dog died to determine the amount of hydrazino nitrogen present. Urine samples collected prior to injection were used to determine baseline values. Within the first 4 hours, 5-11% of the injected hydrazine was recovered in the urine of five surviving dogs. Four survived 6-8 hours, and 12-20% of the nitrogen in the injected hydrazine was found in their urine. Excretion of hydrazine in the urine of unanesthetized dogs was also

studied. Two dogs were given hydrazine sulfate iv and two others received hydrazine sulfate sc at a dose of 15 mg/kg. At 5 days, 38.6 and 58.4% of the hydrazino nitrogen was recovered in the urine of the dogs given iv injections. The other two dogs died after 1.5-2 days, at which time 21.3 and 29.8% of the hydrazine had been recovered. By reacting the urine with benzaldehyde, the authors were able to determine that 82% (range 66-93%) of the hydrazino nitrogen was from hydrazine or a simple derivative.

In a followup to the study [66] just discussed, McKennis et al [67] also studied the metabolism of hydrazine in rabbits. Unanesthetized rabbits were given hydrazine ip at 24 mg/kg. A total of 12.5% of the hydrazino nitrogen was recovered in the urine; 18.4% of this hydrazino nitrogen (2.3% of the total dose) was identified as 1,2-diacetylhydrazine and the rest was hydrazine. Since 1,2-diacetylhydrazine was found to be nontoxic at up to 87 mg/kg by ip injection, it was viewed as a detoxication product produced by the rabbits. The dogs did not produce this metabolite.

Dambrauskas and Cornish [68], in 1964, reported on the distribution, metabolism, and excretion of hydrazine in rats and mice. Male albino Swiss ICR mice, 22-30 g, were given hydrazine iv or sc at 40-100 mg/kg. Male Sprague-Dawley rats, 330-450 g, were given sc doses of 60 mg/kg. Cumulative urine samples from each animal were collected, and after 0.5, 1, 2, 20, and 48 hours at least three mice were killed. Their carcasses were homogenized in a para-dimethylaminobenzaldehyde solution. The amount of hydrazine in the solution was then determined spectrophotometrically. In rats, blood was collected when the animals were killed and the kidneys, spleen, lungs, heart, liver, skin, stomach, intestinal tract, muscle, brain, and fat were then removed. Each organ was homogenized separately in

para-dimethylaminobenzaldehyde for spectrophotometric analysis.

In those mice given hydrazine at 40 or 60 mg/kg, 31-37% was excreted in the urine within 20 hours and 47-48% within 48 hours [68]. Only 0.3 and 1.4% of the 40 and 60 mg/kg doses, respectively, were found in the carcasses after 48 hours; no hydrazine was found in the carcasses after 72 hours. In rats killed 2 hours after injection, 8.4% of the injected hydrazine was excreted in the urine [68]. Of the organs analyzed, the kidneys had the highest hydrazine concentration, 56 $\mu\text{g/g}$. The other tissues had hydrazine concentrations ranging from 5.5 to 18.6 $\mu\text{g/g}$, except the fat, which contained 0.8 $\mu\text{g/g}$. Twenty hours after injection, 27.4% of the injected hydrazine had been excreted in the urine. The distribution of hydrazine in various organs was qualitatively the same, but it was much lower than that seen 2 hours after injection.

The authors [68] compared their findings with those of McKennis et al [66] in dogs and found that the chemical form and the amounts of hydrazine excreted in the urine agreed; no diacetylhydrazine was identified.

Although the distribution of the injected hydrazine was studied in detail, more than half of the injected dose was still unaccounted for. It appears that metabolites not detectable by para-dimethylaminobenzaldehyde were present and that the release of metabolized hydrazine through exhaled air should also be investigated.

(3) Carcinogenicity and Effects Related to Reproduction

Hydrazine was administered to animals in a 6-month inhalation study and the systemic effects as reported by Haun and Kinkead [56] were described previously. At the end of the exposure period, 10 mice from each group were retained for further study, and these results were reported by

MacEwen [69]. One year after the last exposure, 60-90% of the mice in each group were still alive at which time they were killed and examined. There were five alveologenic carcinomas, two lymphosarcomas, and one hepatoma in six of nine mice (67%) exposed continuously at 1 ppm. Of the group exposed at 5 ppm, 6 hours/day, 5 days/week, five of six (83%) had alveologenic carcinomas. In the groups exposed at 0.2 ppm continuously and at 1 ppm intermittently, three of eight (38%) and two of six (33%), respectively, developed alveologenic carcinomas; the incidence was one of eight (13%) in the control group. MacEwen commented on the importance of these findings in considering an etiologic factor because the incidence of alveologenic carcinoma was dose-related and the other tumors observed in experimental animals did not occur in controls.

In a series of experiments designed to examine carcinogenicity, one investigative group has reported extensively on the effects of hydrazine sulfate when given by intubation to several animal species, including the BALB/c and CBA strains of mice. In one study on BALB/c mice [70], hydrazine sulfate was administered 150 times in daily doses of 1.13, 0.56, 0.28, and 0.14 mg. Another group of BALB/c mice also received 1.13 mg daily but for a total dose of 32 mg given over 4 weeks. There were 39-51 mice, apparently equally divided by sex, in each group, including controls. The mice were 8 weeks old at the beginning of the experiment. Of the males given 1.13, 0.56, 0.28, and 0.14 mg for 150 doses, 90, 65, 62, and 54%, respectively, developed lung tumors. Eighty-five percent of the males receiving a total of 32 mg of hydrazine sulfate also had lung tumors. The tumor-bearing males in the two groups given 1.13 mg/day died at an average age of 67-74 weeks, while the other experimental males died at 80-82 weeks.

The control males lived to 92 weeks and had a lung tumor incidence of 24%. In females given 150 of the aforementioned doses, 90, 76, 89, and 32%, respectively, developed lung tumors. Females receiving 32 mg had a lung tumor incidence of 75%. The average age at death of the females receiving 1.13 mg/day was 74-76 weeks, while the others died at an average age of 84-86 weeks. Only 4% of the female controls developed lung tumors and they reportedly died around 100 weeks of age. Liver tumors were seen in 8% of all mice given hydrazine sulfate at 0.56 mg/kg daily and in 8% of the males given 0.28 mg/kg doses. Mice with liver tumors died at an average age of 88 weeks. Microscopically, the lung tumors were classified as either adenomas or carcinomas, while the liver tumors were vascularized hepatocarcinomas.

The authors [70] suggested that, although lung tumors were the major tumor found, liver tumors would have developed also if the mice had survived longer.

Several other studies [71-73] have reported carcinogenic effects in BALB/c mice when daily doses of 1.13 mg of hydrazine sulfate were given by intubation. When hydrazine sulfate was administered over a 4-week period, the tumorigenic effects were nearly identical to those in the group described above and all lung tumors were classified as adenomas; however, the normal incidence in female controls was 21% [71]. When hydrazine sulfate was given daily until the animals were killed, the first tumor did not appear until the 150th day. By the 200th day, the incidence of lung tumors increased to nearly 100% and the number of tumors/tumor-bearing mouse increased to a maximum of seven when 350 mg of hydrazine sulfate had been given [72]. In females given 150 doses, 90% of the intact virgins and

60% of the gonadectomized mice developed lung tumors, 96% of which were adenomas [73]. However, all breeders developed lung tumors and 47.2% of the tumors were malignant, suggesting to the author that a hormonal factor influenced both the induction and malignancy of these tumors.

In 1966, Milia [74] described the tumorigenic action of hydrazine sulfate on newborn BALB/c mice. One group of 50 mice, starting at 12 hours of age, was given hydrazine by intubation 2-3 times/day in doses increasing in proportion to body weight. In 60 days, each mouse had received about 16.7 mg of hydrazine sulfate, equivalent to 4.15 mg of hydrazine. A second group received sodium bicarbonate according to the same schedule, and a third group was unexposed. These last two groups of 50 mice each served as controls.

Fifty-nine days after the last dose, two mice given hydrazine sulfate were near death [74]. They were found to have adenomas of the lungs, and an additional 13 mice given hydrazine sulfate and 15 mice from each control group were killed for examination of all organs with lesions. While there was no evidence of tumor induction in any control animal, the 15 mice given hydrazine sulfate had a total of 45 lung tumors. Sixty-two percent of the tumors were classified as adenomas, 36% were described as adenomas becoming malignant, and 2% were carcinomas.

The induction time for lung tumors in these newborn mice, less than 17 weeks, was significantly less than that found during other experiments in that laboratory [72], although the doses used in the other study were much higher. Milia believed that an incidence of three tumors/mouse was very high, since mice of such an age usually show no spontaneous lung tumor development.

Biancifiori [75] reported in 1970 on the effect of dose on the incidence of hepatomas in CBA mice induced by hydrazine sulfate. Eight-week-old mice of both sexes were divided into 5 groups, each containing 40-59 animals. In the four experimental groups, each mouse received 1.13, 0.56, 0.28, or 0.14 mg of buffered hydrazine sulfate by gastric intubation daily for 150 days. Since the mice weighed about 25 g, the daily doses were approximately 45, 22, 11, and 5.6 mg/kg. All mice were examined after natural death or when killed while moribund. The lungs, liver, and various endocrine glands were removed for microscopic examinations.

The percentages of male mice dying with hepatomas were 60, 48, 28, and 3.8 in the groups given 1.13, 0.56, 0.28, and 0.14 mg of hydrazine sulfate/day, respectively, while corresponding percentages for females were 62.5, 66.6, 8.0, and 0.0 [75]. Control mice had hepatoma incidences of 10.0 for males and 3.4% for females. For mice with hepatomas, the average age at death was 60-71 weeks at the three highest doses, 80 weeks at the lowest dose, and 87-90 weeks in the controls. All other mice died at 57-83 weeks of age. Most of the tumors seen were characterized as highly vascularized hepatocarcinomas. In the 1.13-mg/day group, there were four instances of lung metastases. The author reported that multiple tumors were present in the lungs of many of the exposed mice, but he did not elaborate on this finding.

Biancifiori [75] found that daily administration of hydrazine sulfate at doses of 1.13 and 0.56 mg was carcinogenic to the liver of CBA mice of both sexes, while 0.28 mg/day had less carcinogenic activity and 0.14 mg/day did not cause cancer.

Other reports by Biancifiiori et al [71,76] provide additional information on the effects of hydrazine sulfate administered in CBA mice, particularly on lung tumor incidence. Twenty-one males and 21 females were each given hydrazine sulfate by intubation at a daily dose of 1.13 mg for 36 weeks starting at 8 weeks of age. Sixteen experimental males (76%) developed an average of 3 lung tumors/mouse, and 19 experimental females (90%) had an average of 6 lung tumors/mouse. Of the 176 lung tumors, 138 were adenomas. Five of the adenomas in males and 20 in females were described as adenomas becoming malignant. Seven tumors in males and six in females were classified as carcinomas. In three females, metastasis to the lymph nodes was observed. The controls had a 3% incidence of lung adenomas in 37 males and 9% in 47 females. In addition, hepatomas were found in 62% of the males (13) and in 71% of the females (15). The spontaneous incidence of hepatomas in the controls was 11% in males and 4% in females.

As he had reported for the BALB/c strain of mice [73], Biancifiiori [77] thought that incidence of lung tumors in CBA mice could be hormonally influenced, since with hydrazine sulfate given 150 times at doses of 0.14-1.13 mg/kg, the lung tumor incidence in females, but not in males, for virgins was always higher than that for gonadectomized mice.

Another group of investigators, Roe et al [78], reported the incidence of lung tumors in Swiss mice given hydrazine compounds. A group of 25 virgin females was given 0.25 mg of hydrazine by gavage 5 days/week, for 40 weeks. Eighty-five mice served as controls. At 40-50 weeks, there were 3 tumors in 2 of the 9 mice examined, while 4 mice examined at 50-60 weeks had a total of 20 tumors. In controls, there were 1 tumor each in 2 of 37 mice examined at 40-50 weeks and 9 tumors in 6 of 42 mice examined at

50-60 weeks. The tumors were alveologenic or bronchiologenic adenomas or adenocarcinomas. Mice given hydrazine showed a significantly greater incidence ($P < 0.001$) of lung tumors than did the controls. The authors believed that the appearance of multiple tumors in mice supported the view that hydrazine was carcinogenic. They did not describe what happened to the 12 unexamined mice.

In 1969, Toth [79] described a study of lung tumor induction and breast adenocarcinoma inhibition by hydrazine sulfate. Three strains of mice, Swiss, AKR, and C3H, were given hydrazine sulfate in drinking water at a concentration of 0.012% for life starting at 6 weeks of age. For the Swiss strain, 50 random-bred mice of each sex had an average daily intake of hydrazine sulfate of 0.65 mg for females and 0.74 mg for males. A total of 110 males and 110 females were used as controls. For AKR mice, 40 males and 40 females were given hydrazine sulfate at an average daily intake of 0.63 mg. The AKR control consisted of 30 females and 30 males. For C3H mice, the average daily intake of hydrazine sulfate was 0.84 mg for 40 females and 0.98 mg for 41 males. Thirty males and 30 females were kept as controls. Complete necropsies were performed on all animals.

In Swiss mice given hydrazine sulfate, 50% of the males and 48% of the females developed lung tumors at average ages of 73 and 77 weeks [79]. Of these tumor-bearing mice, about 72.5% had adenomas, 16% had adenomas and adenocarcinomas, and the rest had either adenocarcinomas or squamous cell carcinomas. The lung tumor incidences in the controls were 10% in the males and 12.7% in the females. In the experimental group, 6% of the males and 8% of the females had malignant lymphomas, compared with 1.8 and 14.5% of the controls, respectively. In addition, the breast cancer incidence in

females was 4% in the experimental group and 8.1% in the control group. A number of other tumors, generally only one of each type, were found in both groups.

Of the AKR mice given hydrazine sulfate, 33 females (82%) and 30 males (75%) developed malignant lymphomas [79]. However, 96% (29) of the control females and 76% (23) of the control males also developed this type of tumor. A few other tumors unrelated to exposure were found. Of the C3H mice, 15 females (37.5%) given hydrazine sulfate developed breast adenocarcinomas compared with 23 control females (76.6%). In addition, four experimental females and two males had lung adenomas.

In 1972, Toth [80] described the effects of long-term ingestion of hydrazine on randomly bred Swiss mice. The mice, 6 weeks of age at the start of experiment, were given hydrazine in their drinking water at a concentration of 0.001% for life. The average daily consumption of hydrazine was 0.056 mg for the females and 0.069 mg for the males. Data for the control group, consisting of 110 mice of each sex from a similar colony, were obtained previously [79]. Of the 50 females that received hydrazine, 27 (54%) developed a total of 47 lung tumors at an average age of 91 weeks (range 26-119) [80]. The female controls had a lung tumor incidence of 12.7%. Nine females developed malignant lymphomas (18% incidence) at an average age of 92 weeks, compared with 16 in the controls (14.5% incidence). Of the 50 males that received hydrazine, 24 (48%) developed 39 lung tumors at an average age of 97 weeks (range 56-119). The incidence of lung tumors in the male controls was 10%. Seven males (14%) developed malignant lymphomas at an average age of 77 weeks, compared with two tumors in male controls (1.8%). The reported data did not indicate

whether or not the same animals with lung tumors had lymphomas. Miscellaneous tumors were also found in 4.2% of the mice receiving hydrazine and in 7% of the controls. Thus, hydrazine administered to mice in drinking water at a concentration of 0.001% throughout life increased the incidence of lung tumors, but apparently did not increase the incidence of malignant lymphomas, at least not in the female mice.

Kelly et al [81], in 1969, compared the carcinogenic activity of hydrazine sulfate and that of several other hydrazine compounds. Thirty male and 30 female offspring of BALB/c x DBA/2 mice (CDF1), 7-8 weeks old, were given weekly doses of hydrazine sulfate for 8 weeks. Males were given 2.6 mg ip injections (about 87 mg/kg) and females were given 5.2 mg oral doses (about 200 mg/kg). Control groups of 10 male and 10 female mice were given saline. All survivors were killed 33 weeks after the initial injection. Necropsy revealed 6 alveologenic carcinomas of the lungs in 6 males (20%) and 25 similar tumors in 13 surviving females (46%) given hydrazine sulfate. In the control groups, 1 of 9 males and 1 of 10 females examined had undescribed lung tumors.

