National Toxicology Program
Toxicity Report Series
Number 42

NTP Technical Report
on Toxicity Studies of

1,3-Diphenylguanidine

(CAS No. 102-06-7)

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

Richard D. Irwin, Ph.D., Study Scientist
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Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 95-3933
September 1995

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

Note to the Reader

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

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CONTRIBUTORS

This NTP report on the toxicity studies of 1,3-diphenylguanidine is based primarily on 2-week studies conducted in June and July 1989 and on 13-week studies that began in February 1990 and ended in June 1990 at Microbiological Associates, Inc., Bethesda, MD.

National Toxicology Program
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PEER REVIEW

The draft report on the toxicity studies of 1,3-diphenylguanidine was evaluated in March 1994 by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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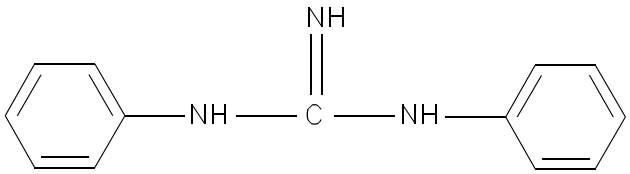
ABSTRACT

1,3-Diphenylguanidine



Molecular Formula	$C_{13}H_{13}N_3$
CAS Number	102-06-7
Molecular Weight	211.26
Synonyms	Diphenylguanidine DPG Melaniline *N,N′*-Diphenylguanidine *sym*-Diphenylguanidine Vulkazit

1,3-Diphenylguanidine (DPG) has been used as a primary and secondary accelerator in the vulcanization of rubber. Exposure to 1,3-diphenylguanidine may occur as a result of dermal contact during rubber manufacture or from contact with the finished products. DPG is poorly absorbed through skin. Therefore, to evaluate the toxicity associated with systemic exposure, 2-week and 13-week toxicologystudies were conducted by administering DPG in feed to groups of male and female F344/N rats and B6C3F$_1$ mice. Genetic toxicity was also evaluated in *Salmonella typhimurium* and in the micronucleus erythrocyte assay in peripheral blood from male and female mice.

During 2-week studies, rats and mice received feed containing 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine. All rats and mice survived to the end of the study. Feed consumption and mean body weights of groupsof rats that received 750, 1,500, or 3,000 ppm were lower than controls. No compound-related gross lesions were observed at the end of the study. The final mean body weight of femalemice that received 3,000 ppm was 6% lower than the controls at the

end of the study; however, no other effects attributable to chemical exposure were observed in mice. Based on these results the same exposure concentrations (0, 250, 500, 750, 1,500, and 3,000 ppm) were selected for the 13-week study; because of the poor palatability of the 750 ppm or higher dosed feed in rats, concentrations greater than 3,000 ppm were not considered appropriate.

Six male rats and all female rats that received feed containing 3,000 ppm died or were killed moribund before the end of the 13-week study. Final mean body weights and feed consumption of male and female rats that received 1,500 or 3,000 ppm were lower than controls throughout the study. The values of several hematologic parameters were significantly different from the controls in groups of rats that received 1,500 or 3,000 ppm; however, these differences were attributable to reduced nutrient intake as a result of reduced feed consumption. Lower total serum protein, cholesterol, triglyceride, and creatinine concentrations were also considered to be the consequence of reduced nutrient intake. Alkaline phosphatase activity and bile acid concentrations were greater than the controls in most groups exposed to DPG and were considered to be an indication of cholestasis.

Secretory depletion of the seminal vesicles and prostate gland, epididymal hypospermia, spermatogenic arrest, and significant reductions in the absolute weights of the prostate gland, seminal vesicles, and testis were observed in male rats in the 3,000 ppm group. Uterine hypoplasia characterized by a reduction in uterine size due to thinner and less developed endometrium was observed in female rats that received diets containing 750 ppm or greater. The mean length of the estrous cycle was greater in female rats that received 750 or 1,500 ppm feed than in the controls.

All mice survived to the end of the 13-week study. Mean body weights of males and females that received feed containing 750, 1,500, or 3,000 ppm were lower than the controls. Reduced organ weights relative to control for mice that received 1,500 or 3,000 ppm were related to low body weights of these groups. In mice that received 3,000 ppm sperm motility was reduced and the number of spermatid heads was greater than for control males, and the estrous cycle length in females was longer than that of the controls.

1,3-Diphenylguanidine was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S9 metabolic activation enzymes. No mutagenic

activity was observed in the absence of S9. With S9, positive responses were observed in strains TA98 and TA100, and an equivocal response was observed in strain TA1537. Results of a peripheral blood micronucleus test in B6C3F$_1$ mice were concluded to be negative in males and equivocal in females.

In summary, consumption of feed containing 1,3-diphenylguanidine for 2 weeks or 13 weeks was not associated with any histologic response which could be attributed to chemical exposure. Instead the observed changes were indicative of reduced nutrient intake and are consistent with similar changes observed in other studies of feed restricted rats and mice.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

1,3-Diphenylguanidine is a white solid that melts at 148° to 150° C. It is soluble in organic solvents such as ethanol, chloroform, or carbon tetrachloride but is sparingly soluble in water. 1,3-Diphenylguanidine has been used as a primary accelerator in the vulcanization of rubber and as a secondary accelerator for sulfur-containing compounds such as thiazoles, sulfenamides, and thiurams. Depending on the specific application, the concentration of 1,3-diphenylguanidine may vary from 0.25% to 2.0% by weight (*Kirk-Othmer*, 1982; *Merck Index*, 1983). Rubber containing 1,3-diphenylguanidine has been used in footwear, tires, and molded goods. Domestic production of 1,3-diphenylguanidine was not reported by the United States International Trade Commission (USITC) for the years 1981 to 1988 (USITC, 1988), and there is currently no known domestic production of the compound.

According to a National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1981 to 1983, an estimated 611 workers at 60 plants were potentially exposed to 1,3-diphenylguanidine (NIOSH, 1990). All of these individuals were employed in the manufacture of rubber and miscellaneous plastic products as blending machine operators, molding and casting machine operators, chemical technicians, or unspecified workers (48 individuals). 1,3-Diphenylguanidine and several other chemicals used in rubber manufacturing cause a form of contact dermatitis referred to as "rubber itch," which is observed most often in workers involved in pigment blending, mixing, milling, and extrusion (Williams *et al.*, 1985).

Exposure to 1,3-diphenylguanidine may also occur as a result of contact with finished rubber products. Buxton *et al.* (1983) reported that a number of patients undergoing regular hemodialysis gave positive patch tests for several rubber chemicals, including 1,3-diphenylguanidine.

Absorption, Disposition, Metabolism, and Excretion

Shah *et al.* (1985) found that 1,3-diphenylguanidine was poorly absorbed through the skin of rats. In this study, 0.3 µmole [^{14}C]-labeled 1,3-diphenylguanidine in acetone was applied to the clipped skin of Sprague-Dawley rats. After 30 minutes, only 0.1% of the applied DPG had been absorbed, and after 120 hours, only 10% of the DPG had been absorbed. Absorption was characterized as a first-order process with a half-time of 33.6 days. Absorbed material was distributed throughout the body, with peak concentrations being attained approximately 6 hours after dosing, at which time the highest concentrations of radioactivity were present in the liver, kidney, and intestine. Within 5 days, 61% of the absorbed dose had been eliminated in the urine and 27% had been eliminated in the feces. During the first 72 hours after dosing, approximately 50% of the radioactivity in the urine was in the form of the parent compound, while 50% was in the form of a single metabolite; 96 hours after dosing, all urinary radioactivity was in the form of the metabolite. No parent compound was identified in the feces, and all radioactivity was in the form of two metabolites that differed from the urinary metabolite.

1,3-Diphenylguanidine disposition in male F344 rats was examined by Ioannou and Matthews (1984). After oral or intravenous administration, [^{14}C]-labeled 1,3-diphenylguanidine-derived radioactivity was rapidly distributed throughout the body, with the highest concentration occurring in the liver at all time points examined. Radioactivity peaked in the liver approximately 45 minutes after intravenous administration and was rapidly cleared from all tissues so that the total body burden 24 hours after dosing was 10% of that at 15 minutes. Clearance was biphasic, with the initial rapid phase accounting for the major portion of the dose. Approximately equal amounts of radioactivity were eliminated in the feces and urine; however, in rats in which the common bile duct had been cannulated, 75% of the administered DPG appeared in bile within 6 hours after dosing, which is consistent with significant enterohepatic recycling of material excreted in bile. The majority of the radioactivity found in bile (95%) was in the form of a single metabolite. Incubation of this metabolite with β-glucuronidase yielded a metabolite identical to the major fecal metabolite, which accounted for 95% of the radioactivity eliminated in the feces. Radioactivity in the urine was in the form of the parent compound (28%), two major metabolites (37%) and (34%), and a minor metabolite (3%). Incubation of the major urinary metabolite with β-glucuronidase also yielded a metabolite that was identical to the major fecal metabolite.