There have been several other studies on the carcinogenic effect of hydrazine or its sulfate salt on mice after ip injection. Hydrazine sulfate was reported to induce lung tumors in SWR, and to a lesser degree, in C57BL/B mice [82]. However, reticular cell sarcomas in the mediastinum and myeloid leukemias were found in another study on hydrazine [83]. Of newborn BALB/C mice injected with a total dose of 19 mg of hydrazine sulfate, all 20 developed an average of 5 lung tumors/mouse compared with only 1 tumor each in 3 of 20 controls [84]. These results agree with those found in other newborn mice given hydrazine sulfate orally [74].

Severi and Biancifiori [76], in 1968, reported the results of a study of the carcinogenicity of hydrazine sulfate in Cb/Se rats. Hydrazine sulfate was given daily via stomach tube to 14 males at a dose of 18 mg and to 18 females at 12 mg over 68 weeks starting when the rats were 8 weeks old. Of these animals, three males (21%) and five females (28%) developed lung tumors, classified as adenomas or adenocarcinomas, with induction periods averaging 75 and 78 weeks, respectively [76]. No lung tumors were found in a control group of 28 males and 22 females. The authors stated that, although they originally intended to study the induction of lung tumors, they observed that the liver had also been affected. Therefore, they examined the livers of 13 experimental rats of each sex. Of these, four males (31%) had hepatic cell carcinomas or sarcomas with an induction time of 85 weeks, but no liver tumors were found in females. Because no spontaneous liver or lung tumors were found, Severi and Biancifiori concluded that hydrazine sulfate was carcinogenic in Cb/Se rats.

In a study of the possible carcinogenic effect of hydrazine sulfate [75], 23 golden hamsters, beginning at 8 weeks of age, were each given 60 doses of 3.0 mg of hydrazine sulfate by intubation for 15 weeks, and 35 were given 2.8 mg 100 times in 20 weeks. There were 56 controls.

Hepatic lesions were present in 60.8% of the those receiving hydrazine sulfate at 3.0 mg/day, in 82.8% of those receiving 2.8 mg/day, and in none of the control hamsters [75]. The liver in almost all animals with hepatic lesions was small, grayish-yellow, and hard. Cirrhosis was confirmed microscopically. The diffuse hepatic lesions were found to be associated with an increase in the fibrous connective tissues. Reticuloendothelial cell proliferation was found in 85.7-96.5% of the

hamsters receiving hydrazine sulfate. Thirty-one percent of the animals that received hydrazine sulfate had bile duct proliferation, and 21% had degeneration of the fibrous cells in hyalinized tissues. The incidence of liver lesions was similar for both sexes, but there was no evidence of tumor induction in either the lungs or the liver.

Toth [85] reported a study in 1972 on the tumorigenic effects of hydrazine sulfate on hamsters. Syrian golden hamsters, 50 males and 50 females, were given drinking water containing 0.012% hydrazine sulfate. The experiment started when the hamsters were 9 weeks old and lasted for their lifespan. The average daily intake of hydrazine sulfate by each hamster was 2.3 mg. All the animals were weighed and checked weekly for gross abnormal changes [85]. A concurrent control group was not maintained, but data [86] previously obtained from a similar colony were used for comparison. Complete necropsies were performed on all animals including those that were killed when in poor condition. All organs were examined, and microscopic studies were performed on any organ that showed gross abnormalities. The author found no detectable tumorigenic effects of hydrazine sulfate in hamsters. Although the 8% incidence of polypoid adenomas of the cecum was somewhat higher than that of the control group, the difference was not statistically significant. Toth pointed out that these findings in hamsters agreed with those reported by Biancifiori [75].

There have been several studies investigating the mutagenicity and possible teratogenicity of hydrazine. In 1972, Rohrborn et al [87] studied the mutagenic potential of hydrazine in a host-mediated assay system. Five to six male NMRI mice, 10-14 weeks old, were each injected ip with a broth containing Salmonella typhimurium G46 and sc with hydrazine sulfate at

doses of 3.5, 3.25, or 3.1 mg/kg. The hydrazine sulfate injections were repeated after 1 and 2 hours. Seven control animals received only the bacteria. One hour after the last hydrazine injection, the animals were killed. The mutated and total bacteria within the peritoneal cavity were counted, and mutation frequency ratios of the hydrazine-administered versus the control animals were calculated. Injection of hydrazine sulfate at doses of 3.5, 3.25, and 3.1 mg/kg resulted in mutation frequency ratios of 362.84, 87.61, and 32.71, respectively. The authors concluded that hydrazine had a dose-dependent mutagenic potential in a host-mediated assay system.

Herbold and Buselmaier [88], in 1976, investigated the mutagenic effects of various substances, including hydrazine. Cultures of Salmonella typhimurium (strains TA 1535, TA 1536, TA 1537, TA 1538, and G46) were incubated in the presence of phenobarbital-activated mouse liver microsomes and 0, 0.12, 1.2, and 12 mg/ml of hydrazine. Forty-eight hours later, revertants were counted and mutation frequencies were determined. Hydrazine caused dose-dependent increases in mutation frequency in both the TA 1535 and G46 strains. The ratios were 1, 1.42, 2.35, and 9.1, respectively, for TA 1535 and 1, 3.6, 3.25, and 312 for G46. The authors concluded that hydrazine was a mutagen based on the finding in these two strains. However, they stated that it was inappropriate to correlate these results to the evaluation of mutagenic risks in humans.

A study of the effect of hydrazine on pregnant rats was described by Lee and Aleyassine [89] in 1970. Seventy-eight Wistar rats at the 11th day of pregnancy were divided into three groups for the experiment. One group received hydrazine sc at 8 mg/kg/day for 10 days, the second group received

hydrazine sc and 200 mg/kg/day of pyridoxine intramuscularly (im) for 10 days, and a control group received sc injections of normal saline at 2 ml/kg/day for 10 days. On the 21st day of pregnancy, 11-12 rats in each group were killed and examined for surviving and dead fetuses and implantation sites. Surviving fetuses were weighed and examined, and selected organs of some fetuses were examined microscopically. The remaining pregnant animals were observed until they delivered, and the live newborn rats were counted 24 hours after delivery.

Repeated injections of hydrazine alone resulted in 2 deaths among 26 pregnant rats, and 1 death occurred in the 26 that received both hydrazine and pyridoxine [89]. No deaths occurred in the controls. Eighty percent of the control rats bore litters of 9-18, and all the newborn subsequently survived. Of the dams that received hydrazine alone, no offspring survived the first 24 hours. Of the dams injected with both hydrazine and pyridoxine, 7 of 13 delivered live newborn, but the litter size was usually smaller (1-14 animals) than that seen in the controls. These newborn rats were very pale and less active than the offspring of the controls. They all showed a moderate degree of dehydration. Twenty-nine of these 33 pups developed and grew normally through weaning. Of the rats killed on the 21st day of pregnancy, two or three in each group had no live fetuses. The fetal survival rates (total fetal survivors/total implantation sites) were 37% for rats given hydrazine, 70% for those given hydrazine and pyridoxine, and 79% for those given saline. The mean body weights of the surviving fetuses were 2.89, 3.44, and 4.70 g for these groups, respectively. Besides being smaller, the fetuses from the rats that received hydrazine were pale and edematous, with occasional petechial hemorrhages. No gross

malformations were observed. Pyridoxine did not improve the appearance of the fetuses. Dams given hydrazine during gestation had weight losses averaging 40-50 g.

In 1976, Greenhouse [90] reported a study on the effect of hydrazine sulfate on the development of South African clawed toad (Xenopus laevis) embryos. The toad embryos were cultured in aquatic media containing various concentrations of hydrazine sulfate. The medium was changed twice every week because of hydrazine degradation, but only initial concentrations were reported. Hydrazine sulfate was not toxic or teratogenic in Xenopus larvae exposed continuously at initial concentrations up to 400 mg/liter, but it was found to be teratogenic at a concentration of 40 mg/liter or higher if exposure started prior to neurulation completion. Malformations seen included foreshortening of the axial skeleton, tail kinks, and edema. If malformed embryos were left in hydrazine sulfate solution, they all died, but they survived if transferred to freshwater. No data were available on whether or not these larvae metamorphosed.

In an additional study [91] on the teratogenicity of hydrazine, clawed toad embryos at the cleavage stage were exposed to hydrazine continuously until hatching. At 10 mg/liter, 35% of the embryos were malformed; at 25, 50, and 100 mg/liter, all exposed embryos were affected. In embryos exposed to hydrazine at 25 mg/liter at different stages of development, only those exposed during neurulation and returned to tap water by the time they had reached the tail bud stage showed teratogenic effects.

(b) Methylhydrazine

(1) Systemic Effects

In a study of the acute toxicity of hydrazines, Jacobson and coworkers [20] found that the toxic signs in rats exposed to methylhydrazine were the same as those reported for hydrazine, and the LC50 values were calculated to be 74 ppm (139 mg/cu m) for rats and 56 ppm (105 mg/cu m) for mice for single, 4-hour exposures. The LC50 for hamsters was reported to be 143 ppm (270 mg/cu m) of methylhydrazine.

Groups of three male dogs were also exposed to methylhydrazine for 4 hours at 15, 21, or 29 ppm and observed for up to 14 days after exposure [20]. Necropsies were performed on the dogs, including those killed when near death, and blood was obtained before and after exposure.

The dogs exposed to methylhydrazine salivated, vomited, panted, choked, and showed incoordinated locomotion and convulsions [20]. At 29 ppm, two of three dogs died during exposure, and at 21 ppm, two of three dogs died the day after exposure. All other dogs survived the 14 days of observation, except for one in the 29-ppm group, which was killed on day 2 for examination. Methylhydrazine exposure caused hemolysis, indicated by a 24% mean decrease in hematocrit value, a 43% decrease in erythrocyte count, and a 41% decrease in hemoglobin content 4 days after exposure. The percentage of reticulocytes in the blood increased from a mean of about 4% before exposure to about 75% 8 days after exposure. A mild bilirubinemia was caused by elevation of the direct-reacting fraction of heme pigments. The sulfobromophthalein (BSP) retention in dogs was unaltered by methylhydrazine exposure, and the ECG's of the dogs were normal. Moderate to marked polymorphonuclear leukocytosis also developed. The hemolytic

effect was most pronounced 4-8 days after exposure, and blood values returned to normal 17-24 days after exposure ended.

In 1969, Haun et al [92] investigated the acute effects of inhalation of methylhydrazine in animals. Groups of 10 male Sprague-Dawley rats, weighing 125-175 g, and 20 male Swiss mice, weighing 17-23 g, were exposed to methylhydrazine for single 30-, 60-, 120-, and 240-minute periods. Twenty-two beagle dogs were exposed at 92 or 104 ppm for 60 minutes, 180-200 ppm for 30 minutes, or 380-400 ppm for 15 minutes. Twenty-five squirrel monkeys were exposed at 75-90 ppm for 60 minutes; 130-170 ppm for 30 minutes, or 300-376 ppm for 15 minutes. Five Rhesus monkeys were exposed to methylhydrazine at 160 or 170 ppm for 60 minutes.

The LC50's, calculated for each exposure interval and species of animal, are listed in Table III-1 [92]. The number and severity of toxic signs in the rats and mice exposed to methylhydrazine were dose-dependent and included nose and eye irritation, diarrhea, frequent urination, rapid and labored breathing, intermittent periods of hyperactivity, tonic-clonic convulsions, and tremors. The rodents appeared to have died during convulsions. Toxic signs observed in the dogs and monkeys were similar to those seen in rodents, but the dogs were also incoordinated and cyanotic.

Of the various tests performed on dogs and Rhesus monkeys (blood counts, liver and kidney function tests), only the hematologic examination showed changes from baseline values [92]. Moderate to severe anemia occurred in all surviving dogs, while mild to moderate anemia was observed in all surviving monkeys. Decreased hematocrit values and hemoglobin concentrations, apparent in both species, were lowest about 7-14 days after exposure. Reticulocyte counts increased in both species and peaked 10 days

after exposure. The authors indicated that the blood taken from the dogs was rusty-brown, which, with observed cyanosis, suggested to them the possibility of methemoglobin formation. About 35 days after exposure, the monkeys had normal blood values, but preexposure levels had not been attained 9 weeks after exposure for dogs.

Microscopic examination of tissues from dogs, rats, and squirrel monkeys after lethal exposure showed pulmonary congestion with hemorrhage, hepatic congestion, and swelling of the renal tubular epithelium [92]. The brains of the dogs and monkeys frequently showed subarachnoid hemorrhages. Renal damage, ranging from mild swelling of the tubular epithelium to vacuolization and coagulative necrosis of tubular epithelial cells, was the most common finding in animals killed 60 days after near-fatal doses of methylhydrazine. Haun et al observed that the amount of visceral congestion and hemorrhage was not sufficient to produce death and that, of the species studied, squirrel monkeys were the most sensitive and rats the least sensitive to the lethal effects of methylhydrazine.

The LC50's determined in this study [92] correlate well with those determined in the other study [20]. Mild to severe anemia appeared to be the major toxic effect on dogs and was observed in both studies.

In 1971, MacEwen and Haun [93] conducted a series of 6-month exposures of animals to methylhydrazine at 0.2, 1, 2, and 5 ppm for 6 hours/day, 5 days/week. Another group was exposed continuously at 0.2 ppm. Each group consisted of 8 beagle dogs, 4 Rhesus monkeys, 50 Wistar rats, and 40 ICR mice. All animals except the rats were female. A series of 15 clinical chemistry and 8 hematologic tests and body weight measurements were conducted every 2 weeks during the study. Surviving animals, except

one-half of the dogs, were killed for examination at the end of exposure, and bone marrow studies were then conducted on the dogs. The remaining dogs were held for 30 days after the end of exposure and were examined for possible reversibility of toxic effects and recovery time.

Death attributed to exposure occurred only in mice at the two highest concentrations, with mortalities of 27% at 5 ppm and 15% at 2 ppm [93]. Growth rate depression in rats was observed, but only in the 2- and 5-ppm groups was that effect sustained. Long-term effects in dogs and monkeys were primarily related to the reaction of methylhydrazine with red blood cells. The hemolytic responses were about the same for the 0.2-ppm continuous exposure group and the 1-ppm intermittent exposure group, as would be expected, since the weekly exposure concentration times, 33.6 and 30 ppm-hours, were essentially the same. Reductions in erythrocyte counts, hemoglobin concentrations, and hematocrit values of 43, 43, and 30%, respectively, were observed in the dogs exposed to methylhydrazine at 150 ppm-hours/week compared with 9, 3, and 2% at 6 ppm-hours/week. A twofold to threefold increase in methemoglobin occurred in the dogs exposed at 5 ppm (150 ppm-hours/week). In monkeys exposed at 150 ppm-hours/week, decreases in hematocrit values, hemoglobin concentration, and erythrocyte counts of 28, 32, and 30%, respectively, were observed. Increased red cell fragility was observed in canine blood. The degree of hemolysis, measured in 0.6% salt solution, was 2.5% at the 6 ppm-hours/week exposure, 15% at 150 ppm-hours/week, and 1% in controls. Samples of canine and primate blood taken at 3-7 months were found to contain 1-5 Heinz bodies/100 red blood cells, and no dose- or species-related effects were found. After the exposure ended, the blood cell counts returned to normal in 2-4 weeks.

Mean bilirubin and alkaline phosphatase values for all groups of exposed dogs were statistically higher than control values at all sampling periods after 3 weeks of exposure, and dose-dependent effects were evident [93]. The increase in total inorganic serum phosphorus was less pronounced, but the authors believed that it indicated, along with the other two tests, intrahepatic cholestasis from liver damage caused by long-term exposure to methylhydrazine. Data on monkeys were not reported. Bone marrow samples from exposed dogs showed a dose-related decrease in the myeloid/erythroid ratio with increasing erythropoietic activity.

The authors [93] concluded that methylhydrazine exposure produced dose-related hemolytic anemia and Heinz body formation without an apparent threshold level and that the anemia was reversible when animals were removed from further exposure, at least up to 5 ppm in intermittent exposure. As a result of their study, they recommended that the TLV of 0.2 ppm be reexamined.

Kroe [94] examined selected tissues from the animals used by MacEwen and Haun [93]. Tissues from the lungs, heart, liver, spleen, and kidneys of all the monkeys and dogs and from 10 rats and 10 mice of each group were examined [94]. There were no lesions in the monkeys and rats. Periportal hepatic hemosiderosis and cholestasis and proximal tubular hemosiderosis were found in the dogs exposed to methylhydrazine at 150 ppm-hours/week. Similar hepatic and renal tubular changes were also seen in the dogs exposed at 60 ppm-hours/week. Hepatic cholestasis was found in dogs exposed at 33.6, 30, and 6 ppm-hours/week. Moderate lymphoid hyperplasia was also noted.

Lung and heart tissues of all exposed mice were normal [94]. The livers of mice exposed at 150 ppm-hours/week had centrilobular cholestasis, bile duct proliferation, and centrilobular hemosiderosis. The kidneys and spleens of the same mice also had hemosiderosis. The liver changes in the mice exposed at 60 ppm-hours/week were similar to those of the 150 ppm-hours/week group, only less pronounced. Splenic and renal tubular hemosiderosis was also less pronounced. In the three lowest exposure groups, hepatic, splenic, and renal tubular hemosiderosis was greatest at the 0.2-ppm continuous level, less at 1 ppm, and still less in the 0.2-ppm intermittent group. There was no cholestasis or bile duct proliferation in the livers of these mice.

The interspecies differences observed in the development of hemosiderosis and cholestasis were attributed to species susceptibility to methylhydrazine-induced hemolysis and to the ability of some species to clear the hemolytic products.

In 1973, Darmer and MacEwen [95] reported the effects of long-term exposure of animals to methylhydrazine vapor. Groups of 8 female beagles, 4 female Rhesus monkeys, and 80 male Sprague-Dawley rats were exposed continuously to methylhydrazine at 0, 0.04, or 0.1 ppm for 90 days. After the animals were exposed for 45 and 90 days, blood samples from 30 rats of each group were examined, while the remaining 20 rats were killed for tissue examination. Blood counts were measured on the dogs and monkeys before the experiment and every 2 weeks thereafter. In addition, total serum inorganic phosphorus, serum alkaline phosphatase, and erythrocyte fragility (dogs only) were determined. The presence of Heinz bodies was noted, and body weight was monitored.

Exposure of rats to methylhydrazine at 0.1 ppm for 90 days (16.8 ppm-hours/week) caused a significant decrease in body weight (about 20 g), but organ-to-body weight ratios were unaffected [95]. Rats exposed at 0.04 ppm (6.7 ppm-hours/week) showed no growth impairment. After 45 days of exposure, rats in both groups had a significant decrease in mean hematocrit value (6%), hemoglobin concentration (4%), and erythrocyte count (8%). After 90 days, rats exposed at 0.04 ppm showed only an increase in serum phosphorus (8%), while those exposed at 0.1 ppm had depressed erythrocyte counts (13%) and increased serum phosphorus levels (13%). In dogs exposed to methylhydrazine at 0.1 ppm, hematocrit value, hemoglobin concentration, and erythrocyte count were found to be decreased by 10, 17, and 24%, respectively, while serum phosphorus was increased by 23% and alkaline phosphatase activity by 465%. Red blood cells from dogs exposed at 0.1 ppm had increased osmotic fragility; changes were insignificant at 0.04 ppm. Reticulocytes increased at both exposure levels. No toxic effects were found in the blood of the monkeys. In all three species, the only gross tissue abnormality was a nutmeg appearance of the livers of dogs exposed at 0.1 ppm considered to be consistent with passive congestion. No microscopic data were reported.

O'Brien et al [96] investigated the acutely toxic effects of several hydrazines, including methylhydrazine, on rats. Thirty-five female rats weighing 180-240 g were given methylhydrazine ip at 10-100 mg/kg. Toxic signs were observed, and the LD50 was determined to be 28 mg/kg. Death generally was preceded by convulsions. Blood glucose, measured before convulsions began in two rats given an LD50 dose, was elevated 2-3 fold in 35 minutes. The authors, however, considered that glucose interference was

not related to the lethal action of methylhydrazine.

In 1971, Gregory et al [97] reported on the effect of varying the route of administration on the LD50's for methylhydrazine in hamsters and Sprague-Dawley rats. A 4.2% solution of methylhydrazine was given orally, ip, and iv, and a 50% solution was applied topically. The LD50's observed in rats and hamsters, respectively, were 70.7 and 22.1 mg/kg for oral doses, 183.4 and 239.4 mg/kg for topical applications, and 20.5 and 21.2 mg/kg for ip injections. The LD50 for iv injections in rats was 17.3 mg/kg. The cause of death was respiratory failure. Those rats alive 3 weeks after exposure had mild to severe muscular incoordination and cerebellar demyelination. The LD50's for methylhydrazine nitrate were similarly calculated. Except for oral administration in hamsters, the nitrate form was slightly more toxic than the free base.

Rothberg and Cope [58] reported LD50's for methylhydrazine of 14.2 μ l/kg (12 mg/kg) for iv injection in rabbits and 93 mg/kg and 47 mg/kg for rabbits and guinea pigs, respectively, following skin absorption. A mild edema appeared on the skin at the site of application, disappearing in 24 hours and leaving a blanched appearance to the skin. Application of 3 μ l of methylhydrazine to the eye of each of two rabbits resulted in only mild conjunctivitis and slight erythema of the eyelid.

In 1969, Smith and Clark [98] reported on the absorption of methylhydrazine through canine skin. Methylhydrazine at doses of 0.32-5.75 millimoles/kg (14.7-264.5 mg/kg) was applied to a 300-sq cm area on the chest of 16 anesthetized male mongrels. The skin at the site of application quickly reddened, the discoloration deepened, the skin became edematous, and eventually the site appeared slightly gray. Swelling

subsided in 6 hours. Only one animal, receiving 175 mg/kg, died during the 6-hour period, but many convulsed despite the anesthesia. Methylhydrazine was detected in the blood within 30 seconds of application. The concentration in blood continued to rise for 30-60 minutes, eventually reaching a plateau; the amount of both methylhydrazine and methemoglobin in the blood was apparently dose-related. After 100-140 minutes, there was a gradual decline in methemoglobin throughout the 6-hour observation period. The authors noted that a dermal dose 5-7 times that of iv injection was necessary to produce an equivalent amount of methemoglobin.

Fortney and Clark [46] investigated the effect of methylhydrazine on methemoglobin production both in vitro and in vivo. For the in vivo experiment, anesthetized dogs were injected with methylhydrazine iv at 0.54 millimole/kg (25 mg/kg). Arterial blood samples were taken 5, 15, and 30 minutes and 1, 1.5, 2.5, and 4 hours after injection to determine methemoglobin concentration. Blood glucose and lactate concentrations were analyzed at 30-minute intervals from 1 hour before to 2 hours after injection, then at hourly intervals for 2 more hours. The amount of hemoglobin present as methemoglobin peaked at a level greater than 30% an hour after injection and declined gradually to 19% in the next 3 hours. The blood lactate rose markedly 1-2 hours after injection and was still elevated at 4 hours. Blood glucose rose slightly the 1st hour and then fell sharply.

In vitro, methemoglobin was formed when methylhydrazine was incubated with either canine blood or purified oxyhemoglobin, although the rate of reaction was faster in whole blood than in oxyhemoglobin [46].

In 1970, Leahy [47] reported the results of a study of the in vitro effect of methylhydrazine on blood. Canine blood was incubated with methylhydrazine under either unlimited aerobic or anaerobic conditions and reaction rates were determined by sequential spectral analyses.

Under anaerobic conditions, hemoglobin was reduced and only 2-3 g/100 ml of methemoglobin was found [47]. Under aerobic conditions, the hemoglobin-methylhydrazine mixture gradually turned from bright red to purple-brown. The rate of methemoglobin formation and the total amount formed were proportional to the original concentration of methylhydrazine, although the conversion was limited to 75-80% of the original hemoglobin. When the molar ratio of methylhydrazine to heme was 1, a methemoglobin concentration of 12 g/100 ml was detected in 60 minutes. When this ratio was higher than 2, rapid denaturation of globulin and precipitation were observed. The blood of humans, rats, and monkeys was also tested under aerobic conditions. The equilibrium levels of methemoglobin reached in the blood samples differed in each species and were 8.5, 4.0, 3.0, and 2.5 g/100 ml for dogs, humans, rats, and monkeys, respectively, when the methylhydrazine-heme ratio was 0.5. Gas-chromatographic analysis of the gas produced during aerobic incubation showed the presence of nitrogen and methane representing about 80% of the nitrogen and 20% of the carbon of the methylhydrazine.

When one considers the equilibrium amount of methemoglobin that can be accumulated in the blood, the argument that human blood is more sensitive to the effects of methylhydrazine than the blood of rats and monkeys but is less sensitive than canine blood is supported.

Van Stee [62] investigated the effects of methylhydrazine on the renal function of dogs in an experiment identical to the one discussed above for hydrazine. Nine anesthetized dogs were given iv injections of methylhydrazine at 0.63 millimoles/kg (29 mg/kg) and tubocurarine chloride to suppress convulsions. Inulin and PAH clearance rates were significantly decreased, although renal plasma flow was not affected. Methemoglobinemia appeared within minutes following injection of methylhydrazine, and methemoglobinuria appeared within a few hours. The author postulated that the mechanism producing impairment in renal function was similar to that for hydrazine, ie, decreased PAH clearance was caused by decreased glomerular filtration and interference with active transport by the proximal renal tubular epithelium.

In 1969, Sopher et al [99] studied the effects of methylhydrazine on dogs. Forty-two beagles were each given a single ip injection of 5-30 mg/kg of methylhydrazine, some with 100-200 mg/kg of pyridoxine to protect against the convulsive effects of methylhydrazine. One hour to 8 days later, the animals were killed and the major organs removed for gross and microscopic examination.

Methylhydrazine at 5 mg/kg caused vomiting and convulsions but no deaths; at 10 mg/kg or more, death occurred within 2 hours [99]. The toxic signs were relieved by pyridoxine, and those animals that received both preparations recovered. In dogs killed 1-2 days after injection with methylhydrazine and not receiving pyridoxine, the most prominent gross findings were in the urinary tract. The kidneys were swollen, and the delineation between the cortex and medulla was obliterated. The bladder contained dark-brown urine and occasional blood clots. All other organs

examined were also congested. In dogs that were killed more than 2 days after injection of both methylhydrazine and pyridoxine, kidney swelling and hyperemia of other organs were diminished or absent. Of the dogs that received a high dose of methylhydrazine, those that convulsed and died had severe congestion and cyanosis in most organs and scattered hemorrhagic areas in the lungs.

The microscopic appearance of the kidney tissues varied with the methylhydrazine dose given and the time between injection and necropsy [99]. The higher the dose, the more pronounced the changes, which included swelling and eosinophilia in the epithelium of the proximal tubules and loops of Henle. Tissues from dogs given methylhydrazine at 5 mg/kg were normal, while the damage in the kidneys of the dogs receiving 7.5-15 mg/kg involved several changes, including overt hemoglobinuria and hyaline droplet degeneration. At 20 or 30 mg/kg, methylhydrazine also caused severe renal epithelial damage characterized by syncytial masses that engulfed the hemoglobin casts. The animals that survived for several days developed hemosiderosis, and the hyaline droplets were either reduced in size and number or no longer present. The authors concluded that methylhydrazine in dogs caused severe erythrocyte damage, leading to severe anemia and formation of methemoglobin and other hemoglobin destruction products, that resulted in hemoglobinuric nephropathy.

The toxic effects of repeated injections of methylhydrazine on monkeys were studied by Back and Pinkerton [100]. In 10 Macaca mulatta monkeys given methylhydrazine ip at 2.5 or 5 mg/kg for 31 days for total doses of 65 or 95 mg/kg, the only clinical or microscopic evidence of toxicity was a decrease in body weight in the 1st week. Back and Pinkerton

therefore administered higher ip doses of methylhydrazine, to induce clinical signs and tissue damage, to three male monkeys weighing from 2.40 to 5.91 kg. Two controls received saline injections. Doses of 7 and 10 mg/kg were alternated daily until the animal died.

No toxic signs were noted in any of the animals on the 1st day, but one animal died on each of the next 3 days; the causes of death were not apparent [100]. Serum enzyme activities, SGOT and alkaline phosphatase, were normal, except that the last blood sample of one monkey had a high SGOT activity. Significant amounts of fatty infiltration and vacuolization of cells were noted in the liver. The kidneys, heart, and bone marrow were normal. One animal had tiny perivascular cerebellar hemorrhages, which the authors attributed to severe convulsions. The authors also noted that there was an extremely narrow range between a no-effect and a lethal concentration of methylhydrazine for monkeys.

Ten male and 10 female Macaca mulatta monkeys, weighing 3-6 kg, were used by George and associates [101] in a study of the nephrotoxicity of methylhydrazine. Eight weeks after translocation of the left kidney to a subcutaneous pocket, baseline values of kidney function and a kidney biopsy were performed.

After another 6 weeks, the monkeys were divided into five groups, apparently of four each [101]. Group I, the control group, was given saline ip daily for 14 days. The other groups received methylhydrazine. Animals in group II received a single injection of 7.5 mg/kg; group III, 2.5 mg/kg/day for 14 days; group IV, 5 mg/kg/day every other day for 14 days; and group V, 5 mg/kg/day for 5-10 days. Forty-eight hours after the

final injection, biopsy specimens were taken for electron microscopic examination.

Although monkeys in groups IV and V developed toxic signs, no methylhydrazine was detected in the blood or urine of any animal 24 hours after the final injection [101]. In group II, there were cellular vacuolization and mitochondrial swelling in both the proximal and distal tubular cells after injections. These changes were neither uniformly distributed nor uniformly severe but were present in all biopsy samples. In the most severe cases, the tubular cells were completely filled with vacuoles and mitochondria were barely recognizable. The monkeys in groups III, IV, and V had similar but less severe changes in renal tubular cells.

George et al [101] noted that the changes observed in renal tubular cell morphology did not cause significant changes in renal function. They hypothesized that there were enough intact, unaffected nephrons to maintain normal function. Comparing their findings in monkeys to those in dogs [62,99], the authors concluded that the nephrotoxic effects on dogs were more severe.

This study [101] appears to complement an earlier study by Back and Pinkerton [100] in which only slight fatty infiltration of the liver was observed using a light microscope. Using an electron microscope, George et al [101] observed changes in renal tubular cells. Both studies agreed that there were no significant changes in renal function of monkeys given ip injections of methylhydrazine at 2.5-7.5 mg/kg.

Reynolds and Back [102] tested the effect of ip injections of methylhydrazine on the learned behavior of macaque monkeys. Four monkeys received methylhydrazine at 2.5 mg/kg and five received 5 mg/kg. Each

monkey was given hourly shock-avoidance tests, eight times/day, for 3 days; injections were given on the 1st and the 3rd days.

Performance did not vary with dose [102]. In over half the tests, a performance decrement preceded or occurred without clinical signs, but clinical signs never preceded a performance decrement. Performance deteriorated 1-2 hours after methylhydrazine injection and returned to normal in 3-30 hours. Clinical signs generally occurred 2-3 hours after injection and disappeared in 3-6 hours. The authors believed that performance tests were a more sensitive index of toxicity than were clinical signs and that operant behavior in monkeys was significantly impaired after ip injection of methylhydrazine at 2.5 or 5 mg/kg.

(2) Metabolism

Male Sprague-Dawley rats, weighing 250 g, in groups of two each, were given ip injections of ¹⁴C-labeled methylhydrazine in a study of methylhydrazine metabolism by Dost et al [103]. The rats were then kept in chambers, and radioactivity in their expired air and their urine was monitored. The total respiratory radioactivity measured 27 hours after injection of methylhydrazine at doses of 5.5, 11, and 22 mg/kg was 37, 31, and 24%, respectively, of the injected amount. Of the radioactivity detected, 20-25% was ¹⁴C carbon dioxide; the remainder was identified as methane. The output of these two gases peaked at about the same time, but the ratio of yields of methane to carbon dioxide decreased from 10 initially to 3 or 4 after 10 hours, suggesting to the authors that different metabolic pathways might be involved.