Toxicity

ANIMAL TOXICITY

The acute toxicity of 1,3-diphenylguanidine hasbeen evaluated in rats, mice, and rabbits (Table 1). The similarity of LD_{50} values among the three species for the oral route of exposure suggests that the absorption, distribution, and metabolism of 1,3-diphenylguanidine may be similar across species.

The toxicity of 1,3-diphenylguanidine has been evaluated in 2-week and 13-week studies. In a 2-week repeated dose toxicity study in Sprague-Dawley rats, groups of five rats per sex received 0, 300, 500, 800, 1,500, or 3,000 ppm 1,3-diphenylguanidine in the diet (Monsanto Company, unpublished). Two males and three females that received 3,000 ppm died before the end of the study. Exposure-related decreases in mean body weights, body weight gains, and feed consumption were observed in all exposed groups. The lower absolute organ weights that were observed in groups receiving 500 ppm or greater were considered a consequence of lower body weight gains. No histopathology was conducted in this study.

During a 13-week toxicity study, groups of 15 Sprague-Dawley rats per sex received 0, 50, 150, or 500 ppm 1,3-diphenylguanidine in the diet (Monsanto Company, unpublished). One male that received 500 ppm and one control female died before the end of the study. Mean body weights, body weight gains, and feed consumption were reduced in groups that received 500 ppm. In addition, after 6 and 13 weeks of exposure, urine volume was decreased and specific gravity was increased in groups receiving 500 ppm. There were no gross or microscopic histologic lesions that could be attributed to 1,3-diphenylguanidine exposure.

TABLE 1 Summary of Selected Animal Toxicity Data for 1,3-Diphenylguanidine

Species	Route of Exposure	LD_{50} (mg/kg)	Reference
Rat	Oral	323	NTP, 1988
	Oral	350	Monsanto, 1986
	Oral	375	NTP, 1988
Mouse	Oral	258	NTP, 1988
Rabbit	Oral	246	NTP, 1988
	Dermal	>794	Monsanto, 1986

TERATOLOGY AND REPRODUCTIVE TOXICITY

The teratogenicity of 1,3-diphenylguanidine was evaluated in a study in which ICR mice received 0, 0.25, 1, 4, or 10 mg/kg by gavage on gestation Days 0 to 18 (Yasuda and Tanimura, 1980). All animals were necropsied on the 18th day of pregnancy. No maternal toxicity was observed. The mean number of implants was reduced in dams that received 10 mg/kg. However, there were no significant differences between treated and control groups in the percentage of dead fetuses, average litter size, sex ratio, or mean body weights. The incidence of external or skeletal abnormalities in treated groups was similar to that of the control group.

In studies performed by Bempong and Hall (1983), male Syrian golden hamsters and C57BL/J6 × DBA_2 mice were administered 0, 4, or 8 mg 1,3-diphenylguanidine per kg body weight in acidified drinking water daily for up to 15 weeks. After approximately 4 weeks of exposure, the incidence of abnormal sperm was increased in exposed hamsters and mice. In addition, reduced sperm counts and decreased testis weights were observed in mice exposed to 1,3-diphenylguanidine for 5 weeks or longer. Histologic examination of the testes of exposed mice revealed irregularly shaped seminiferous tubules with no defined basement membrane, a loss of interstitial cells, and reduced numbers of spermatids and spermatozoa in the tubule lumens.

Bempong and Hall (1983) also investigated the reproductive effects of 1,3-diphenylguanidine in C57BL/J6 × DBA_2 mice. In this study, male mice were exposed to 0, 4, or 8 mg 1,3-diphenylguanidine per kg body weight in acidified drinking water daily for about 13 weeks;

beginning on Day 7 of exposure, males were mated weekly with untreated virgin females. Exposure to 1,3-diphenylguanidine for 4 weeks did not significantly reduce fertility indices or induce dominant lethal effects in mice. However, after 5 weeks of exposure, fertility indices and the number of implants per pregnant mouse were significantly decreased in exposed groups, and the number of dead fetuses per pregnancy was significantly increased.

In a recent study by Koëter *et al.* (1992), male Swiss (CD-1®) mice were administered 1,3-diphenylguanidine by gavage at doses of 0, 0.06, 0.25, 1, 4, or 16 mg/kg per day for 8 weeks prior to mating. At the end of the dosing period, approximately 50% of the males from the control and 16 mg/kg groups were randomly selected and evaluated for sperm morphology; the remaining males in the control, 4, and 16 mg/kg groups were mated with unexposed females. No differences in weight gain, clinical observations, or organ weights were observed between the control group and any of the treated groups (0.06, 0.25, 1, 4, and 16 mg/kg). Microscopic examination of the testes of mice in the control and 16 mg/kg groups did not reveal any treatment-related abnormalities. In addition, the fertility and reproductive performance of males exposed to 1,3-diphenylguanidine were similar to those of the control group.

GENETIC TOXICITY

1,3-Diphenylguanidine is chemically related to the aromatic amides which can be nitrosated to potentially mutagenic metabolites. 1,3-Diphenylguanidine was weakly mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when testing was performed in the presence of induced hamster liver S9 (Mortelmans *et al.*, 1986). A preincubation protocol was used in this assay, and effective concentrations were generally at the 100 µg/plate level and higher. Published *Salmonella* data indicate an absence of mutagenic activity in tests using induced rat liver S9 enzymes (Rannug *et al.*, 1984; Crebelli *et al.*, 1985); this enzyme mix is apparently less effective than hamster-derived enzymes in producing mutagenic metabolites of 1,3-diphenylguanidine (Mortelmans *et al.*, 1986). Bempong and Mantley (1985) reported that 1,3-diphenylguanidine (at concentrations below 36 µg/plate) was mutagenic in *Salmonella* in the absence of S9; however, the report did not contain sufficient data or protocol detail for a critical evaluation of the results.

No increases were observed in the frequency of gene mutations at the HGPRT locus in Chinese hamster V79 cells treated with up to 500 µg/mL 1,3-diphenylguanidine, with and without S9

(Donner *et al.*, 1983). However, the S9 source used in this study was induced rat liver, and the use of this enzyme mix may not have been appropriate for the chemical. Chromosomal damage was reportedly detected in *Vicia faba* root tip cells exposed to 5 or 10 µg/mL 1,3-diphenylguanidine (Bempong and McCoy, 1972). However, this latter study presented insufficient information for a critical interpretation of the results.

Data derived from the testing of structurally related compounds showed no evidence for mutagenicity. Negative results were obtained in *Salmonella* mutation tests for *N*,*N*′-bis(2-methylphenyl)guanidine (CAS No. 97-39-2) (Rannug *et al.*, 1984), carbanilide (CAS No. 102-07-8) (Zeiger *et al.*, 1988), and thiocarbanilide (CAS No. 102-08-9) (Haworth *et al.*, 1983); all testing was performed with and without S9. In addition, no induction of sex-linked recessive lethal mutations occurred in germ cells of male *Drosophila melanogaster* administered thiocarbanilide by feeding (1,000 ppm) or by injection (100 ppm) (Zimmering *et al.*, 1985).

Study Rationale and Design

1,3-Diphenylguanidine was nominated for toxicity testing by the National Cancer Institute because of the potential for human exposure to the compound, the lack of adequate data to characterize the toxicity of diarylguanidines as a chemical class, and the possibility that 1,3-diphenylguanidine could be converted to a strong mutagen by nitrosation. This report presents the results of 2-week and 13-week feed studies in rats and mice and mutagenicity studies in *Salmonella typhimurium* and in the micronucleus erythrocyte assay in peripheral blood from male and female mice.

MATERIALS AND METHODS

Procurement and Characterization of 1,3-Diphenylguanidine

1,3-Diphenylguanidine was obtained in one lot (Lot 239474) from Fluka Chemical, Inc (Ronkonkoma, NY). Identity and purity analyses were performed by Midwest Research Institute (MRI, Kansas City, MO). The chemical, an off-white, grayish powder, was identified as 1,3-diphenylguanidine by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; the spectra were consistent with the structure of 1,3-diphenylguanidine and an available literature reference (*Sadtler Standard Spectra*). The determined melting point of the chemical was also consistent with an available literature reference (*Merck Index*, 1983).