Twenty-seven hours after administration of methylhydrazine, the rats given 5.5, 11, or 22 mg/kg had excreted 41, 39, and 22%, respectively, of

the total doses in the urine [103]. The difference between the amount of radioactivity injected and that found in the urine and exhaled air at 27 hours was considered to have been retained by the tissues. These amounts were calculated to be 1.2, 3.2, and 12 mg/kg, respectively. The authors believed that the increased percentage of tissue-retained radioactivity at the highest dose was caused by an impairment of the excretion mechanisms.

Pinkerton et al [104] studied the distribution and excretion of methylhydrazine in four species. Twenty Sprague-Dawley rats, 20 Swiss mice, 17 mongrel dogs, and 16 Macaca mulatta monkeys received ¹⁴C-labeled methylhydrazine ip at 15, 22, 10, and 10 mg/kg, respectively. The animals, fasted overnight, were killed 2, 4, 8, or 24 hours after injection, and urine and blood samples were collected for analysis of methylhydrazine. Whole organs were removed and weighed, and radioactivity in each organ was measured individually, except for mice, where organs were pooled in groups of five to obtain sufficient material.

In the more than 20 samples of tissues and serum analyzed, the serum, liver, kidneys, and bladder in all 4 species had the highest concentration of radioactive material [104]. These concentrations in samples from dogs and mice peaked at 4 hours but peaked at 2 hours in those from the monkeys. The radioactivity in samples from rats did not have any apparent pattern. Urinary excretion of methylhydrazine was most rapid in mice, followed by that in rats, monkeys, and dogs. Two hours after injection, dogs excreted only one-half as much of the injected dose as the monkeys, rats, or mice did. Twenty-four hours after injection, 25.6, 31.3, and 39.9% of the injected methylhydrazine had been excreted in the urine of the dogs, monkeys, and rats, respectively.

In a later study of several metabolic effects of methylhydrazine in male Sprague-Dawley rats, Dost [105] examined glucose catabolism during acute and subacute methylhydrazine intoxication. The ¹⁴C-labeled glucose was infused intraintestinally at 150 mg/hour, a concentration adequate to prevent glycogen depletion. In acute studies, a single ip injection of 0.45 millimole/kg (21 mg/kg) of methylhydrazine was given 7 hours later. In subacute studies, 0.036 millimoles/kg/hour (1.66 mg/kg/hour) of methylhydrazine was given by iv infusion, starting 4 hours before glucose administration. In both cases, respired ¹⁴C carbon dioxide was measured as an index of glucose catabolism. In a third experiment, glucose infusion was started, 7 hours later methylhydrazine infusion began, and blood glucose concentrations were monitored.

Following both acute and subacute methylhydrazine intoxication, there was a substantial depression of glucose catabolism, as measured by the concentration of ¹⁴C carbon dioxide in the respired air. In the subacute studies, it was possible to distinguish that for glucose labeled in the first position, oxidation was much less depressed compared with that observed when the label was in the second, third, fourth, or sixth position. Pyridoxine was effective in reversing this depression. In the third experiment, blood glucose concentrations increased following administration of methylhydrazine and they continued to increase for about an hour after the onset of convulsions even though methylhydrazine administration was stopped. Both pyridoxine and insulin were effective in counteracting the hyperglycemia induced by methylhydrazine.

(3) Carcinogenicity and Effects Related to Reproduction

In 1972, Toth [80] reported a study of the tumorigenicity of methylhydrazine in Swiss mice. Fifty males and 50 females, 6 weeks of age, were given 0.01% methylhydrazine in drinking water for life. The average daily intake of methylhydrazine was 0.66 mg/male and 0.71 mg/female. Data from a control group of 110 female and 110 male mice were obtained previously from a similar colony [79].

Methylhydrazine shortened survival, ie, all experimental mice died before they were 80 weeks old while the last of the control animals was still alive at 120 weeks [80]. Of the 50 females given methylhydrazine, 12 (24%) developed 17 lung tumors classified as adenomas at an average age of 51 weeks (range 36-67). The female controls had a lung tumor incidence of 12.7%. Of the 50 males given methylhydrazine, 11 (22%) developed 12 lung adenomas at an average age of 51 weeks (range 34-70), compared with 10% in the male controls. Two malignant lymphocytic lymphomas were observed in females. Fifteen liver cell tumors, both benign and malignant, eight cholangiomas and two cholangiocarcinomas were diagnosed in animals given methylhydrazine.

As part of the same study, 0.001% methylhydrazine sulfate was administered to another group of mice of the same age in an identical manner [80]. The average daily intake of methylhydrazine sulfate for the males was 0.102 mg and 0.078 mg for the females, an equivalent of 0.033 and 0.025 mg of methylhydrazine, respectively. Of the 50 female mice, 23 (46%) developed 46 lung tumors at an average age of 95 weeks (range 71-119). Of these 23 mice, 11 had a total of 17 adenomas, 4 had 5 adenocarcinomas, and 8 had 15 adenomas and 9 adenocarcinomas. Ten females (20%) each had a

malignant lymphoma. Two of these lymphomas were lymphocytic, seven were histocytic, and one could not be classified. Of the males receiving methylhydrazine sulfate, 23 (46%) developed 43 lung tumors at an average age of 87 weeks (range 56-117). Of these 23 mice, 18 had a total of 24 adenomas, 2 had 1 adenocarcinoma each, and 3 had a total of 10 adenomas and 7 adenocarcinomas. Eight males (16%) developed malignant lymphomas. Seven of the lymphomas were histocytic, and one could not be classified. Fifteen controls developed tumors; five were hemangiomas, but none was a lymphoma.

The incidence of lung tumors caused by methylhydrazine sulfate was higher than that caused by methylhydrazine, although the latter was given at a dose 10 times higher than the former [80]. On the average, the mice given methylhydrazine survived 51 weeks while those given the sulfate salt survived 91 weeks. Furthermore, methylhydrazine is less stable than its sulfate salt, and it is possible that methylhydrazine given in the feed water may have degraded.

The previously described study by Roe et al [78] on the carcinogenicity of hydrazine and several of its derivatives included methylhydrazine sulfate. Twenty-five female virgin Swiss mice were each given 0.5 mg of methylhydrazine sulfate by gavage for 5 days/week for 40 weeks. There were 85 controls. The mice were examined 40-50 or 50-60 weeks after exposure began. By 60 weeks, 1 (5%) of the 19 experimental mice examined had evidence of tumor formation, and that mouse had 6 lung tumors, while 8 surviving control mice (10%) had 11 tumors. The authors concluded that methylhydrazine sulfate was not carcinogenic in mice, but they noted that they were unable to administer higher doses because of the toxicity of the compound.

Kelly et al [81] examined the incidence of lung tumors in mice given methylhydrazine. Thirty male and 30 female CDF1 mice, 7-8 weeks old, were given 8 weekly doses of methylhydrazine. Males received 0.23 mg/dose (7 mg/kg) ip and females received 0.46 mg/dose (17 mg/kg) orally. Ten males and 10 females given saline served as controls.

Three experimental males (10%) had developed lung tumors when killed at 33 weeks, but there were no tumors in the nine females examined [81]. The control group had a tumor incidence of 11% in males and 10% in females. Since the difference was not significant, the authors concluded that methylhydrazine was not carcinogenic in mice.

Several weaknesses are apparent in these two studies [78,81]. Considering the number of animals, total dose used, and the latent period observed by Toth [80], the validity of the conclusion by Kelly et al [81] and Roe et al [78] that methylhydrazine is not carcinogenic in mice is questionable. Furthermore, the authors did not mention if tumors had occurred in tissues other than the lungs. In both cases, a significant number of animals apparently were not examined.

In 1973, Toth and Shimizu [106] studied the tumorigenicity of methylhydrazine in Syrian golden hamsters. Groups of 50 male and 50 female hamsters were given 0.01% methylhydrazine, prepared three times a week, in their drinking water for life starting at 6 weeks of age. On the average, the males received 1.1 mg/day of methylhydrazine and the females received 1.3 mg/day. There were 100 males and 100 females in the control group.

Sixteen female hamsters (32%) developed malignant histiocytomas of the liver (Kupffer cell sarcoma) at an average age of 70 weeks (range 46-92) [106]. In males, 27 (54%) developed malignant histiocytomas of the

liver at an average age of 78 weeks with a range of 47-103 weeks. In addition, these animals had six tumors in the lungs, two in the lymph nodes, and two in the spleen. Nine females (18%) developed tumors of the cecum at an average age of 64 weeks (range 50-76). Seven of these animals had nine polypoid adenomas, one had a polypoid adenoma and an adenocarcinoma, and one had two adenocarcinomas. In males, seven (14%) developed nine tumors of the cecum: five had a total of six polypoid adenomas, one had a polypoid adenoma and an adenocarcinoma, and another had an adenocarcinoma. In the controls, one female and one male each developed one polypoid adenoma.

In 1975, a study [107] on the effects of methylhydrazine in Syrian golden hamsters was reported. Methylhydrazine, prepared daily, was administered in the drinking water to 5-month-old hamsters for life. Preliminary experiments indicated that methylhydrazine was not stable in tapwater unless the pH was adjusted. Therefore, 30 hamsters received 0.01% methylhydrazine in tapwater, 30 received it in tapwater adjusted to pH 3.5, and 17 had their drinking water adjusted to pH 3.5.

Four tumors, all adrenocortical, were found in three controls (23% of 13 survivors) [107]. Four tumors in 4 animals given the unbuffered solution (16% of 25 survivors) consisted of an adrenocortical carcinoma, a hemangioendothelioma, and 2 carcinomas of the liver. Six tumors in 5 animals given the buffered solution (20% of 25 survivors) were classified as adrenocortical carcinomas, a melanoma and a cutaneous histiocytoma. By comparing the overall tumor incidences, the authors concluded that methylhydrazine was noncarcinogenic under these experimental conditions.

This conclusion [107] is in disagreement with that reached by Toth and Shimizu [106]. There were differences in the preparation of the chemicals and the ages of hamsters at the beginning of the experiments, which could have contributed to the conflicting results.

In a study of the teratogenic effects of some hydrazine derivatives, Chaube and Murphy [108] gave single ip injections of methylhydrazine sulfate at various doses to 23 pregnant rats on the 12th day of gestation and killed them on the 21st day. The authors estimated an LD50 in dams to be 80 mg/kg. In the pups, there were no abnormalities in the palate, appendages, paws, tail, or jaws. The authors concluded that methylhydrazine was not teratogenic. No additional details of the experiment were reported, so there is insufficient information to draw any conclusions beyond those of the authors.

In a 1976 study by Greenhouse [91], previously discussed for hydrazine, the teratogenic effects of methylhydrazine on South African clawed toad embryos were investigated. Embryos were cultured in an aqueous solution of methylhydrazine at various concentrations up to 15 mg/liter. At 3, 5, 10, and 15 mg/liter, 1, 52, 93, and 100%, respectively, of the exposed embryos were malformed. The malformations observed were similar to those caused by hydrazine, and their appearance was independent of concentration. Greenhouse concluded that methylhydrazine was teratogenic in toad embryos.

Brusick and Matheson [109], in 1976, described the results of four tests of the mutagenicity of methylhydrazine: (1) in vitro microbial assays (Ames tests) with five mutant strains of Salmonella typhimurium, an Escherichia coli strain, and a strain of Saccharomyces cerevisiae; (2) an

in vitro mutation assay with cultured mouse cells; (3) an assay for unscheduled DNA synthesis in cultured human diploid cells; and (4) a dominant-lethal assay in mice. In all but the dominant-lethal assay, the tests were run both with and without mouse liver microsomes, a procedure to evaluate the possible effect of metabolic activation of methylhydrazine to a more powerful mutagen. Both positive and negative controls were run in all assays.

In the Ames test, concentrations of methylhydrazine ranging from 0.0001 to 5.0 μ l/plate produced negative results with the mutant S. typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100, and with the E. coli and S. cerevisiae strains in standard plate tests [109]. These negative results were obtained both with and without microsomal activation. However, when TA-1535 cells were incubated with liver microsomes and 1 or 5 μ l/ml of methylhydrazine in suspension tests and assayed for revertant cells, mutagenic activity was demonstrated. When L5178Y mouse lymphoma cells were incubated with methylhydrazine in nonactivation and activation tests, no mutations were found.

Unscheduled DNA synthesis was evaluated in normal human diploid WI-38 cells in tissue culture, and 3H thymidine was incorporated into DNA to follow the synthesis [109]. Methylhydrazine had no mutagenic activity in either nonactivation or activation assays at concentrations of 0.1, 0.5, and 1.0 μ l/ml.

In the dominant-lethal test, 10 male ICR mice each were given methylhydrazine ip at 0.26, 0.86, and 2.60 mg/kg for 5 days and 10 male rats (strain unstated) were similarly given methylhydrazine at 0.215, 0.72, and 2.5 mg/kg. Two days after the last dose, each mouse was caged with two

virgin females for 5 days. The mating schedule was repeated with two new females each week for 7 weeks. Fourteen days after the middle of the mating period, each female was killed and examined for number of living and dead fetuses. There were no significant trends showing that methylhydrazine produced mutation by this test.

The authors [109] concluded that methylhydrazine showed no mutagenic activity in any of the tests used, except in an activation-suspension assay with Salmonella typhimurium T-1535.

(c) 1,1-Dimethylhydrazine

(1) Systemic Effects

Jacobson et al [20], in 1955, investigated the acute inhalation toxicity of some methylated hydrazine derivatives. Rats, mice, and hamsters were exposed to 1,1-dimethylhydrazine for a single 4-hour exposure in an experiment identical to that reported earlier for hydrazine. Toxic signs were the same as those produced by exposure to hydrazine, and LC50 values were calculated to be 252 ppm (618 mg/cu m) for rats, 172 ppm (423 mg/cu m) for mice, and 392 ppm (962 mg/cu m) for hamsters.

Groups of three male beagles were also exposed to 1,1-dimethylhydrazine at 24-111 ppm (59-272 mg/cu m) [20]. Two dogs exposed to 1,1-dimethylhydrazine at 111 ppm had convulsions and died within 192 minutes, and the third dog, near death, was killed for examination. All three dogs vomited and convulsed, two panted, and one had diarrhea. One dog exposed to 1,1-dimethylhydrazine at 52 ppm for 4 hours was killed when near death 1 day after exposure. The other two animals survived; one showed panting, nausea, and incoordination, while the other showed no toxic signs. All dogs exposed at 24 ppm for 4 hours survived, but one vomited

and convulsed. All five surviving dogs were killed on day 14 and necropsies were performed. Results of blood counts, sulfobromophthalein retention, and prothrombin time were normal. Gross examinations of tissue from dogs, rats, and mice showed pulmonary edema and some instances of patchy pulmonary hemorrhage. No other significant change was found.

In 1960, Rinehart et al [110] reported the effects of long-term inhalation of 1,1-dimethylhydrazine on male Wistar rats, female CF-1 mice, and male beagle dogs. All exposures were for 6 hours/day, 5 days/week. Twenty rats and 30 mice were exposed to 1,1-dimethylhydrazine at 140 ppm (342 mg/cu m) for 6 weeks (4,200 ppm-hours/week), and 30 rats and 30 mice were exposed at 75 ppm (183 mg/cu m) for 7 weeks (2,250 ppm-hours/week). Two groups of three dogs, each animal weighing about 11.4 kg, were exposed at 25 ppm (61 mg/cu m) for 13 weeks (750 ppm-hours/week) or 5 ppm (12.2 mg/cu m) for 26 weeks (150 ppm-hours/week).

Rats and mice had occasional tremors during exposure, and those that died had tonic-clonic convulsions [110]. At 342 mg/cu m, 29 of 30 mice and 1 of 20 rats died, and the others gained weight at a slower rate than did controls during the first 2 weeks. At 183 mg/cu m, 8 of 20 mice died within 5 weeks, while the only toxic signs observed in rats were breathing difficulty and lethargy.

During the 3rd day of exposure to 1,1-dimethylhydrazine at 61 mg/cu m, the dogs showed signs of toxicity, including depression, increased salivation, vomiting, diarrhea, incoordination of the hindlegs, tonic-clonic convulsions, hyperemic oral and conjunctival membranes, bradycardia, and fever [110]. One dog died, one had all the toxic signs, and the third

showed only depression and increased salivation. During 13 weeks of exposure, the two survivors lost 2.5 kg of weight, compared with 0.2 kg in three control dogs. No severe toxic signs were observed in the dogs exposed to 1,1-dimethylhydrazine at 12.2 mg/cu m, but an average weight loss of 0.8 kg compared with the controls was noted.

After 4 weeks of exposure to 1,1-dimethylhydrazine at 61 mg/cu m, dogs appeared to have hemolytic anemia, since erythrocyte counts were decreased by 58%, hematocrit values by 28%, and hemoglobin concentrations by 34% [110]. Hematocrit and hemoglobin values later approached preexposure values, but erythrocyte counts remained depressed. Since the blood was first examined at the 4th week of exposure, the time of onset of the blood abnormalities was uncertain. Bilirubin, blood nonprotein nitrogen, blood glucose level, and sulfobromophthalein retention time were all normal throughout the experiment. Dogs exposed at 12.2 mg/cu m had similar evidence of hemolytic anemia after 24 weeks, a 26% decrease in hemoglobin concentration, an 18% decrease in hematocrit value, and a 17% decrease in erythrocyte count. The mean bilirubin level was elevated.

Microscopic examination of the rodent tissues showed no morphologic alteration that could be attributed to 1,1-dimethylhydrazine [110]. Dogs that survived the exposure at 61 mg/cu m showed hemosiderosis of the reticuloendothelial system, including the spleen, lymph nodes, bone marrow, and Kupffer cells of the liver. The bone marrow had significantly increased erythrocytic activity. The lung tissue of the dog that died during exposure at 61 mg/cu m showed alveolar hemorrhaging, emphysema, and collapse but no hemosiderosis. Dogs exposed at 12.2 mg/cu m showed hemosiderosis only in the spleen; no other tissue abnormalities were noted.

The authors [110] concluded that 1,1-dimethylhydrazine at concentrations of 5 ppm (12.2 mg/cu m) or greater was toxic, that the most prominent sign of toxicity in dogs was hemolytic anemia, and that, for humans, 1,1-dimethylhydrazine concentrations should be kept well below 5 ppm. They suggested 0.5 ppm (1.22 mg/cu m) as a guideline for industrial practice.

The toxicity from single, brief exposures to 1,1-dimethylhydrazine vapor for rats and dogs was examined by Weeks et al [111]. Male rats and mongrel dogs were exposed for 5, 15, or 60 minutes and rats alone were exposed for 30 minutes. Selected rats from each group were killed for examination immediately after exposure or 1, 3, or 7 days later. Similarly, dogs were killed immediately or after 7, 14, or 21 days. No microscopic changes were found in tissue samples from either the dogs or the rats. The LC50's for all the groups are given in Table III-1. Ten additional rats were exposed to 1,1-dimethylhydrazine at 1,000 ppm for 60 minutes, and their blood counts were normal following exposure.

The authors [111] noticed that sharp noises made the dogs exposed to 1,1-dimethylhydrazine shiver and cower. Consequently, dogs were exposed for single 5-, 15-, and 60-minute periods at various fractions of the LC50 values of 1,1-dimethylhydrazine. Auditory, visual, and electrical stimuli were added at 15 minutes, 1 hour, and 2 hours thereafter to evaluate the role of external stimulation. The external stimuli added stress to the exposed animals and seemed to magnify or perhaps hasten the development of toxic signs.

Weeks et al [111] also estimated the retention of 1,1-dimethylhydrazine in six pentobarbital-anesthetized dogs. Each animal was exposed through an endotracheal tube or a face mask to 1,1-dimethylhydrazine at 2,900-19,600 mg/cu m for 60 minutes. It was reported that 71-93% of the 1,1-dimethylhydrazine inhaled was retained in the respiratory tract by the dogs.

Weeks et al [111] further investigated the effects of multiple exposure to 1,1-dimethylhydrazine on conditioned avoidance tests. Groups of four dogs each were exposed twice a week for 6 weeks to 1,1-dimethylhydrazine at 50, 200, or 600 ppm for 60, 15, and 5 minutes, respectively. All animals were observed for toxic signs and reflex reactions. No changes from normal were noted in the conditioned avoidance test, and there were no alterations in the patellar, extensor thrust, and hopping reflexes. Doubling the exposure concentrations of the three groups of dogs for 2 additional weeks had no effect on the conditioned avoidance responses, and no changes were noted in neurologic and physical examinations, even though signs of intoxication appeared after the first exposure.

Back et al [112], in 1977, reported the results of 6-month inhalation exposures of C57 black mice, Fischer 344 rats, Syrian golden hamsters, and beagle dogs to 1,1-dimethylhydrazine at 5, 0.5, or 0.05 ppm. At each concentration, 400 female mice, 200 male rats, 200 male hamsters, and 4 dogs of each sex were exposed for 6 hours/day, 5 days/week. An equal number of animals of each species was maintained as controls. Necropsies were performed on all animals that died, and a series of blood measurements and clinical tests were conducted on dogs.

No toxic sign was observed in any animal exposed to 1,1-dimethylhydrazine [112]. The results of clinical chemistry tests were all normal except for SGPT activity and BSP retention. The SGPT values were significantly elevated ($P < 0.01$) in dogs exposed at 5 ppm, but they returned to normal 6 months after exposure ended. At 0.5 ppm, fairly frequent increases ($P < 0.05$) in SGPT were observed, although the degree of elevation was less than that seen in the 5-ppm group. BSP retention was measured at the end of exposure, and only those exposed at 5 ppm had significantly elevated values ($P < 0.05$). These values returned to normal 9 months after exposure ended. The authors [112] stated that the significant effects of 1,1-dimethylhydrazine were limited to slight to moderate hepatotoxicity in dogs exposed at 5 ppm (150 ppm-hours/week) after 6 months of exposure.

Haun [113] reported that in the previous study [112], 1,1-dimethylhydrazine was contaminated with 0.12% nitrosodimethylamine. The author examined the effects of this contaminant by preparing pure 1,1-dimethylhydrazine. Four beagles were exposed to this purified compound at 5 ppm, 6 hours/day, 5 days/week for 8.5 weeks. Four dogs were used as controls. Liver biopsies were taken and the dogs rested 5 days. Then the dogs were exposed to the purified 1,1-dimethylhydrazine at 5 ppm continuously for 13 days. Immediately following the second exposure, two controls were exposed to 1,1-dimethylhydrazine to which 0.12% nitrosodimethylamine had been added. Two dogs, previously exposed to pure 1,1-dimethylhydrazine, were used as controls. Various clinical tests, including measurement of SGPT activity, were performed both before and during exposure. BSP retention times were determined at the beginning and end of the study.

There was no elevation of SGPT activity from either intermittent or continuous exposure to 1,1-dimethylhydrazine, except when the contaminant, nitrosodimethylamine, was also present [113]. In all cases, BSP retention times were unaffected. The author concluded that nitrosodimethylamine was the active agent producing increased SGPT levels, even though the level tested was insufficient to cause discernible hepatocellular changes or alterations in liver function.

A study by Rothberg and Cope [58] on the acute toxicity of hydrazines included the effects of 1,1-dimethylhydrazine. The LD50's for 1,1-dimethylhydrazine in rabbits were found to be 89.2 μ l/kg (69.8 mg/kg) by the iv route and 1.05 g/kg by percutaneous absorption. In guinea pigs, the LD50 for skin absorption was 1.31 g/kg. There was no evidence of skin damage. When 3 μ l of 1,1-dimethylhydrazine was applied to the eyes of two rabbits, only mild conjunctivitis and slight erythema of the eyelid developed.

Hodge [114], in 1954, reported the results of acute toxicity tests of 1,1-dimethylhydrazine. The oral LD50 in female rats was found to be 0.46 ml/kg (360 mg/kg). 1,1-Dimethylhydrazine was applied to the clipped bellies of rabbits and prevented from evaporating by a watch glass. It was lethal at 156 mg/kg but was tolerated at 23 mg/kg. When six rats inhaled 1,1-dimethylhydrazine at a concentration of 18.4% (v/v), all died within 35 minutes. The effects of 1,1-dimethylhydrazine on eyes were investigated by instilling 2 drops (about 0.05 ml) of the compound into the right eye of a rabbit; only slight vascularization of eyelids, without any evidence of corneal injury, was observed. When 0.01 ml (7.8 mg) of 1,1-dimethyl-

hydrazine was given intracutaneously to a rabbit, no skin irritation was found.

Smith and Clark [115] investigated the dermal absorption of 1,1-dimethylhydrazine in dogs. 1,1-Dimethylhydrazine at doses of 5-30 millimoles/kg (300-1,800 mg/kg) was applied to a 15- x 20-cm shaved area on the chest of 13 anesthetized mongrels. Glucose and 1,1-dimethylhydrazine concentrations in the blood and urine were measured hourly for 6 hours after 1,1-dimethylhydrazine application. The reduced glutathione content and glutathione peroxidase activity of erythrocytes were also estimated at hourly intervals. Two control animals were used to determine normal blood and urinary glucose concentrations.

1,1-Dimethylhydrazine spread rapidly and evenly over the surface [115]. A slight reddening developed within 10-15 minutes of application and quickly disappeared, leaving no sign of skin damage. Six of the dogs died about 6 hours after application. The dermal LD50 for 1,1-dimethylhydrazine was estimated to be 1,200-1,680 mg/kg. Mild clonic convulsions were seen in three dogs, and these convulsions were not always followed by death.

Skin application of 1,1-dimethylhydrazine at all doses tested produced detectable concentrations of the compound in the blood within 30 seconds; however, neither blood nor urine concentrations of 1,1-dimethylhydrazine were dose-dependent [115]. All tested doses of 1,1-dimethylhydrazine caused mild hyperglycemia for 5-6 hours with a corresponding increase in urinary glucose. No effect was seen on reduced glutathione content in erythrocytes. Glutathione peroxidase activity decreased after the two lowest doses were given, remained stable at the

midlevel dose, and increased at the highest dose and during the hour preceding death, regardless of the dose. The authors concluded that 1,1-dimethylhydrazine was toxic if applied dermally but that its mode of action was in biochemical systems other than those tested in their experiment.

O'Brien et al [96] determined the LD50 of 1,1-dimethylhydrazine in rats and studied its effects on carbohydrate metabolism. Ninety-four female rats, weighing 180-240 g, were given 1,1-dimethylhydrazine ip at doses of 50-408 mg/kg. The LD50 was estimated to be 102 mg/kg; this dose also induced the maximum number of convulsions. Higher doses led to a decrease in the time to onset of convulsions, the time between convulsions, and the time to death. Hyperglycemia was also found in two rats given an LD50 dose of 1,1-dimethylhydrazine, the blood glucose increasing from 80 to 160 mg/100 ml in 80 minutes.

In 1964, Cornish and Barth [116] studied the effects of practical grade 1,1-dimethylhydrazine on urinary amino acid and creatinine excretion in male Sprague-Dawley rats. In groups of four rats each receiving 1,1-dimethylhydrazine ip at 40, 60, or 80 mg/kg, creatinine nitrogen values were relatively constant for any given rat and were not affected by 1,1-dimethylhydrazine. Amino acid nitrogen excretion, however, was increased on the 1st day after injection. When the amino acid nitrogen-to-creatinine ratios were calculated, the initial enhanced excretion of amino acid was followed by a period of decreased excretion; by day 5, the ratios were only 74-77% of those of controls. Paper chromatography showed that there were no abnormalities in the relative amounts of individual amino acids excreted. The authors speculated that the observed increase in amino acid excretion could have been caused by interference with amino acid

metabolism, protein synthesis, or gluconeogenesis. Toxic effects on the kidneys could not be ruled out, but examined tissue samples and SGOT activity were normal, so the authors did not believe that the effect was produced by tissue damage.

Patrick and Back [60] described the toxicologic effects of repeated injections of practical grade 1,1-dimethylhydrazine in 1965. Seven Rhesus monkeys, weighing 3.2-3.5 kg, each received 10 mg/kg of 1,1-dimethylhydrazine ip daily, 5 days/week, for 4 weeks. The only observed toxic effect was an initial weight loss of 0.2-0.8 kg, and the only abnormal finding in the blood was a 90% increase in the plasma glucose level. There was slight lipid deposition near the central vein in the liver of one monkey and in the tubular membranes of the kidneys of a second animal. Significant amounts of lipids were found in the heart muscle of two monkeys; there were trace amounts in two others.

In 1969, Cornish and Hartung [117] reported the effects of repeated administration of 1,1-dimethylhydrazine to rats. Groups of 10 female Sprague-Dawley rats with an average weight of 225 g were given daily ip injections of 0, 10, 30, 50, or 70 mg/kg of 1,1-dimethylhydrazine for 3 weeks. Body weights and urine from seven animals in each group were taken daily; at necropsy, organs were weighed and tissue samples from two rats in each group were examined.

All the rats receiving 18 daily injections of 1,1-dimethylhydrazine at 10 mg/kg survived. The numbers of animals surviving 21 injections of 30, 50, and 70 mg/kg were 5, 4, and 1, respectively [117]. All deaths occurred during the first 3 days. There was an initial dosage-related weight loss of 10-20 g in the animals that received 10, 30, or 50

mg/kg/day, but the organ-to-body weight ratio remained normal. Daily injections of 30 mg/kg or more of 1,1-dimethylhydrazine resulted in substantial and sustained diuresis throughout the experiment; the total urine output in these rats was more than twice that of the controls. A low white cell count occurred only in the one survivor of the 70-mg/kg group. Animals receiving 10 or 30 mg/kg/day had mean blood urea nitrogen (BUN) values similar to those of controls (about 15 mg/100 ml), but in the animals given 50 mg/kg/day, the BUN value increased about 70%. 1,1-Dimethylhydrazine administration caused a dose-dependent increase in SGOT activity. The average SGOT activities for the animals receiving 0, 10, 30, 50, and 70 mg/kg/day were 47.2, 63.7, 79.8, 80.5, and 124 units, respectively. Cloudy swelling and lipid infiltration were found in the renal tubules of the only surviving animal that received 70 mg/kg/day; less pronounced changes were observed at 50 mg/kg/day. Early degenerative fatty infiltration was found in the liver of the 70-mg/kg/day survivor; some control animals also showed similar changes.

In 1966, Wong [61] examined the effects of 1,1-dimethylhydrazine on the renal function of female mongrels. Creatinine and glucose were administered as described earlier for hydrazine to both the control and experimental groups, each containing six dogs [61]. 1,1-Dimethylhydrazine was given iv to the experimental animals at 45 mg/kg. From 20-120 minutes after administration, the control and experimental creatinine clearance values were roughly the same, approximately 56 ml/minute. From 120-240 minutes, the experimental group showed about a 10% elevation in creatinine clearance. No significant effect on urinary glucose resorption rates (approximately 170 mg/minute) was seen. The author found that 45 mg/kg of

unbuffered 1,1-dimethylhydrazine produced no harmful effects in the kidneys that could be seen by the two tests used.

Another study by Van Stee [62], in which inulin and para-aminohippurate clearance rates were measured, also indicated that 1,1-dimethylhydrazine caused no significant changes in the renal function of dogs. However, three of eight dogs died with severe pulmonary edema and subsequent circulatory failure within 2 hours after being given 1,1-dimethylhydrazine.

In 1962, Reynolds et al [118] performed three experiments on Java monkeys to assess the effects of 1,1-dimethylhydrazine on shock avoidance. Before the experiments, the monkeys were trained in a shock avoidance test and matched according to performance. In the first experiment, two monkeys received 1,1-dimethylhydrazine ip at 30 mg/kg, the threshold dose for vomiting. Two control animals received saline injections. Eight 15-minute test sessions, given hourly, began 20 minutes after injection. In the second experiment, 3 weeks later, the same test procedure was repeated with the control and experimental groups reversed. In another experiment, performed 60 days after the second experiment, three of the above monkeys and one new one served as the experimental group, and the other monkey and two new ones were controls. In all experiments, the number of lever-presses/minute was used to measure shock avoidance. McNemar's comparison of change statistic was used to evaluate the results.