The results of elemental analyses for carbon, hydrogen, and nitrogen agreed with theoretical values. Karl Fischer analysis indicated a water content of 0.05% ± 0.03%, and functional group titration indicated a purity of 98.9% ± 0.6%. Analysis by thin-layer chromatography indicated a major spot, a minor impurity, and a trace impurity by one solvent system and a major spot, three minor impurities, and a trace impurity by a second solvent system. High performance liquid chromatography (HPLC) indicated one impurity with an area of 0.2% relative to the major peak. The cumulative analytical data indicated an overall purity of approximately 99%.

Accelerated stability studies of the bulk chemical were also performed by MRI. Analyses with HPLC indicated that the bulk chemical was stable for 2 weeks at temperatures up to 25° C when stored protected from light; some evidence of decomposition was noted in the chemical sample stored at 60° C. At the study laboratory, the bulk chemical was stored at 5° C protected from light and moisture; reanalyses by the study laboratory using HPLC indicated no detectable decomposition of the bulk chemical.

Dose Formulations

For the 2-week and 13-week studies, 1,3-diphenylguanidine was mixed with NIH-07 Open Formula Diet (Zeigler Brothers,Inc., Gardners, PA) in powder form. A premix was prepared for each exposure concentration by mixing a weighed amount of 1,3-diphenylguanidine in a 6-

beaker with an equal weight of feed. The premix was then blended with a weighed amount of feed in a twin-shell blender for 15 minutes, with the intensifier bar on for the first 5 minutes.

Homogeneity analyses of a 250 ppm 1,3-diphenylguanidine feed mixture were conducted at MRI; analyses with HPLC indicated a maximum variation in concentration of 1.6% among three sampling points. Analyses conducted by the study laboratory on feed mixtures of 1,3-diphenylguanidine with reverse-phase HPLC confirmed the homogeneity of the mixtures.

The stability of 1,3-diphenylguanidine feed mixtures was evaluated by HPLC. These analyses indicated that a 30 ppm 1,3-diphenylguanidine feed mixture was stable for 3 weeks when stored in the dark at 5° C, and a feed mixture of 250 ppm 1,3-diphenylguanidine was stable for 3 weeks in the dark at −20° C. Analyses of 250 ppm feed mixtures after 7 and 14 days of storage in the dark at −20° C indicated minor (2.8% and 5.2% respectively) but statistically significant chemical losses at both time points. These small differences were attributed to analytical variation. Both the 30 and 250 ppm feed mixtures showed significant losses of 1,3-diphenylguanidine (6% and 11% respectively) after 1 day of storage under animal room conditions. Feeders were changed daily, 7 days per week in the 2-week and 13-week studies.

The feed mixtures used in the 2-week and 13-week studies were stored in plastic bags or jars in the dark at −20° C and were discarded 3 weeks after preparation. The study laboratory periodically analyzed the 1,3-diphenylguanidine feed mixtures by reverse-phase HPLC. All feed mixtures administered to animals were within 10% of the target concentrations. The results of a referee analysis performed by MRI on a 500 ppm 1,3-diphenylguanidine feed mixture prepared at the start of the 13-week studies were in agreement with the study laboratory results.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice used in the 2-week and 13-week studies were obtained from Taconic Farms (Germantown, NY) and were approximately 4 weeks old at receipt. The animals were quarantined for 11 to 15 days and were 6 to 7 weeks old when the studies began. At the end of the 13-week studies, blood samples were collected from five sentinel rats and five control mice per sex, and the sera were analyzed for antibody titers to rodent viruses (Boorman *et*

al., 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning study design and performance are listed in Table 2.

In the 2-week studies, groups of five male and five female rats and mice were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available *ad libitum*. The exposure levels selected for the 13-week studies were based on the results of the 2-week studies. In the 13-week studies, groups of 10 male and 10 female rats and mice were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available *ad libitum*. Additional rats (10 males and 10 females per exposure group) were used in a supplemental clinical pathology study.

During the 2-week and 13-week studies, rats were housed five per cage and mice were housed individually. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available *ad libitum*. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week, throughout the 2-week and 13-week studies.

Complete necropsies were performed on all animals in the 2-week and 13-week base studies. For rats and mice, the heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

In the 2-week studies, histopathologic examinations were performed on all tissues with gross lesions. In the 13-week studies, complete histopathologic examinations were performed on all rats and mice in the 0 and 3,000 ppm groups, on all rats in the 1,500 ppm groups, and on all animals that died early. Gross lesions and selected tissues were examined in the lower exposure groups. Tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet

tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

In the 13-week study in rats, hematology and clinical chemistry evaluations were performed on 10 male and 10 female supplemental rats per group at Days 5 and 21 and on base-study rats at study termination (Week 13). For these evaluations, rats were anesthetized with CO_2, and blood samples were collected from the retroorbital sinus. Samples for hematology analysis were placed in tubes containing potassium EDTA, and samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant. The latter samples were allowed to clot at room temperature; the samples were then centrifuged and serum was removed.

All automated hematologic determinations were made with a Serono-Baker 9000 automated cell counter (Serono-Baker Diagnostics, Allentown, PA); the parameters evaluated are listed in Table 2. Manual hematocrit determinations were made using a Damon/IEC microcapillary reader (International Equipment Company, Needham Heights, MA). Differential leukocyte counts were determined from blood films stained with a modified Wright's stain in an Ames Hema-Tek II slide stainer, (Miles Laboratory, Ames Division, Elkhart, IN), and methemoglobin was measured with an IL CO-Oximeter 282 (Instrumentation Laboratory, Inc., Lexington, MA). Smears for reticulocyte determination were stained with freshly prepared methylene blue.

All clinical chemistry parameters were determined on a Serono-Baker Encore II chemistry system. Reagents for total bile acid determinations were obtained from Nyegeard and Company Diagnostics (Oslo, Norway); reagents for all other clinical chemistry determinations were obtained from Serono-Baker Diagnostics. The clinical chemistry parameters evaluated are listed in Table 2.

Sperm Motility and Vaginal Cytology Evaluations

At the end of the 13-week studies, vaginal cytology and sperm motility evaluations were performed on all base-study rats in the 0, 500, 750, and 1,500 ppm groups and all mice in the 0, 250, 750, and 3,000 ppm groups. The parameters evaluated are listed in Table 2. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). Beginning 12 days prior to sacrifice, the vaginal vaults of 10 females from each exposure group were lavaged, and then aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the corpus epididymis and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

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

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Feed Studies of 1,3-Diphenylguanidine

2-Week Studies	13-Week Studies
EXPERIMENTAL DESIGN	
Study Laboratory Microbiological Associates, Inc. (Bethesda, MD)	Same as 2-week studies
Strain and Species F344/N rats B6C3F$_1$ mice	Same as 2-week studies
Animal Source Taconic Farms (Germantown, NY)	Same as 2-week studies
Size of Study Groups Five males and five females	10 males and 10 females
Exposure Concentrations 0, 250, 500, 750, 1,500, or 3,000 ppm in feed, available *ad libitum*	Same as 2-week studies
Exposure Durations 2 weeks	13 weeks
Date of First Exposure Rats: 23 June 1989 Mice: 22 June 1989	Rats: 1 March 1990 (males), 2 March 1990 (females) Mice: 26 February 1990
Date of Last Exposure Rats: 6 July 1989 Mice: 5 July 1989	Rats: 30 May 1990 (males), 31 May 1990 (females) Mice: 28 May 1990 (males), 29 May 1990 (females)
Date of Necropsy Rats: 7 July 1989 Mice: 6 July 1989	Rats: 31 May 1990 (males), 1 June 1990 (females) Mice: 29 May 1990 (males), 30 May 1990 (females)
Type and Frequency of Observation Observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations and individual body weights were recorded on Days 1 and 8 and at the end of the studies. Feed consumption was recorded 5 consecutive days per week for 2 weeks.	Observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations were recorded weekly. Individual body weights were recorded at the start of the studies, weekly thereafter, and at the end of the studies. Feed consumption was recorded daily for 5 consecutive days per week for 13 weeks.
Necropsy Necropsies were performed on all animals. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were weighed at necropsy.	Necropsies were performed on all animals in the base studies. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were weighed at necropsy.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Feed Studies of 1,3-Diphenylguanidine (continued)