No controls in any experiment showed significant performance changes after saline injection [118]. The first experimental group had significantly more lever-presses/minute ($P < 0.001$) than did either the controls or the experimental group themselves before injection. In the

second and third experiments, no significant differences between the control and experimental groups were observed. One monkey in the third experiment had a significantly poorer performance ($P < 0.05$); all others were normal.

In a second experiment, Reynolds and coworkers [119] administered 1,1-dimethylhydrazine ip at 30 mg/kg to four adult male Java monkeys trained to perform different and more difficult tasks from those in the previous study [118]. Two 2-day tests were conducted, with an intervening 1-day rest period [119]. The monkeys were given a saline injection on the 1st day and 1,1-dimethylhydrazine on the 2nd day. On each test day, 3-minute work and 2-minute rest periods were alternated and repeated for six to nine sessions. Performances on lever press, discrete avoidance, auditory monitoring, and visual monitoring were tested.

Although all monkeys developed toxic signs such as gagging, coughing, and vomiting, there were wide differences in individual responses on the performance tests [119]. Of the 32 possible performance combinations of 4 monkeys, 2 injections, and 4 performance tasks, only 8 cases of significant performance decrement ($P < 0.05$) were observed. Six of eight cases occurred 3-3.5 hours after the second test replication, when the monkeys were ill. In all other cases, the monkeys performed normally, although some showed toxic signs. Both clinical illness and performance impairment disappeared between 6 and 9 hours after injection.

Thus, it appears that the impaired performance from 1,1-dimethylhydrazine intoxication was probably associated with the illness because it occurred earlier than did the performance decrement. It may be that repeated exposure to 1,1-dimethylhydrazine would more greatly affect

primate behavior, since almost all impaired performances were observed after the second injection.

(2) Metabolism

In 1962, Mitz et al [120] studied the metabolism of 1,1-dimethylhydrazine. Six female Sprague-Dawley rats were given ¹⁴C-labeled 1,1-dimethylhydrazine ip at 40 mg/kg. Three animals were killed 30 minutes after injection and the remaining three after 4 hours. The brain, kidneys, liver, heart tissue, and carcass, as well as blood and urine samples, were analyzed for radioactivity. Approximately 19% of the injected dose was found in the urine after 4 hours. The liver and blood after 4 hours contained 3.7 and 2.7% of the injected 1,1-dimethylhydrazine, respectively; the other organs tested each had less than 1%. Since the remaining carcass contained only 51.2% of the radioactivity, a total recovery of 77.7% was reported. The distribution of 1,1-dimethylhydrazine 30 minutes after injection was similar but there was less radioactivity in the urine (5.7%), and it was higher in the carcass (75.8%). The authors did not give specific data for dogs but stated that dogs showed the same pattern observed in the rats. The authors also found that only about 2% of the injected radioactivity was lost by respiration. Analysis of the radioactive compounds in the urine confirmed the presence of three major metabolites. However, only two metabolites were identified. One was 1,1-dimethylhydrazine constituting 50-60% of the total radioactivity, and another 3-10% was glucose dimethylhydrazone.

Reed and associates [121] studied the metabolism and distribution of 1,1-dimethylhydrazine and the effects of this agent on glucose catabolism in Sprague-Dawley rats. For the metabolic studies, rats were injected ip

with ^{14}C -labeled 1,1-dimethylhydrazine at 20, 40, and 60 mg/kg and at 11 mg/kg for the distribution studies. For glucose catabolism studies, rats were administered 1.5 grams of glucose twice, 9 hours apart, by stomach tube. For the second administration, glucose was labeled with ^{14}C at the first, second, third, fourth, or sixth position. The rats were simultaneously injected ip with 1,1-dimethylhydrazine at 40-60 mg/kg.

Seven hours after labeled 1,1-dimethylhydrazine administration, 23% of the original dose of 20 mg/kg was recovered as ^{14}C carbon dioxide in the respired air, while 19 and 12% were recovered from the 40 and 60 mg/kg injection, respectively [121]. When rats were injected with labeled 1,1-dimethylhydrazine at 11 mg/kg, 12% was converted to respired carbon dioxide in 4 hours. About 25% of this dose was distributed in the body, while 43% was excreted in the urine in 4 hours. There was little, if any, preferential tissue uptake of 1,1-dimethylhydrazine.

In the glucose catabolism studies, ^{14}C carbon dioxide formation from different carbon atoms of glucose was altered by the administration of 1,1-dimethylhydrazine [121]. 1,1-Dimethylhydrazine preferentially inhibited glucose catabolism to carbon dioxide via glycolysis and the pentose phosphate pathway. A decarboxylation process of the sixth carbon, the glucuronate pathway, appeared to be unaffected by 1,1-dimethylhydrazine.

There appears to be some conflicting data reported by Mitz et al [120] and by Reed et al [121]. Although rats were similarly given an ip injection of 1,1-dimethylhydrazine at 40 mg/kg, Mitz et al found that only 2% was excreted in respired air, while Reed et al found that 19% was expired as carbon dioxide in 7 hours. Reed et al used a more elaborate system to measure labeled carbon dioxide and used more rats in the

experiment, so their data would seem to be more reliable.

Back et al [122] studied the absorption, distribution, and excretion of 1,1-dimethylhydrazine in a number of species. Twelve albino rabbits weighing 1.7-4.4 kg each were given ¹⁴C-labeled 1,1-dimethylhydrazine iv at 50 mg/kg. Two rabbits each were killed at intervals from 2 to 24 hours after injection to determine the amount of radioactivity in major organs. There was no preferential concentration of 1,1-dimethylhydrazine in any of these organs, and retention remained high even at 24 hours. At 2 hours, 28.3% of the dose could be accounted for, while 14.7% was accounted for at 24 hours. Urine and tissue representing the bulk of the body weight were not analyzed, and this factor probably accounted for the low recovery.

To study the concentration of 1,1-dimethylhydrazine in the blood and urine, the authors gave ¹⁴C-labeled 1,1-dimethylhydrazine ip at 50 mg/kg to two dogs and two cats [122]. Fifteen to 60 minutes after injection, the amount of radioactivity in the blood reached a maximum that was about 13-14% of the original dose for dogs and 7-9% of that for cats. About half of this radioactive material was unchanged 1,1-dimethylhydrazine. As much as 30-50% of the radioactive compound, believed to be unchanged 1,1-dimethylhydrazine, was excreted in the urine during the first 5 hours after injection. The percentage of the dose recovered in urine was similar for cats and dogs given 50 mg/kg of unlabeled 1,1-dimethylhydrazine iv, but in cats given 10 mg/kg only 11-28% was recovered in the urine in 6 hours.

As part of the same study [122], a number of rats, cats, dogs, and monkeys were injected ip with unlabeled 1,1-dimethylhydrazine at 1-100 mg/kg, and the plasma concentrations of 1,1-dimethylhydrazine were determined colorimetrically. 1,1-Dimethylhydrazine at doses of less than

10 mg/kg could not be detected in the plasma of monkeys. There was considerable individual variation, so that plasma concentrations were not a good indicator of dose. For example, 1 hour after injection, plasma concentrations of 1,1-dimethylhydrazine were 0.5-11.5 $\mu\text{g/ml}$ and 6.5-16.0 $\mu\text{g/ml}$ in rats given 10 and 30 mg/kg, respectively. There was, however, a good correlation of the average concentration for a group with time. For example, in 15 monkeys given 100 mg/kg of 1,1-dimethylhydrazine, the average plasma concentrations at 1, 2, and 4 hours after injection were 70.4, 52.9, and 33.9 $\mu\text{g/ml}$, respectively.

These experiments showed that 1,1-dimethylhydrazine was not preferentially concentrated in specific organs. Exposure was not accurately determinable from the 1,1-dimethylhydrazine concentration in blood; urinary concentration was a more sensitive indicator of exposure to 1,1-dimethylhydrazine.

(3) Carcinogenicity and Effects Related to Reproduction

In 1973, Toth [123] reported on tumor formation in random-bred Swiss mice after oral administration of 1,1-dimethylhydrazine for life. A 0.01% solution of 1,1-dimethylhydrazine in drinking water was given ad libitum to 50 male and 50 female mice starting at 5 weeks of age. The average daily intake of 1,1-dimethylhydrazine was 0.7 mg. The controls were 110 male and 110 female mice from a similar colony as reported in an earlier study [79].

The ingestion of 1,1-dimethylhydrazine in the drinking water significantly shortened the survival time of the experimental group [123]. At 60 weeks, only 13 male (26%) and 23 female (46%) mice given 1,1-dimethylhydrazine were still alive, compared with 55 male (50%) and 89

female (81%) control mice. Of the females given 1,1-dimethylhydrazine, 37 (74%) developed blood vessel tumors at an average age of 59 weeks (range 41-76). Forty-two of the experimental males (84%) developed blood vessel tumors at an average age of 42 weeks (range 35-66). There were 78 blood vessel tumors, characterized as angiosarcomas, found in the liver, 18 in the muscles, 11 in the heart, 7 in the lungs, 4 in the fat, 3 in the subcutis, and 1 each in the glandular stomach, the pancreas, and pararenal tissue.

Thirty-two (64%) of the female mice given 1,1-dimethylhydrazine developed 103 lung tumors, 96 of which were adenomas; 6 of these animals had, in addition, 7 adenocarcinomas [123]. The average age for tumor development was 62 weeks (range 44-76). In the exposed males, 39 (78%) developed 119 lung tumors; they had a total of 115 adenomas and 4 of them also had an adenocarcinoma. The average age at which lung tumors were observed was 53 weeks (range 40-66).

Only one female mouse given 1,1-dimethylhydrazine developed a kidney tumor, which appeared at 60 weeks of age [123]. Of the experimental males, 9 developed 11 kidney tumors at an average age of 59 weeks (range 42-66). Microscopically, some of these tumors were classified as cystic-papillary adenomas; others had developed into the solid form. Six males given 1,1-dimethylhydrazine developed benign hepatomas at an average age of 58 weeks (range 46-66).

In exposed females, seven malignant lymphomas were observed, an incidence of 14% [123]. These tumors occurred when the mice were 26-74 weeks of age, with an average of 55 weeks. Microscopically, six lymphomas were classified as one lymphocytic type, one mixed lymphocytic and

histocytic type, and four histocytic types; one could not be classified. One such tumor, first seen in exposed males aged 61 weeks, was classified microscopically as lymphocytic.

In summary, the tumor incidences in the blood vessels, lungs, kidneys, and liver were 79, 71, 10, and 6%, respectively [123]. Corresponding incidences in nonconcurrent controls were 2, 11, 0, and 0%.

Roe et al [78] also examined the carcinogenicity of 1,1-dimethylhydrazine. Virgin female Swiss mice were given 1,1-dimethylhydrazine by gavage at 0.5 mg/day, 5 days/week, for 40-60 weeks. There were 28 experimental mice and 85 controls. Necropsies were performed on some mice at 40-50 weeks, and there were two lung tumors in one of eight animals examined. There were 24 tumors in 4 of 9 animals examined at 50-60 weeks. The control group had 2 tumors in 2 of 37 animals at 40-50 weeks and 9 tumors in 6 of 42 animals at 50-60 weeks. The tumors were classified as alveologenic or bronchiologenic adenomas or adenocarcinomas. The incidence of tumors in mice given 1,1-dimethylhydrazine was not statistically greater at the 95% confidence level than that observed in the controls. However, some mice developed multiple tumors, and the authors concluded that this finding supported the view that 1,1-dimethylhydrazine was tumorigenic. No mention was made of any studies on the fate of the remaining nine mice, so their cause of death is unknown, and possible tumors in other organs were not identified.

Kelly et al [81], in 1969, reported the results of a study on the carcinogenicity of hydrazine compounds, including 1,1-dimethylhydrazine. Thirty CDF1 male mice, aged 7-8 weeks, were given 1,1-dimethylhydrazine in 8 weekly ip injections totaling 3.6 mg (120 mg/kg), and 30 females of the

same age were given 8 oral doses totaling 7.2 mg (277 mg/kg). A group of 10 males and 10 females given saline served as controls. After 28-32 weeks, 1 of the 25 females (4%) examined and 1 of the 30 males (3%) developed lung tumors, while the controls had a tumor incidence of about 10% in both sexes. The authors, therefore, concluded that 1,1-dimethylhydrazine was not carcinogenic in mice. While these results would appear to conflict with those of Toth [123] and Roe et al [78], the first lung tumor did not occur in Toth's study [123] until 35 weeks after administration, with the average latent period being 48 weeks, an experimental period longer than that used by Kelly et al [81]. The total dose used by Toth [123] was also much higher than that used by Kelly et al [81].

In 1976, Greenhouse [90] investigated the effects of several hydrazines, including 1,1-dimethylhydrazine, on the development of embryos of the South African clawed toad (Xenopus laevis). The animals were raised in aquatic media containing various concentrations of the test compounds. 1,1-Dimethylhydrazine at concentrations up to 1 mg/liter was neither toxic nor teratogenic to the exposed embryos, but at 10 mg/liter, 1,1-dimethylhydrazine was teratogenic to all embryonic stages (cleavage, gastrulation, neurulation, and tailbud). At 100 mg/liter, 1,1-dimethylhydrazine was lethal. The most common malformations observed were foreshortening of the body and tail, tail kinks, and edema. A number of embryos also had abnormally small heads and brains. In a continuation of this study, Greenhouse [91] found that the embryos were susceptible to 1,1-dimethylhydrazine-induced teratogenicity only at the neurulation stage.

Brusick and Matheson [109] investigated the mutagenicity of 1,1-dimethylhydrazine using the same assay techniques employed for methylhydrazine. In the Ames test, 1,1-dimethylhydrazine at concentrations of 0.01, 0.1, 1, 0, and 5.0 μ l/plate produced negative results with the mutant Salmonella typhimurium strains TA-1535, TA-1537, TA 1538, TA-98, and TA-100, and with Escherichia coli and Saccharomyces cerevisiae strains. These negative results were obtained without activation by liver microsomes. Except for marginally positive responses with TA-98 and possibly with TA-1538, negative responses occurred under activation conditions as well.

L5178Y mouse lymphoma cells were incubated with 0.01, 0.05, 0.1, and 0.25 μ l/ml of 1,1-dimethylhydrazine in the nonactivation tests and 0.005, 0.01, 0.05, and 0.1 μ l/ml of 1,1-dimethylhydrazine in the activation tests [109]. A moderate dose-related response occurred in the nonactivation trials. In the activation tests, a 15-fold increase in mutation frequency occurred at the highest concentration of 1,1-dimethylhydrazine compared with negative controls.

Unscheduled DNA synthesis was evaluated in normal human diploid WI-38 cells in tissue culture, and tritiated thymidine was incorporated into DNA to follow the synthesis [109]. There was no response without the addition of liver microsomes at 1,1-dimethylhydrazine concentrations of 0.1, 0.5, and 1.0 μ l/ml, but there was a positive effect in the microsomal activation tests. In the activation tests, the positive control, 2-acetylaminofluorene, produced a 430% response while 1,1-dimethylhydrazine produced responses of 186-237%, the lowest response resulting from the

highest concentrations. The authors attributed this inverted relationship to cellular toxicity.

In the dominant-lethal test, 3 groups of 10 male ICR mice, 7-8 weeks of age, were given 1,1-dimethylhydrazine ip at 1.25, 4.2, or 12.5 mg/kg daily for 5 days [109]. Two days after the last dose, each mouse was caged with two virgin females for 5 days. The mating schedule was repeated with two new females each week for 8 weeks. Fourteen days after the middle of the mating period, each female was killed and examined for numbers of living and dead fetuses. There were no significant trends showing that 1,1-dimethylhydrazine produced dominant-lethal mutation.

(d) 1,2-Dimethylhydrazine

(1) Systemic Effects

In 1955, Jacobson et al [20] reported on the acute toxicity of 1,2-dimethylhydrazine. The LC50 was estimated to lie between 280 and 400 ppm (686 and 980 mg/cu m). The toxic signs observed were similar to those previously described for hydrazine. The authors reported that short-term exposure caused primarily respiratory irritation and convulsions.

Rothberg and Cope [58] investigated the toxicity of 1,2-dimethylhydrazine in a manner identical to that already described for hydrazine. The LD50's in rabbits were 53.0 μ l/kg (43.8 mg/kg) from iv injection and 466 mg/kg by the percutaneous route. For guinea pigs, the LD50 for skin absorption was 131 mg/kg. There was no skin damage. When 3 μ l of 1,2-dimethylhydrazine was placed in the eyes of two rabbits, only a mild conjunctivitis and slight erythema of the eyelid developed.

Weir et al [124], in 1964, reported on the acute toxicity of 1,2-dimethylhydrazine. Male Swiss-Webster mice were given 1,2-

dimethylhydrazine dihydrochloride ip at pH 7 to determine mortality at 24 and 168 hours. The LD50's were 940 mg/kg (425 mg/kg free base) at 24 hours and 47 mg/kg (21 mg/kg free base) at 168 hours. Some animals were hyperactive at low doses, but convulsions were observed only at doses above 750 mg/kg. Convulsions alone and violent aggression usually started 90-170 minutes after injection but death was delayed until about 48 hours except at very high doses. Mice that suffered delayed death showed decreased responsiveness and less spontaneous movement before dying. Because of the immediate and delayed signs of toxicity, the authors suggested that there might be two different toxic mechanisms involved.

Weir et al [125] later expanded their study to include the effect of the solution's pH on the toxicity of 1,2-dimethylhydrazine in mice, the LD50's for other species, and a study of hepatotoxicity. There was little difference between the LD50's in mice at 24 hours for hydrochloric acid as a control, 89 mg/kg, and unbuffered 1,2-dimethylhydrazine dihydrochloride, 83 mg/kg as the free base dose. Both solutions had a pH of less than 1.0. However, with the pH of 1,2-dimethylhydrazine dihydrochloride adjusted to 3, 7, and 11, the 24-hour LD50's were increased to 621, 462, and 245 mg/kg, respectively. This marked difference in toxicity led the authors to believe that the 24-hour toxicity of unbuffered 1,2-dimethylhydrazine dihydrochloride was largely caused by the hydrochloric acid. The 168-hour LD50's of hydrochloric acid and 1,2-dimethylhydrazine dihydrochloride, buffered or unbuffered, were similar, ranging from 32 to 54 mg/kg as the free base dose. A different mechanism from that of the 24-hour toxicity was suspected.

Weir and coworkers [125] then compared the toxicity of 1,2-dimethylhydrazine in rats and dogs to that found in mice. When male Long-Evans rats, 150-225 g, and male mongrels, 5-8 kg, were given 1,2-dimethylhydrazine dihydrochloride ip at pH 7, the immediate and delayed toxicity in mice, previously observed, was not seen. The LD50's at 24 and 168 hours, as free base doses, were 297 and 275 mg/kg for rats and 63 and 53 mg/kg for dogs.

To examine hepatotoxicity, 10 male Long-Evans rats were given 1,2-dimethylhydrazine dihydrochloride, adjusted to pH 7, ip at 495 mg/kg [125]. In addition, 2 groups of 10 male Swiss Webster mice were given 1,2-dimethylhydrazine dihydrochloride ip at 54 mg/kg unadjusted for pH or at 77 mg/kg adjusted to pH 7. Control groups were injected with distilled water. At 24, 48, 72, and 96 hours after injection, two animals from each group were killed, and liver sections were removed and studied.

The livers of experimental rats showed moderate focal necrosis of an indefinite pattern and leukocyte infiltration [125]; the degree of change was not time-dependent. The livers of mice killed 24 hours after injection of buffered 1,2-dimethylhydrazine dihydrochloride showed marked vacuolization and granularity of the cytoplasm. At 48 hours, in addition to these effects, widespread areas of focal necrosis were noted. Liver tissues of mice killed 72 and 96 hours after injection showed areas of regeneration and vacuolization but no necrosis. Unbuffered 1,2-dimethylhydrazine dihydrochloride caused more intense and persistent liver damage in mice than did the buffered form. Controls showed no liver lesions at any time.

The hepatotoxicity of 1,2-dimethylhydrazine dihydrochloride in several species was examined in 1976 by Wilson [126]. 1,2-Dimethylhydrazine dihydrochloride, at pH 7.0, was given sc and, at an unadjusted pH, it was given by gastric intubation. Four groups of 6 to 8 male miniature swine were given 8-10 weekly doses of 30 or 60 mg/kg with and without pH adjustment. Four swine served as controls. Twenty-eight male dogs received oral or sc doses of 5-60 mg/kg for 2-10 weeks for a total dose of either 50, 105, or 120 mg/kg. Two dogs served as controls. Groups of 6 male Hartley strain guinea pigs each received 60 mg/kg for 7 weekly doses or 30 mg/kg for 10 weekly doses by either route. Six guinea pigs were used as controls. Two groups of 10 male Sprague-Dawley rats were given oral doses of 30 mg/kg for 8 or 4 weekly doses. Six rats were used as controls.

In swine given 1,2-dimethylhydrazine dihydrochloride at 60 mg/kg, only three animals survived past 10 weeks [126]. All those that died earlier had hemorrhagic, hepatic degeneration and necrosis. Jaundice, bile duct proliferation, and megalocytosis were common. Most of the swine given 30 mg/kg survived, and when they were killed at 18 months, focal megalocytosis and postnecrotic fibrosis were observed in their livers. All dogs receiving 30-60 mg/kg died in the 2nd week, and all had jaundice, weight loss, hepatic degeneration, and hemorrhagic necrosis. Karyolysis in the hepatocytes was common. At 5 mg/kg, all dogs survived, but they had a transitory loss of appetite and jaundice. At 18 months, necropsies revealed postnecrotic hepatic fibrosis, hemosiderosis, and mild ascites. In guinea pigs, only one in each group receiving 60 mg/kg survived 8 months, and each had developed bile duct carcinomas. Those dying early had

extreme bile duct hyperplasia and hepatic necrosis. In the groups given 30 mg/kg, all survived at least 11 months. They were killed when near death during the next 7 months, and all had hepatic fibrosis and ascites. Nine of these animals had bile duct carcinomas, and two had hepatomas. In all groups, the experimental rats survived, but 16 of 20 developed 56 tumors of the colon. Three had carcinomas of the ear canal. The development of colon tumors was inversely related to hepatotoxicity, although the tumors might have developed in other species had they survived longer.

(2) Carcinogenicity and Effects Related to Reproduction

Kelly et al [81], in 1969, studied the effects of the hydrazines, including 1,2-dimethylhydrazine dihydrochloride, on lung tumor formation. Thirty male CDF1 mice, 7-8 weeks old, were given 1,2-dimethylhydrazine dihydrochloride in 8 weekly ip injections totaling 5.3 mg (189 mg/kg), and 30 females of the same age were given a total of 10.6 mg (424 mg/kg) by gavage over 8 weeks. A group of 10 males and 10 females given saline served as controls. Ninety percent of the females died, and at 33 weeks, one (33%) of the remaining three had lung tumors. One male died and 3 (10%) of the remaining 29 developed lung tumors. About 10% of the control males and females developed similar tumors. The authors concluded that 1,2-dimethylhydrazine was not carcinogenic in mice. However, the number of females surviving the toxicity of the compound was insufficient to draw any conclusion on long-term tumorigenic effects, and only lung tumors were examined.

In 1971, Toth and Wilson [127] reported on blood vessel tumors induced in mice by 1,2-dimethylhydrazine dihydrochloride. A 0.001% solution of the compound in drinking water was given to 50 male and 50

female randomly bred Swiss albino mice for life, starting at 7 weeks of age. The average daily intake of 1,2-dimethylhydrazine dihydrochloride was 0.058 mg for females and 0.087 mg for males. Controls consisted of 110 males and 110 females. All organs were examined macroscopically, and microscopic studies were performed on any gross, abnormal changes.

The lifespan of mice given 1,2-dimethylhydrazine dihydrochloride was considerably shortened when compared with that of the previously studied controls; 81% of the female and 50% of the male controls were alive at 60 weeks of age, while all of the exposed mice had died [127]. Subcutaneous generalized edema, hemoperitoneum, and anemia were noted in experimental mice. Forty-nine of the 50 exposed females (98%) developed blood vessel tumors at an average age of 45 weeks (range 28-58 weeks). Forty-six of the 50 males (92%) developed blood vessel tumors at an average of 42 weeks (range 29-58 weeks). The control group had a blood vessel tumor incidence of 3.6% in females and 1.8% in males at average ages of 68 and 76 weeks, respectively. The major locations of blood vessel tumors in females, in order of decreasing frequency, were 40 in the muscle, 37 in the liver, 36 in pararenal tissue, 32 in the fat, and 15 in parametrial tissues, while in the males the corresponding numbers were 39 in pararenal tissue, 37 in the muscle, 36 in fat, 28 in paraepididymal tissues, 26 in the liver, 15 in the subcutis, and 12 in the lymph nodes. The blood vessel tumors were classified as angiosarcomas.

Of the 50 females given 1,2-dimethylhydrazine dihydrochloride, 22 (44%) developed 35 lung tumors, all adenomas, at an average age of 49 weeks (range 31-58) [127]. Twelve of the 50 males (24%) developed 17 lung tumors, all adenomas except for 1 adenocarcinoma, at an age of 44 weeks

(range 29-58). Controls had lung tumor incidences of 13% in the females and 10% in males, at an average age of 90 and 74 weeks, respectively. The average latent period for lung tumor formation, about 40 weeks, in this experiment [127] was longer than the 33-week observation period of Kelly and coworkers [81], so that the results of the two papers are not necessarily inconsistent.

In another study of identical experimental design, conducted by the same investigative group [85], blood vessel tumors (85% incidence), classified as angiosarcomas, were induced in Syrian golden hamsters given 1,2-dimethylhydrazine dihydrochloride at about 0.16 mg/day. An increased incidence of cecal tumors (23%), mostly adenomas, and liver tumors (17%), both benign and malignant, was also found; however, lung tumors were not present.

In 1976, Toth and coworkers [128] reported on the induction of tumors in mice following sc injection of 1,2-dimethylhydrazine dihydrochloride. One group of 50 male and 50 female Swiss mice, 5 weeks old, were injected sc at a single dose of 20 mg/kg; a similar group received 10 injections. One hundred male and 100 female mice reported on earlier [129] were used as controls.

Repeated injection of 1,2-dimethylhydrazine dihydrochloride drastically reduced the survival rate, but single injection reduced it only slightly [128]. Of the mice given a single injection, one female (2%) and one male (2%) each developed a tumor of the cecum. Of the animals given repeated injections, 41 of 50 females (82%) developed 130 tumors of the large intestine at an age of 29-90 weeks, while of the males, 45 (90%) developed 156 tumors of the large intestine at 32-77 weeks of age. In both

males and females, polypoid adenomas and adenocarcinomas of the cecum, colon, and rectum were found, with the tumors occurring most frequently in the cecum adjacent to the ileum, in the lower part of the colon, and in the rectum. There were no tumors of the large intestine in the control group.

Blood vessel tumors, classified as angiomas or angiosarcomas, were found in 10 females (20%) and 12 males (24%) of the single injection group [128]. Most of the tumors were in the liver. Of the mice given 10 injections, 23 females (46%) and 25 males (50%) developed blood vessel tumors. Eight females had angiomas and 15 had angiosarcomas. The tumors were found, in decreasing order of frequency, in the following organs: liver, lungs, muscle, fat, lymph nodes, uterus, ovaries, and kidneys. In the males, 22 had angiosarcomas, and 3 had angiomas. In decreasing order of frequency, the liver, paraepididymial tissues and muscle, fat, pararenal tissues, subcutaneous tissues, lymph nodes, kidneys, brain, lungs, and spleen were affected. In the control group, 5% of the females and 6% of the males developed blood vessel tumors.

Fourteen of the females (28%) given a single injection of 1,2-dimethylhydrazine dihydrochloride developed 16 lung tumors, and 15 of the males (30%) developed 24 lung tumors [128]. All tumors were adenomas except for four cases in which the mice had a total of six adenocarcinomas. Of the animals receiving repeated injections, 24 females (48%) developed 62 lung tumors, 3 of which were adenocarcinomas. In addition, 19 males (38%) developed 26 adenomas of the lungs. In the control groups, 21% of the females and 23% of males developed lung tumors.

Of the animals given 10 weekly injections of 1,2-dimethylhydrazine dihydrochloride, 3 (6%) females and 24 (48%) males developed kidney tumors,

mostly adenomas [128]. Tumors of the anus were also found in 12% of the females and in 16% of the males.

Toth and Wilson [127] had reported in another study that 1,2-dimethylhydrazine dihydrochloride in drinking water given to mice caused mainly blood vessel tumors. In this study [128], however, injected 1,2-dimethylhydrazine dihydrochloride induced tumors of the large intestine and lungs as well. The authors suggested that this was caused by differences in routes of administration, resulting in different metabolism.

In a study designed to detect the effects of vitamin A on colon cancer, Rogers et al [130] induced tumors by gastric intubation of 1,2-dimethylhydrazine in male Sprague-Dawley rats. Three groups of rats were given 1,2-dimethylhydrazine at total doses of 420, 275, or 197 mg/kg over 18 weeks. An equal number of controls were given 0.9% saline. In addition, animals of each group were fed normal diets, diets deficient in vitamin A, or diets supplemented with vitamin A. At 420 mg/kg, 10 of 14 rats developed carcinomas of the gastrointestinal tract and the colon. At 275 mg/kg, 48 of 62 rats had the same types of tumors. At the lowest dose, 8 of 10 rats had carcinomas of the gastrointestinal tract and 6 had carcinomas of the colon. In all 86 rats, there were, in addition, 3 hemangiosarcomas, 19 carcinomas of the ear canal, 2 hepatocarcinomas, and 1 embryonal nephroma. The results were reported only on rats retained 18-30 weeks after administration of the first dose, and there was no effect on tumor incidence attributable to vitamin A.

Druckrey [131], in 1970, reported a study of the production of carcinomas by 1,2-dimethylhydrazine dihydrochloride. The compound was given to 2 groups of 13 BD rats sc at a weekly dose of 15.6 or 47 mg/kg for

36 weeks. An additional group of 14 BD rats was given 47 mg/kg by intubation weekly for 11 weeks, and another was given 6.7 mg/kg in drinking water daily for 5 days/week, for 11 weeks.

After 3-4 months, the rats given 1,2-dimethylhydrazine dihydrochloride sc at 47 mg/kg developed diarrhea, weight loss, and jaundice; some rats developed a prolapse of the tumorous rectum [131]. All 13 rats died with what was described as multiple malignant intestinal cancer; 5 had multiple tumors of both the colon and rectum, 5 had colon tumors, and 3 had rectal tumors. The median latent period was 26 weeks, at which time a total dose of 517 mg/kg had been given. Additional tumors were found in the duodenum of seven rats, in the small intestine of four, and in the liver of two. Twelve rats that received 15.6 mg/kg developed tumors of the colon, 6 had rectal tumors, and 2 had duodenal tumors. The median latent period for these tumors was 48 weeks. All tumors were classified as adenocarcinomas, often associated with polyps in all stages of progressing malignancy. They reportedly bore striking resemblance to human colonic and rectal carcinomas.

One of 14 rats given 1,2-dimethylhydrazine by stomach tube died of pneumonia; the remaining 13 died with multiple colonic carcinomas [131]. In addition, four had rectal carcinomas, three had duodenal tumors, one had a nephroblastoma, and six had pronounced cystic degeneration of the liver. In the rats given 1,2-dimethylhydrazine in drinking water, only hemangioendotheliomas of the liver with multiple metastases to the lungs were found. The difference in organ response caused by sc and oral administration of 1,2-dimethylhydrazine was attributed to metabolic activation in the liver. Druckrey suggested that, in animals, 1,2-

dimethylhydrazine was first enzymatically oxidized to methylazoxymethanol and then converted to methylating carcinogens, including methyldiazohydroxide and methyldiazonium hydroxide. Azoxymethane, a derivative of 1,2-dimethylhydrazine, was also studied and was found to induce tumors similar to those caused by 1,2-dimethylhydrazine.