2-Week Studies	13-Week Studies
Histopathologic Examinations	
Histopathologic examinations were performed on all tissues showing gross lesions.	Histopathologic examinations were performed on all rats and mice in the control and 3,000 ppm groups, all rats in the 1,500 ppm groups, and all animals that died early. The following tissues were examined: adrenal glands, brain (three sections), esophagus, femur with marrow, gallbladder (mice only), gross lesions, heart, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland with adjacent skin, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, skin, spleen, spinal cord/sciatic nerve (rats only), stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thigh muscle (rats only), thymus, thyroid glands, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). For rats, the uterus and prostate gland were examined in the lower exposure groups; for mice, no tissues were designated for examination in the lower exposure groups.
Clinical Pathology Studies	
None	Hematology and clinical chemistry evaluations were performed on 10 supplemental-study rats per sex and exposure level at Days 5 and 21 and on base-study rats at study termination (Week 13). Hematology parameters evaluated included: automated and manual hematocrit (Hct), hemoglobin (Hgb), erythrocytes (RBCs), reticulocytes, nucleated erythrocytes, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, leukocyte (WBC) count and differential, and methemoglobin. Clinical chemistry parameters evaluated included: urea nitrogen (UN), creatinine, total protein, albumin, total and direct bilirubin, cholesterol, triglycerides, alkaline phosphatase (AP), alanine aminotransferase (ALT), creatine kinase (CK), sorbitol dehydrogenase (SDH), and bile acids.
Sperm Motility and Vaginal Cytology Evaluations	
None	Sperm motility and vaginal cytology evaluations were performed on base-study rats in the 0, 500, 750, and 1,500 ppm groups and mice in the 0, 250, 750, and 3,000 ppm groups. Males were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Feed Studies of 1,3-Diphenylguanidine (continued)

2-Week Studies	13-Week Studies
ANIMAL MAINTENANCE	
Time Held Before Study Rats: 15 days Mice: 13 days	Rats: 14 to 15 days Mice: 11 days
Age When Study Began 6½ to 7 weeks	6 to 7 weeks
Age at Necropsy 8½ to 9 weeks	19 to 20 weeks
Method of Animal Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Diet NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in powder form and drinking water (Washington Suburban Sanitary Commission Potomac Plant) available *ad libitum*.	Same as 2-week studies
Animal Room Environment Rats were housed five per cage and mice were housed individually. Temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as 2-week studies

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). 1,3-Diphenylguanidine was sent to the laboratory as a coded aliquot. It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days incubation at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of 1,3-diphenylguanidine. High dose was limited by experimental design to 10,000 µg/plate. All assays were repeated.

PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A modification of the technique described by MacGregor *et al.* (1990) was used. At the termination of the 13-week toxicity study, blood was obtained from male and female mice and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. The frequency of micronuclei was determined in 2,000 normochromatic erythrocytes (NCEs) in each of 5 animals per dose group. The criteria of Schmid (1976) were used in defining micronuclei.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

For the 13-week studies, two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry, hematology, spermatid, and spermatozoal data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

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

ANALYSIS OF VAGINAL CYTOLOGY DATA

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

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

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

ANALYSIS OF PERIPHERAL BLOOD MICRONUCLEUS DATA

The frequency of micronucleated PCEs was analyzed by a statistical software package (ILS, 1990) that employed a one-tailed trend test across dose groups and a *t*-test for pairwise comparisons of each dose group to the concurrent control.

Quality Assurance

The animal studies of 1,3-diphenylguanidine were performed in compliance with United States FDA Good Laboratory Practices regulations (21CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Feed Study in F344/N Rats

All rats survived to the end of the 2-week study (Table 3). The final mean body weights and body weight gains of males and females exposed to 1,500 or 3,000 ppm 1,3-diphenylguanidine were notably less than those of the control groups (Table 3). During the second week of the study, clinical signs of toxicity were observed in males and females in the 3,000 ppm groups and included ruffled fur and thin appearance.

TABLE 3 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for F344/N Rats in the 2-Week Feed Study of 1,3-Diphenylguanidine

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Feed Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	5/5	165	224	59		20.6	
250	5/5	165	232	67	104	19.3	22
500	5/5	165	230	65	103	19.3	45
750	5/5	164	219	55	98	17.7	64
1,500	5/5	163	202	39	90	15.5	121
3,000	5/5	161	170	9	76	10.9	200
FEMALE							
0	5/5	116	144	28		12.5	
250	5/5	113	141	28	98	12.3	23
500	5/5	117	143	26	99	11.9	44
750	5/5	110	137	27	95	11.4	65
1,500	5/5	112	134	22	93	10.8	127
3,000	5/5	115	120	5	83	6.2	166

[1] Number surviving at 2 weeks/number of animals per group.
[2] (Exposure group mean/control group mean) x 100.
[3] Average of individual consumption values for Days 6 and 13.

During the first week of the study rats exposed to 3,000 ppm consumed 35% less feed than controls and the final mean bodyweights of these groups remained the same or decreased slightly from their initial values. During the second study week, feed consumption by the 3,000 ppm groups increased relative to controls and body weight gains increased but the final mean body weights remained lower than controls. Feed consumption and final mean body weights of groups receiving 750 or 1,500 ppm were also lower than the controls during both study weeks; however, animals in these groups gained weight continuously during the study.

The pattern of organ weight changes observed during the 2-week study was not indicative of chemical-related toxicity (data on file at NIEHS). Absolute organ weights of male rats that received 3,000 ppm were uniformly lower than controls due to the markedly reduced final mean body weights of this group. Ovarian weights of females that received 750 or 1,500 ppm were lower than controls, however final mean body weights of both groups were also lower than the controls. Relative liver weights of males that received 500 or 1,500 ppm and relative kidney weight of females that received 750 ppm weregreater than those of the controls but the influences were small in magnitude, not exposure related, and not considered biologically meaningful.

No gross lesions associated with exposure to 1,3-diphenylguanidine were observed in male or female rats. No microscopic examination was conducted.

Because of the absence of chemical associated toxicity, exposure concentrations selected for the 13-week study were the same as those used in the 2-weekstudy. Although lower body weights and feed consumption were observed in the 3,000 ppm groups, animals in these groups began to eat and gained weight during the second week of the study.

13-Week Feed Study in F344/N Rats

Six males and all females in the 3,000 ppm groups died or were killed moribund before the end of the 13-week study (Table 4); all rats in the lower exposure groups survived to the end of the study. Mean body weights of male and female rats that were exposed to 1,500 or 3,000 ppm were markedly lower than those of controls throughout the 13-week study (Figure 1). Mean body weights of the 3,000 ppm groups decreased during the first week of the study for males and the first 2 weeks of the study for females before starting to increase. No final mean body weight or body weight gain was determined for female rats administered 3,000 ppm 1,3-diphenylguanidine due to 100% mortality in this exposure group.

TABLE 4 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Feed Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	147	368	221		17.3	
250	10/10	142	351	210	96	17.0	17
500	10/10	150	350	200	95	16.7	32
750	10/10	141	340	198	92	16.4	50
1,500	10/10	145	290	145	79	14.8	100
3,000	4/10[4]	142	192	49	52	10.3	181
FEMALE							
0	10/10	118	202	84		11.4	
250	10/10	120	195	75	96	11.2	17
500	10/10	120	191	71	95	10.6	32
750	10/10	116	187	71	93	10.4	49
1,500	10/10	116	174	58	86	9.5	95
3,000	0/10[5]	119	–	–	–	7.5	184

[1] Number surviving at 13 weeks/number of animals per group. For groups with no survivors, no final mean body weights or body weight changes are given.
[2] (Exposure group mean/control group mean) x 100.
[3] For all groups except 3,000 ppm females, consumption values are averaged for Weeks 1-13. For 3,000 ppm females, consumption values are averaged for Weeks 1-12.
[4] Week of death: 9, 9, 9, 10, 10, 12.
[5] Week of death: 4, 4, 5, 7, 7, 8, 9, 12, 12, 12.

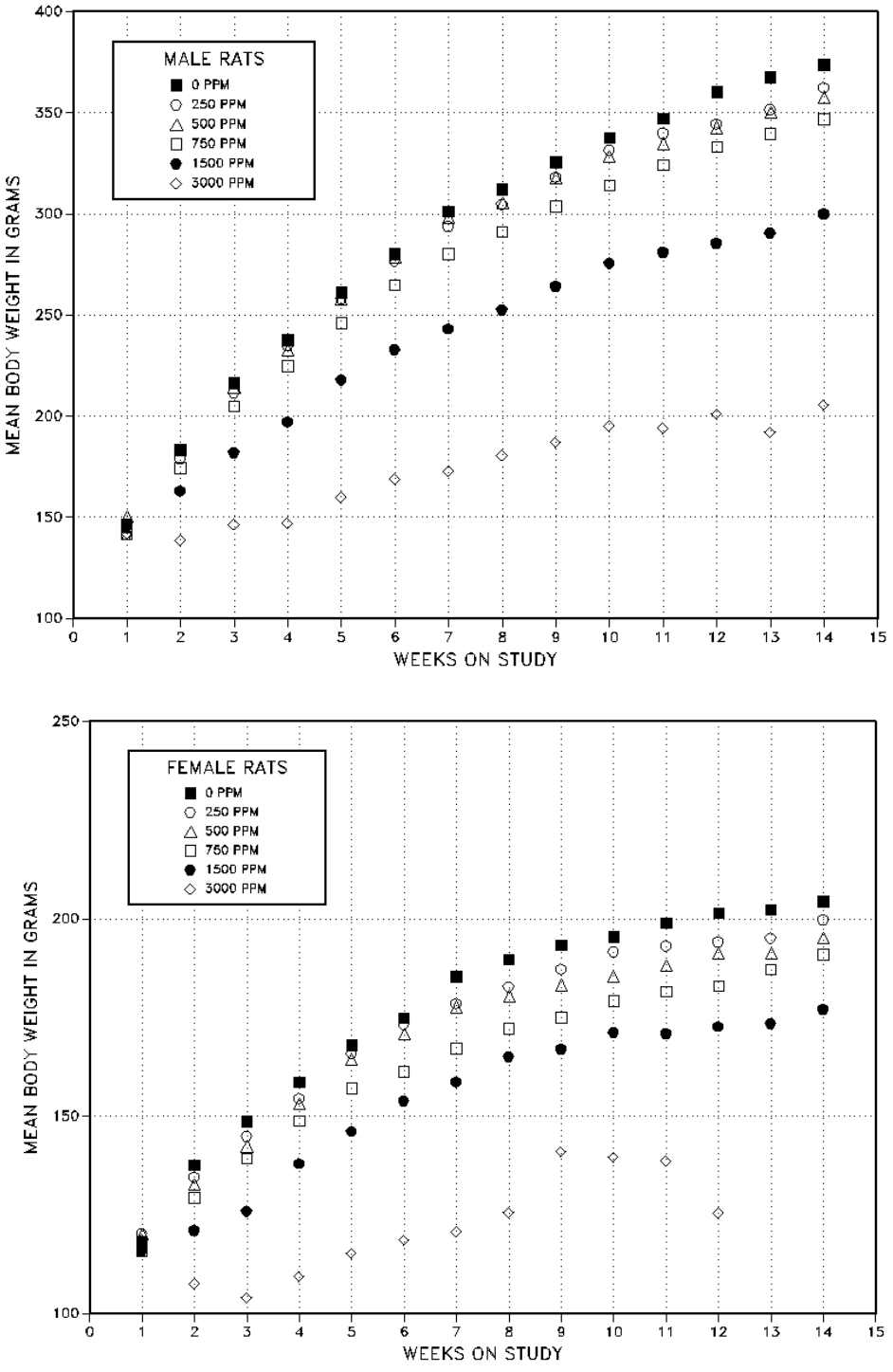


FIGURE 1 Body Weights of F344/N Rats Administered 1,3-Diphenylguanidine in Feed for 13 Weeks

Clinical signs of toxicity were noted primarily in rats in the 1,500 and 3,000 ppm groups beginning at Week 2. The majority of rats in these groups appeared thin and had ruffled fur, with discolorations of the tail, ears, and scrotum or vaginal area. Salivation, hypoactivity, and convulsions and seizures were also observed in some male and female rats in these groups, and abnormal posture (staggering) was noted in most males and females. Other clinical signs observed in these groups included hyperactivity, hunched posture, ptosis, ataxia, dyspnea, and bristly hair.

Average feed consumption decreased as exposure concentrations increased above 500 ppm with feed consumption 34% to 40% less than the controls during the 13-week study period in males and females that received 3,000 ppm (Table 4). During the first week of the study feed consumption by groups receiving 3,000 ppm were 57% and 63% lower than control for males and females respectively, indicating poor palatability at this exposure concentration.

Organ weights for groups receiving 750 ppm or greater were significantly lower than those of the controls and were the result of low body weights and low feed consumption by these groups rather than a specific toxic response to 1,3-diphenylguanidine (Table A1).

In general, changes in hematology parameters were limited to rats receiving 1,500 and 3,000 ppm (Table B1). A mild polycythemia occurred at Day 5 in the 3,000 ppm male and female rats, and to a lesser extent in the 1,500 ppm females. This was indicated by greater erythrocyte counts, hematocrit values, and hemoglobin concentrations than controls and would be consistent with a relative polycythemia related to dehydration and hemoconcentration. There were slightly lower reticulocyte counts at Day 5 in 3,000 ppm male and female rats and 1,500 ppm females. Other changes in hematology parameters were minor, sporadic, and did not suggest a treatment effect.

Changes in clinical chemistry parameters occurred primarily in the 1,500 and 3,000 ppm groups, although some minor changes were observed in other groups (Tables 5 and B2). Greater alkaline phosphatase activity and bile acid concentration than controls occurred in an exposure-related manner in male and female rats. Males exhibited greater increases in activity and at earlier time periods. By Week 13, alkaline phosphatase activity and bile acid concentration were greater than the controls in all groups of exposed rats; these changes are consistent with cholestasis. The lack of an increase of alkaline phosphatase activity in groups that received 3,000 ppm was probably

related to inanition and a decreased contribution of the intestinal fraction of alkaline phosphatase to the total serum activity. Total protein, creatinine, cholesterol, and triglyceride concentrations in the 1,500 and 3,000 ppm groups were lower than the controls and these differences are consistent with inanition.

TABLE 5 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

	Concentration (ppm)					
	0	250	500	750	1,500	3,000
MALE						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	10	4
Creatinine (mg/dL)						
Day 5	0.68 ± 0.02	0.62 ± 0.03	0.68 ± 0.01	0.63 ± 0.03	0.65 ± 0.02	0.58 ± 0.01**
Day 21	0.80 ± 0.03	0.75 ± 0.02	0.78 ± 0.03	0.76 ± 0.02	0.75 ± 0.02	0.65 ± 0.03**
Week 13	0.69 ± 0.02	0.68 ± 0.02	0.65 ± 0.02	0.64 ± 0.02	0.62 ± 0.03*	0.60 ± 0.04*
Total protein (g/dL)						
Day 5	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Day 21	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.1**	5.6 ± 0.1**
Week 13	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.1*	5.8 ± 0.2**
Cholesterol (mg/dL)						
Day 5	100 ± 4	100 ± 2	100 ± 3	91 ± 2*	91 ± 2	92 ± 3
Day 21	93 ± 2	90 ± 2	87 ± 2	88 ± 2	88 ± 2	94 ± 5
Week 13	88 ± 2	87 ± 3	91 ± 2	88 ± 2	78 ± 1**	70 ± 2**
Triglycerides (mg/dL)						
Day 5	197 ± 11	227 ± 10	197 ± 7	210 ± 9	201 ± 12	105 ± 13**
Day 21	316 ± 21	308 ± 19	294 ± 20	301 ± 17	239 ± 26*	133 ± 25**
Week 13	237 ± 17	239 ± 17	241 ± 21	262 ± 24	210 ± 18	72 ± 9*
Alkaline phosphatase (IU/L)						
Day 5	535 ± 17	640 ± 10	710 ± 11**	693 ± 12**	729 ± 11**	517 ± 19
Day 21	391 ± 7	452 ± 6	506 ± 12**[2]	528 ± 24**	537 ± 21**[2]	376 ± 18
Week 13	199 ± 7	258 ± 8**	282 ± 7**	291 ± 9**	359 ± 11**	349 ± 31**
Bile acids (µmol/L)						
Day 5	4.9 ± 1.5[2]	5.0 ± 1.1	6.5 ± 1.2	6.6 ± 1.4	23.6 ± 2.8**[2]	37.7 ± 4.0**
Day 21	5.4 ± 2.3[3]	1.3 ± 0.3[4]	6.4 ± 1.9[3]	7.6 ± 3.9[3]	17.8 ± 4.5*[5]	75.2 ± 15.4**[2]
Week 13	5.1 ± 0.8	12.0 ± 1.9**[5]	13.8 ± 2.4**	11.1 ± 2.5*[6]	22.8 ± 3.0**	36.0 ± 1.7**[4]