Many other investigators [132-143] have used sc injections of 1,2-dimethylhydrazine to study the induction of colon cancers. Colonic tumors have been induced in CF1 [132,133], NMRI [134,135], and Swiss [136] strains of mice and in BD rats [137], but one group of investigators [136] was unable to induce tumors in C57/B mice. A strain specificity in germ-free rats was also reported [138], Sprague-Dawley rats being more susceptible to colon tumor induction than Buffalo rats; the Wistar strain was the least susceptible. Differences in tumor induction between rats and mice have been noted [139]. Germ-free rats have been used to determine the effects of bacterial flora in the intestine, and Reddy et al [140] reported a 20% incidence of colonic tumors in germ-free rats as compared with a 93% incidence in conventionally raised animals. Tumors of the ear canal, kidneys, and small intestine were also found in the conventional rats. The effects of diet have also been considered. Rats fed a high-fat diet had a higher incidence of colonic tumors than those on normal or low-fat diets [138,141]. Rats fed cholestyramine developed more colonic tumors with a greater concentration in the distal end of the colon than did regularly fed rats [142]. Mice fed disulfiram concurrently with 1,2-dimethylhydrazine injection failed to develop colonic tumors, whereas 100% of the other animals did [143].

The previously mentioned study by Greenhouse [90] on the effect of hydrazines on the embryonic development of the South African clawed toad included 1,2-dimethylhydrazine. Continuous exposure of the embryos and larvae to 1,2-dimethylhydrazine at concentrations up to 10 mg/liter had no toxic effects. Continuous exposure at 100 mg/liter beginning at the blastula stage was toxic to all exposed embryos; by 2 weeks, 50% of the embryos were dead and the rest had tail malformations. Cleavage stage embryos were then exposed to aqueous media containing 1,2-dimethylhydrazine at various concentrations and malformations were counted at the hatching stage [91]. At concentrations of 10, 20, 40, 50, and 80 mg/liter, 4, 4, 5, 100, and 100%, respectively, of the exposed embryos were malformed.

(3) Metabolism

Fiala et al [144] identified metabolites of 1,2-dimethylhydrazine exhaled by rats. Male F344 rats, kept in metabolism cages, were given ¹⁴C-labeled 1,2-dimethylhydrazine sc at 21 or 200 mg/kg, and expired air was analyzed for azomethane and carbon dioxide.

At 21 mg/kg, 11% of the administered dose was metabolized to carbon dioxide and 14% to azomethane in 24 hours [144]. The corresponding figures at 200-mg/kg were 4 and 23%, respectively. Azomethane, detected almost immediately after injection, reached about 90% of its 24-hour value in 4-5 hours. In the same time, carbon dioxide recovery was only 60% of its 24-hour level. This lag in carbon dioxide production was interpreted as being caused by the time required for complete metabolism of 1,2-dimethylhydrazine.

That azomethane was found to be a major metabolite of 1,2-dimethylhydrazine, and that it was present in the exhaled air gives support

to Druckrey's hypothesis [131] that 1,2-dimethylhydrazine is metabolically activated. Fiala et al [144] also noted that, if azomethane itself is a carcinogen, investigators handling large numbers of animals given 1,2-dimethylhydrazine should take special precautions to avoid exposure.

(e) Phenylhydrazine

(1) Systemic Effects

In 1935, Bolton [145] described changes in the blood cells of a dog weighing 16.2 kg and given phenylhydrazine hydrochloride at 18.5 mg/kg by stomach tube daily for 4 days. Blood counts were determined before phenylhydrazine administration and intermittently for the next 16 days. On the day of the last phenylhydrazine administration, the erythrocyte count had decreased 25% from the baseline value. Four days later it was reduced by 79%, and the hemoglobin concentration was less than 18% of the initial value. On the next day, the leukocyte count had increased from the preexposure level of 12,000/cu mm to 45,000/cu mm. Two weeks after the last dose was given, the leukocyte count was normal and the erythrocyte count was 90% of the baseline value.

In 1936, Von Oettingen and Deichmann-Gruebler [146] reported their study on the pharmacologic action of phenylhydrazine and its derivatives in mice and rats. Groups of mice were injected sc with phenylhydrazine at 170, 180, and 200 mg/kg. At 170 mg/kg, 45% of the mice died within 70 minutes. Phenylhydrazine at 180 and 200 mg/kg killed all the exposed mice in 50 and 40 minutes, respectively. The authors stated that the minimum lethal dose for rats, determined essentially the same way, was the same although they did not present supporting data. Phenylhydrazine in toxic doses produced progressive cyanosis, irregular and spasmodic respiration,

progressive depression, and asphyxial convulsions in mice and rats. In another part of this study, an ointment containing 1% phenylhydrazine was applied to a shaved area on the backs of five rats every other day. The rats lost an average of 15 g of body weight in 7 days, but microscopically the internal organs appeared normal. In a third phase of this study, one-tenth the minimum lethal dose injected sc into rats once a day for 3 days produced a 63% reduction in the number of erythrocytes by day 5.

In 1965, Ekshtat [16] determined the oral LD50 of phenylhydrazine in six guinea pigs, six rabbits, and an unstated number of mice and rats. The LD50's reported were 175 and 188 mg/kg for mice and rats, respectively, and 80 mg/kg for both rabbits and guinea pigs. Toxic signs reported were motor excitation and tonic-clonic convulsions.

Witchett [147], in 1975, reported the effects of phenylhydrazine on erythrocytes. Three male beagles were given phenylhydrazine sc at 20, 30, or 40 mg/kg for 2 consecutive days. Two dogs were used as controls. Blood was drawn daily from 4 days before exposure until death. On the 5th day, all survivors were killed.

Hemoglobin concentration, hematocrit value, and erythrocyte count were significantly reduced at the two lower doses [147]. The dog given the highest dose died shortly after the second injection. Methemoglobin was present in the blood of all three dogs, but leukocyte count and blood glucose concentration were normal. Reduced glutathione levels were decreased 2 hours after the first injection and 30-40% of the erythrocytes contained Heinz bodies; by 24 hours, they were present in 95-100% of the erythrocytes. Reticulocytes could not be counted because of the presence of Heinz bodies. The day after the second injection, the urine of the two

surviving dogs contained blood, and nearly 100% of their erythrocytes contained Heinz bodies. At necropsy, the internal organs were dark brown and the spleen, liver, and kidneys were severely congested. Large amounts of blood pigments were found in these organs, and the spleen was three to five times the normal size. The Kupffer cells in the liver and the epithelium of the convoluted tubules of the kidneys were hypertrophied and filled with blood pigment, apparently hemoglobin. There was also a striking reduction of spermatogenesis.

Chen and Weiss [148] investigated the effects of phenylhydrazine administration on the spleen of rats. Male Sprague-Dawley rats were given phenylhydrazine hydrochloride ip at 100 mg/kg and saline injected rats served as controls. Groups of five rats were killed 4 and 12 hours and 1, 3, 5, and 7 days after injection. Blood samples were collected when the animals were killed, and the spleen was examined by electron microscopy.

All experimental rats developed acute anemia [148]. By day 3, the hematocrit value had decreased from 45 to 25%, but it subsequently increased to near normal by the 7th day. Spleen weights in experimental rats were three times those in controls by day 5 and remained higher than usual throughout the experiment. Cells containing Heinz bodies were found to impede the passage of normal erythrocytes through the walls of the splenic sinuses. When the passageways were occupied because of delayed clearance of damaged erythrocytes, circulation through the spleen was impaired. Damaged cells accumulated in the cords, and coincidentally the number of macrophages also increased. The authors concluded that these factors contributed to the development of splenomegaly.

Saterborg [149] investigated bone marrow response to phenylhydrazine-induced anemia. Colloidal ^{198}Au was used to determine the transformation of fatty, inactive marrow to active marrow capable of hematopoiesis. Eighteen rabbits, given phenylhydrazine hydrochloride at a daily iv dose of 2.5 mg, were killed at various intervals up to 45 days. Before they were killed, the animals were injected with colloidal ^{198}Au and blood samples were taken. In 4-6 days, the reticulocyte count was raised, and it eventually increased to 50%. By day 10, the uptake of colloidal gold in the tibia was three times that of the controls. Hyperemia and capillary proliferation were found in an additional group of rabbits that received phenylhydrazine for 6-14 days. In those rabbits given phenylhydrazine for 14 days, a small area of fatty marrow, dilatated vessels and bleeding were seen. Immature hematopoietic cells were frequently found in dilatated blood vessels. The author concluded that phenylhydrazine was effective in causing the blood destruction that stimulated the transformation of yellow marrow to red active marrow. Toxic effects, not otherwise defined, were also observed in the liver, but hemolysis, not the secondary hepatic injury, was considered the cause of the bone marrow stimulation.

(2) Metabolism

In 1958, McIsaac et al [150] reported a study of the metabolism of phenylhydrazine. Groups of Chinchilla doe rabbits were given ^{14}C -labeled phenylhydrazine hydrochloride at 50 mg/kg orally, and their urine was collected. Urinary excretion was measured in seven rabbits for up to 10 days and two were killed after 4 days to determine the distribution of phenylhydrazine in tissues. The metabolites in the urine were purified by extraction and descending chromatography, located by

autoradiography, and identified by spray reagents. The amount of each metabolite in 2-day urine samples from four rabbits was then determined by isotope dilution. All radioactivity was measured on infinitely thick samples using an end window counter.

The urine of rabbits given phenylhydrazine hydrochloride at 50 mg/kg was dark brown, had a pH of 9, and reduced Benedict's solution [150]. Thirty-four percent of the dose was excreted in the urine in 1 day. By the 4th day, 50% was recovered, and excretion continued more slowly for at least 10 days. Only small amounts were found in any tissue. On the 4th day of a separate experiment, 10% of the phenylhydrazine was found in the erythrocytes, compared to 59% in the urine.

The authors determined from autoradiography that pyruvic acid phenylhydrazone and oxoglutaric acid phenylhydrazone were present in the urine [150]. They confirmed this finding by adding a known amount of each compound, labeled with ^{14}C , to the urine of animals which received unlabeled phenylhydrazine hydrochloride. After considering the total weight and percentage of radioactivity recovered after purification, the authors determined that these two hydrazones were in the urine. A third compound was identified as p-hydroxyphenylhydrazine glucuronide. Isolation of a derivative prepared from a urine sample supported this finding. A fourth component found by autoradiography was not identified. Isotope dilution studies were conducted and showed that 17.2% of the given dose was excreted within 2 days in the urine as p-hydroxyphenylhydrazine, 8.5% as pyruvic acid phenylhydrazone, and 5.2% as oxoglutaric acid phenylhydrazone; these compounds accounted for 79% of the total radioactivity in the urine.

The authors [150] concluded that the main metabolic reactions of phenylhydrazine in rabbits were hydroxylation of the ring and subsequent conjugation and reaction with keto acids. They also noted that there was no evidence of acetylation or decomposition to aniline or benzene.

(3) Carcinogenicity and Effects Related to Reproduction

In 1966, Clayson et al [151] reported the results of a study on the induction of lung tumors in BALB/c mice by phenylhydrazine. Phenylhydrazine hydrochloride was administered to 30 mice of both sexes at a daily dose of 1 mg via stomach tube 7 times a week for 42 weeks; the total dose reported was 200 mg instead of the expected 294 mg because administration was suspended if the mice showed signs of toxicity. Thirty mice were used as controls; a control mouse of similar age was killed whenever an exposed mouse died and both were examined.

Sixteen (53%) of the mice given phenylhydrazine hydrochloride developed lung tumors, compared with 4 (13%) of the control group [151]. The average number of lung tumors in each tumor-bearing mouse was 1 in the control group and 1.5 in the exposed group. Of the 24 tumors found in the exposed group, 17% (4) were carcinomas, 42% (10) were adenomas, and 42% (10) were described as adenomas becoming malignant. Clayson et al concluded that phenylhydrazine hydrochloride was a weak carcinogen, although they pointed out that a relatively high percentage of the tumors induced were malignant.

In 1967, Roe and coworkers [78] reported a study on the carcinogenicity of several hydrazines, including phenylhydrazine, on groups of 25 virgin female Swiss mice. Phenylhydrazine in doses of 0.5-0.25 mg/kg was given by gavage 5 days/week for 40 weeks. At weeks 40-50 or 50-60, 17

surviving mice were killed, and no lung tumors were found. The authors concluded that phenylhydrazine was not carcinogenic. However, six mice were apparently not examined and only lung tumors were considered.

Kelly et al [81] conducted similar experiments on the carcinogenicity of phenylhydrazine. Thirty male CDF1 mice, 7-8 weeks old, were given 8 weekly ip injections totaling 11.6 mg (387 mg/kg) of phenylhydrazine hydrochloride. Thirty females of the same age were given phenylhydrazine hydrochloride in 8 weekly oral doses totaling 23.2 mg (892 mg/kg). Ten male and 10 female mice given saline served as controls. Of the surviving experimental females, 14% developed lung tumors by 28 weeks, while 13% of the surviving males had lung tumors at 26 weeks. The corresponding incidences in the controls were 10 and 11%, respectively. Since the difference in the incidence of lung tumors was not significant, Kelly et al concluded that phenylhydrazine was not carcinogenic in mice. This conclusion is weakened by the fact that all animals were killed by 33 weeks and only the possibility of lung tumors was examined.

Shimizu and Toth [152], in 1976, reported the tumorigenic effects of phenylhydrazine hydrochloride in Swiss mice given a 0.01% solution in drinking water for life starting at an age of 5 weeks. The average daily consumption of phenylhydrazine hydrochloride was 0.63 mg for 50 females and 0.81 mg for 50 males. Data obtained previously from 100 controls of each sex were used for comparison [129].

Of the experimental mice, 11 females (22%) developed blood vessel tumors at an average age of 71 weeks, and 10 males (20%) developed such tumors at an average age of 87 weeks [152]. In the controls, five females (5%) and six males (6%) developed blood vessel tumors. The increased tumor

incidence in the experimental group was statistically significant, with $P < 0.008$ for females and $P < 0.02$ for males. These blood vessel tumors, classified as angiosarcomas and angiomas, were found in the liver, spleen, and ovaries. Lung tumors, malignant lymphomas, hepatomas, and a few other tumor types were also found, but their incidences were not significantly different than those of the controls.

Tamaki et al [153] investigated the functional disturbances of offspring of rats given phenylhydrazine hydrochloride during pregnancy. Some pregnant Wistar rats received phenylhydrazine hydrochloride ip at 10 mg/kg on days 17-19 of pregnancy, while others received 20 mg/kg ip on days 18 and 19. Twelve male offspring with severe jaundice and anemia at birth were chosen for subsequent experiments. Nine normal males served as controls. Testing began when the pups were 9-22 weeks of age. The general reflexes of 7 control and 8 experimental rats were examined, the spontaneous activity of 8 control and 12 experimental animals was monitored over 24 hours, and 4 control and 4 experimental rats were used to test the acquisition and extinction of conditioned avoidance-escape behavior.

No performance differences were observed between the offspring of the two groups of experimental dams, and there were no significant differences between control and experimental groups in tests of general reflexes or of spontaneous activity. In the conditioned avoidance tests, the experimental group was significantly retarded ($P < 0.05$) in response acquisition and speed of acquisition, and, during the extinction phase of the test, it lost the response significantly faster ($P < 0.05$). This extinction factor was

interpreted as evidence that acquired behavior was less stable in the experimental than in the control rats.

The authors [153] concluded that phenylhydrazine injected during pregnancy may adversely affect the performance of the offspring in certain areas of learning by inducing severe neonatal jaundice and anemia. The possibility that phenylhydrazine might act directly on the developing CNS, or that the learning deficit might be a result of the combination of the direct and secondary effects are questions not discussed by the author. It seems that anemia accompanied by jaundice more likely represents fetal toxicity and not teratogenicity; however, to induce terata experimentally, injection should occur at an earlier stage of pregnancy.

Correlation of Exposure and Effect

Little information is available on humans exposed to the hydrazines, so that the toxic effects that would be expected to occur in humans must be established from animal studies. There are both striking similarities and dissimilarities in the effects produced by these structurally related compounds. Judging from animal studies, one finds that the major sites affected appear to be the skin and eyes, the CNS, the liver, the blood, and the kidneys. These effects, along with odor thresholds, metabolism, and changes in biochemical function, are compared for each compound in the following sections, and relevant human information is presented where available.

(a) Skin and Eyes

Dermatitis has been observed in humans who had contact with hydrazine hydrate [38,39], its monohydrochloride [40], sulfate [37], and hydrobromide