TABLE 5 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	Concentration (ppm)					
	0	250	500	750	1,500	3,000
FEMALE						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	10	0
Creatinine (mg/dL)						
Day 5	0.63 ± 0.02	0.61 ± 0.01	0.58 ± 0.03	0.59 ± 0.03	0.57 ± 0.02	0.58 ± 0.03
Day 21	0.62 ± 0.02	0.62 ± 0.02	0.64 ± 0.02	0.61 ± 0.02	0.57 ± 0.02	0.43 ± 0.03**
Week 13	0.63 ± 0.03	0.63 ± 0.02	0.62 ± 0.01	0.66 ± 0.02	0.56 ± 0.03	)
Total protein (g/dL)						
Day 5	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1*	5.3 ± 0.1**
Day 21	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.6 ± 0.1**	5.2 ± 0.1**
Week 13	6.7 ± 0.1	6.7 ± 0.1	6.3 ± 0.1**	5.9 ± 0.1**	5.5 ± 0.0**	)
Cholesterol (mg/dL)						
Day 5	107 ± 2	104 ± 3	104 ± 3	98 ± 3*	92 ± 2**	100 ± 2**
Day 21	112 ± 3	106 ± 2	103 ± 2*	110 ± 3	107 ± 4	85 ± 4**
Week 13	110 ± 4	109 ± 2	100 ± 3	88 ± 2**	86 ± 3**	)
Triglycerides (mg/dL)						
Day 5	136 ± 7	159 ± 12	135 ± 10	138 ± 22	70 ± 6**	70 ± 10**
Day 21	168 ± 12	136 ± 12*	124 ± 13*	145 ± 18	103 ± 11**	69 ± 7**
Week 13	104 ± 11	113 ± 10	106 ± 11	100 ± 18	74 ± 7	)
Alkaline phosphatase (IU/L)						
Day 5	373 ± 5	477 ± 13	469 ± 7	469 ± 13	383 ± 13	308 ± 10
Day 21	305 ± 6	357 ± 12	356 ± 5*	385 ± 11**	406 ± 16**	313 ± 18
Week 13	164 ± 6	185 ± 4**	249 ± 5**	251 ± 11**	254 ± 11**	)
Bile acids (µmol/L)						
Day 5	14.0 ± 3.1	9.7 ± 2.4	12.5 ± 1.2	19.4 ± 3.4	38.5 ± 3.6**	31.7 ± 3.5**
Day 21	11.3 ± 2.9	9.3 ± 2.8[2]	25.4 ± 2.3**	13.6 ± 3.0	50.6 ± 3.8**	214.4 ± 27.0**
Week 13	14.6 ± 2.1	12.3 ± 2.0	18.3 ± 2.8	17.8 ± 3.0	51.9 ± 4.1**	)

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

Gross necropsy observations related to 1,3-diphenylguanidine treatment were limited to thinness of the carcass in higher exposure rats. Microscopic changes associated with chemical administration were observed in the bone marrow, thymus, uterus, testes, prostate gland/seminal vesicle, and salivary glands. All of the gross and microscopic changes occurred in the two highest exposure groups and were attributed to the lower feed intake, reduced weight gains, and poor body condition of these animals.

In the thymus, lymphoid depletion and necrosis were present in several 3,000 ppm females which were found dead or were killed in moribund condition. Depletion of hematopoietic cells in the femoral bone marrow was also variably present in the 3,000 ppm females which died early. Both of these lesions are common in moribund animals and are not considered to be direct toxic effects of chemical administration.

An exposure-related effect in the uterus of females was characterized by an overall reduction in size and was diagnosed as hypoplasia. This finding occurred with greater incidence and severity in the three highest exposure groups. In general, this change was attributed to poor body condition and delayed development due to lower feed consumption; the younger age of those females which died or were killed during the study may have been a reason for the smaller size of the uterus.

Several lesions were noted sporadically in the reproductive organs of 3,000 ppm males. In two of ten 3,000 ppm males, lower numbers of mature spermatozoa were present in the seminiferous tubules than in the controls; lower numbers of spermatozoa were also noted in the epididymal tubules than in the controls. Secretory depletion of the prostate gland and seminal vesicles was observed in several 3,000 ppm males; this difference was characterized by alveolar size smaller than controls and smaller amounts of secretory material within the lumen. Decreased spermatogenesis and secretory depletion of the accessory sex glands were considered secondary to poor body condition. In the salivary glands of several 3,000 ppm males and females, a change diagnosed as cytologic alteration was observed, characterized by smaller size and increased basophilia of the secretory acini. This change was interpreted to be a reflection of physiological atrophy due to reduced feed intake. No specific cause of death could be determined for the early death animals from the 3,000 ppm groups.

Evaluation of male reproductive tissues in groups that received 500, 750, or 1,500 ppm revealed a significant reduction in sperm motility in 1,500 ppm males (Table C1). Among 750 and 1,500 ppm group females the length of the estrous cycle was greater than the controls (Table C2).

2-Week Feed Study in B6C3F$_1$ Mice

All mice survived to the end of the 2-week study (Table 6). The final mean body weight of female mice in the 3,000 ppm group was 6% lower than the controls; final mean body weights of other exposed groups were similar to controls. Clinical signs of toxicity were observed in a few female mice during the latter part of the study; one female in the 1,500 ppm group appeared thin, and one female each in the 750 and 3,000 ppm groups had hunched posture and appeared thin. As shown in Table 6, the average amounts of feed consumed by females in the 750 and 1,500 ppm groups were slightly lower than the control value; the average amounts of feed consumed by all other exposed groups were similar to control values.

TABLE 6 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 2-Week Feed Study of 1,3-Diphenylguanidine

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Feed Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	5/5	22.1	24.1	2.0		3.9	
250	5/5	22.1	24.4	2.3	101	4.6	48
500	5/5	22.2	24.7	2.5	102	4.5	92
750	5/5	22.5	24.3	1.8	101	4.3	133
1,500	5/5	22.5	24.0	1.5	100	4.2	266
3,000	5/5	22.1	24.0	1.9	100	4.5	573
FEMALE							
0	5/5	18.7	21.5	2.8		4.7	
250	5/5	18.2	21.2	3.0	99	4.3	53
500	5/5	18.3	20.9	2.6	97	4.6	112
750	5/5	18.3	21.1	2.8	98	4.1	150
1,500	5/5	18.4	20.6	2.2	96	4.0	303
3,000	5/5	18.4	20.3	1.9	94	4.6	691

[1] Number surviving at 2 weeks/number of animals per group.
[2] (Exposure group mean/control group mean) x 100.
[3] Average of individual consumption values for Days 6 and 13.

Only a few significant organ weight changes were observed (data on file at NIEHS). Absolute and relative liver weights of males and females in the 1,500 and 3,000 ppm groups were lower than those of the control groups, and the relative heart weight of females in the 500 ppm group was greater than that of the control group.

No gross or microscopic lesions related to 1,3-diphenylguanidine exposure were observed in male or female mice.

Because of the absence of chemical related toxicity in this study, the exposure concentrations selected for the 13-week study in mice were the same as those administered in the 2-week study.

13-Week Feed Study in B6C3F$_1$ Mice

All mice survived to the end of the study (Table 7). Mean body weights of both males and females in the three highest exposure groups (750, 1,500, and 3,000 ppm) were lower than those of the control groups especially during the latter part of the study (Figure 2). Thin appearance was the most frequently reported clinical sign for female mice and was most often observed in the three highest exposure groups. Thin appearance was also observed in male mice in the 3,000 ppm group. Other clinical signs observed in mice in the higher exposure groups included alopecia, abnormal posture, ptosis, and bristly hair.

TABLE 7 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Feed Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	24.0	34.9	10.9		4.2	
250	10/10	23.6	33.8	10.2	97	4.4	38
500	10/10	23.4	33.1	9.6	95	4.3	75
750	10/10	23.8	32.4	8.6	93	4.3	114
1,500	10/10	23.3	30.2	6.9	86	4.1	231
3,000	10/10	23.5	28.2	4.8	81	3.9	457
FEMALE							
0	10/10	17.1	28.4	11.4		4.2	
250	10/10	17.4	28.4	11.0	100	4.3	46
500	10/10	17.3	27.2	9.9	96	4.3	93
750	10/10	17.2	26.4	9.2	93	4.2	141
1,500	10/10	16.8	25.0	8.2	88	4.1	285
3,000	10/10	17.3	22.8	5.5	80	3.9	577

[1] Number surviving at 13 weeks/number of animals per group.
[2] (Exposure group mean/control group mean) x 100.
[3] Average of individual consumption values for Weeks 1-13.

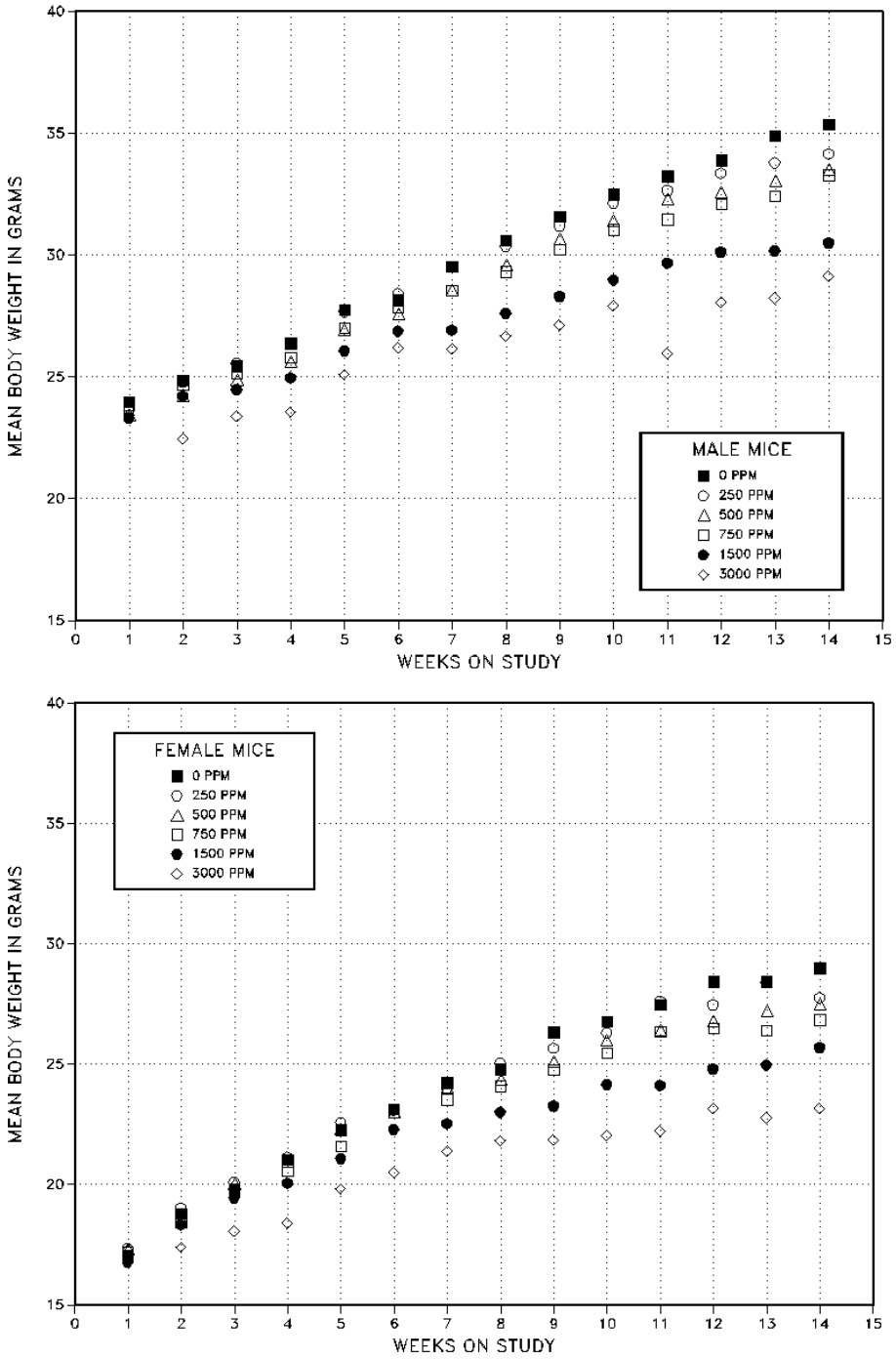


FIGURE 2 Body Weights of B6C3F$_1$ Mice Administered 1,3-Diphenylguanidine in Feed for 13 Weeks

The average amounts of feed consumed by males and females in all exposed groups were similar to the average amounts consumed by the control groups (Table 7).

Significantly lower absolute organ weights and greater relative organ weights than controls were observed for several organs in the 1,500 or 3,000 ppm groups (Table A2). These differences are not indicative of a specific toxic response but appear to be the result of the lower body weights of these groups.

No treatment-related gross or microscopic lesions were observed in male or female mice exposed to 1,3-diphenylguanidine.

Evaluation of male reproductive tissue from animals revealed greater numbers of spermatid heads and lower sperm motility than in the controls in the 3,000 ppm group. In females, estrous cycle length in the 3,000 ppm group was greater than controls (Tables C3 and C4).

Genetic Toxicity Studies

1,3-Diphenylguanidine (1 to 10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the presence of induced hamster or rat liver S9 (Mortelmans *et al.*, 1986; Table D1), and an equivocal response was obtained in strain TA1537 with rat liver S9. No indication of mutagenic activity was noted in the absence of S9.

The frequency of micronucleated normochromatic erythrocytes was determined in peripheral blood samples obtained from male and female mice administered 1,3-diphenylguanidine in dosed feed for 90 days. No effect was noted in male mice, but in females, a significant increase in micronucleated normochromatic erythrocytes was noted in the 750 ppm group (Table D2) Because the trend test for the female data did not yield a significant P value ($P>0.025$) and the increase in micronucleated normochromatic erythrocytes was noted in only one exposure group, the female mouse data were judged to be equivocal.

DISCUSSION

1,3-Diphenylguanidine (DPG) is used extensively as both a primary and secondary accelerator in the vulcanization of rubber prepared for the manufacture of tires, footwear, and other molded products. Human exposure to DPG may occur as a result of dermal contact, inhalation of particulates, or unintended oral ingestion. Although DPG has been reported to cause contact dermatitis, it is poorly absorbed through skin. DPG is easily absorbed through the gastrointestinal tract, and in the present studies it was administered orally in feed to characterize the toxicity resulting from systemic exposure.

During 2-week studies, rats and mice received feed containing 0, 250, 500, 750, 1,500, or 3,000 ppm DPG. These exposure levels were selected on the basis of acute toxicity data which reported an oral LD_{50} values of 300 to 500 mg/kg for rats and 200 to 300 mg/kg for mice. In an unpublished prechronic study conducted by the Monsanto Company, administration of 3,000 ppm in the feed for two weeks resulted in the death of 5 rats; no histopathology was conducted, so the cause of death could not be determined for these animals.

Consumption of feed containing 1,3-diphenylguanidine for 2 weeks was not associated with any organ specific toxicity in either rats or mice. Lower feed consumption indicative of poor palatability, especially during the first week of the study, was observed among groups of rats that received feed containing 750, 1,500, or 3,000 ppm. This resulted in body weights and organ weights that were lower than the controls particularly for groups that received 3,000 ppm. During the second week of the study, feed consumption and mean body weights of the 3,000 ppm group began to increase. Groups of mice that received feed containing 1,3-diphenylguanidine for 2 weeks exhibited only minimal effects of chemical exposure. The final mean body weight of females that received 3,000 ppm was 6% lower than that of the controls and the mean weight gain by this group was 32% lower than the weight gain of controls; however, final mean body weights of other groups were similar to the controls.

The same range of exposure concentrations used in the 2-week studies was selected for the 13-week studies for both rats and mice. The indications of poor palatability of feed containing 3,000 ppm precluded the use of higher concentrations for rats. During the second week of the 2-week

study rats in the 3,000 ppm groups began to consume more feed and gained weight; therefore, it was judged that over the duration of a 13-week study the body weights could possibly recover nearly to control body weights. Mice that received 3,000 ppm exhibited only minimally toxic responses in the 2-week study; however, a doubling of the highest exposure to 6,000 ppm would probably have resulted in poor palatability and was considered too high for the 13-week study.

During the 13-week study, feed consumption by rats in the 1,500 and 3,000 ppm groups was lower than in controls and resulted in low body weights and low absolute organ weights. Sixteen rats receiving 3,000 ppm died or were killed moribund. The lesions present in these animals were those frequently associated with low body weights and reduced feed consumption; no organ-specific toxicity attributable to 1,3-diphenylguanidine exposure was apparent.

The values of several hematology parameters measured on Day 5 for the 1,500 and 3,000 ppm groups were consistent with general dehydration, most likely the result of lower water intake associated with the markedly lower feed consumption of these groups during the first week of the study. Significantly lower total serum protein, cholesterol, and triglycerides values were observed in these groups, consistent with lower feed consumption. Alkaline phosphatase activity and bile acid concentration were significantly greater than the controls for several groups of males and females, especially at the Day 21 and Week 13 sampling points. High serum concentrations of normal bile constituents are frequently associated with reduced bile formation and/or flow (cholestasis). In the absence of chemical-related lesions in the liver which might impair bile formation, the observation of cholestasis in the groups receiving the highest exposures is probably associated with lower nutrient and water intake.

Many of the hematology and clinical chemistry differences observed in the present study in F344/N rats are similar to observations made by Levin *et al.* (1993) in Sprague-Dawley rats in which feed was restricted to 25%, 50%, or 75% of control body weights for 15 days. These differences include reduced organ weights, hematologic differences indicative of hemoconcentration, lower platelet counts, and greater serum activity of alkaline phosphatase (bile acid concentration was not measured), lower total serum protein and cholesterol.

In the 13-week study sperm motility of male rats that received 1,500 ppm was significantly lower than for the controls. Depletion of the prostate gland, hypospermia, and spermatogenic arrest, as well as significant reductions in the absolute weights of the prostate gland, seminal vesicles, and testis were observed in male rats that received 3,000 ppm. Chapin *et al.* (1993a) examined the effect of feed restriction on reproductive function in Sprague-Dawley rats and observed similar changes in males in which feed was restricted to 70% of control body weight. In addition, Levin *et al.* (1993) noted that mild testicular degeneration was observed in the severely feed restricted groups.

Uterine hypoplasia, characterized by a reduction in uterine size due to thinner and less developed endometrium, was observed in female rats in the 750 ppm or greater groups. In addition the mean length of the estrous cycle in female rats that received 1,500 ppm was significantly greater than the controls. Although the uterine hypoplasia is probably a consequence of reduced feed consumption in the 750 ppm or greater groups, Chapin *et al.* (1993a) observed that feed restriction caused transient increases in the length of the estrous cycle in Sprague-Dawley rats. Although this suggests a potential chemical-specific effect, the data from the present study do not allow a more definitive conclusion.

Consumption of feed containing 1,3-diphenylguanidine for 13 weeks was associated with low body weights, reduced feed consumption, and low organ weights in the 1,500 and 3,000 ppm groups of mice but there were no gross or microscopic lesions attributable to chemical exposure.

At the end of the 13-week exposure period, the number of spermatid heads per gram of testis in the 3,000 ppm group of male mice was greater than the controls but the sperm density (per gram of cauda) and sperm motility in the 3,000 ppm group were lower than the controls. The concentration of spermatid heads in the testis is a measure of the ability of the testis to produce sperm while the density of sperm in the epididymis is a measure of the release of sperm by the testis. The increase in the number of spermatid heads in the testis coupled with a lower number of sperm in the epididymis suggests that 1,3-diphenylguanidine does not impair the sperm formation in the testis but may affect the release of sperm into the epididymis.

In Swiss (CD-1®) mice feed restricted to 70% of control body weight for 15 weeks, Chapin *et al.* (1993b) observed lower numbers of testicular spermatids and lower epididymal sperm density than the controls; however, these measures were not reduced by restriction to 80% or 90% of control body weight. In the present study mean body weights of male mice in the 3,000 ppm group were generally between 80% to 90% of control and mean feed consumption for the study was 93% of control. Although this suggests a potential chemical-related effect, the response to feed restriction in male B6C3F$_1$ mice may differ somewhat from that of male Swiss (CD-1®) mice.

The length of the estrous cycle in female mice that received 3,000 ppm 1,3-diphenylguanidine was longer than that of the controls. Chapin *et al.* (1993b) observed a similar lengthening of the estrous cycle in female Swiss (CD-1®) mice restricted to 70% or 80% of control body weight. Therefore, the lengthening of the estrous cycle observed in the 3,000 ppm group appears to be a consequence of the low body weights and reduced feed consumption of this group rather than a chemical-related effect.

Bempong and Hall (1983) reported significantly lower testicular weight, sperm count, and greater numbers of morphologically anomalous sperm in male mice (C57BL/J6 X DBA$_2$) and hamsters given 4 or 8 mg 1,3-diphenylguanidine per kg body weight per day *ad libitum* in drinking water acidified with 0.25% acetic acid for 15 weeks. Testis weights in the 8 mg/kg group were lower than the controls after 5 weeks of chemical exposure. Unfortunately no water consumption or body weight data were reported so it is unclear whether water or feed consumption was reduced in this study. In the present studies male mice in the 3,000 ppm group ingested an average of 457 mg of 1,3-diphenylguanidine per kg body weight per day during the 13-week study; however, testis weight and sperm density were slightly but not significantly lower than those of the controls. Since mice received a significantly greater exposure to 1,3-diphenylguanidine in the present study than in the study by Bempong and Hall, it is difficult to reconcile the different outcomes of the two studies without more information.

In summary, consumption of feed containing 1,3-diphenylguanidine for 2 weeks or 13 weeks was not associated with any histologic response that could be attributed to chemical exposure. Instead the observed changes were indicative of reduced nutrient intake and are consistent with similar changes observed in other studies of feed restricted rats and mice.

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APPENDIX A

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

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE						
n	10	10	10	10	10	4
Necropsy body wt	374 ± 6	362 ± 7	358 ± 5	347 ± 4**	300 ± 7**	206 ± 10**
Heart						
Absolute	1.128 ± 0.041	1.065 ± 0.025	1.085 ± 0.014	1.043 ± 0.020	0.967 ± 0.015**	0.771 ± 0.010**
Relative	3.01 ± 0.08	2.94 ± 0.05	3.04 ± 0.03	3.01 ± 0.03	3.23 ± 0.07	3.77 ± 0.18**
Right kidney						
Absolute	1.317 ± 0.036	1.274 ± 0.037	1.288 ± 0.017	1.185 ± 0.021**	1.151 ± 0.029**	0.900 ± 0.042**
Relative	3.52 ± 0.05	3.51 ± 0.04	3.61 ± 0.06	3.41 ± 0.03	3.84 ± 0.08**	4.39 ± 0.22**
Liver						
Absolute	14.712 ± 0.598	14.433 ± 0.461	14.051 ± 0.379	13.556 ± 0.337	11.670 ± 0.277**	7.202 ± 0.334**
Relative	39.22 ± 1.01	39.83 ± 1.02	39.28 ± 0.88	39.07 ± 0.73	38.97 ± 0.86	35.07 ± 0.85*
Lungs						
Absolute	1.979 ± 0.099	1.742 ± 0.089	1.631 ± 0.046**	1.679 ± 0.068*	1.427 ± 0.059**	1.013 ± 0.043**
Relative	5.29 ± 0.23	4.81 ± 0.24	4.56 ± 0.12	4.85 ± 0.20	4.76 ± 0.20	4.94 ± 0.21
Prostate gland						
Absolute	0.775 ± 0.041	0.733 ± 0.061	0.850 ± 0.062	0.764 ± 0.027	0.647 ± 0.041	0.272 ± 0.065**
Relative	2.09 ± 0.13	2.04 ± 0.19	2.39 ± 0.19	2.20 ± 0.08	2.15 ± 0.13	1.29 ± 0.28*
Seminal vesicles						
Absolute	0.961 ± 0.076	1.044 ± 0.126	0.954 ± 0.110	0.908 ± 0.087	0.894 ± 0.063	0.303 ± 0.097**
Relative	2.57 ± 0.20	2.88 ± 0.34	2.67 ± 0.31	2.62 ± 0.26	2.98 ± 0.20	1.44 ± 0.40
Spleen						
Absolute	0.835 ± 0.018	0.817 ± 0.016	0.818 ± 0.021	0.787 ± 0.032	0.645 ± 0.015**	0.584 ± 0.068**
Relative	2.23 ± 0.04	2.26 ± 0.03	2.29 ± 0.04	2.26 ± 0.07	2.15 ± 0.05	2.84 ± 0.31**
Right testis						
Absolute	1.512 ± 0.014	1.472 ± 0.035	1.501 ± 0.025	1.448 ± 0.030	1.418 ± 0.018	1.112 ± 0.081**
Relative	4.05 ± 0.06	4.06 ± 0.05	4.20 ± 0.06	4.18 ± 0.08	4.74 ± 0.07**	5.39 ± 0.22**
Thymus						
Absolute	0.333 ± 0.018	0.329 ± 0.014	0.307 ± 0.013	0.281 ± 0.005*	0.217 ± 0.011**	0.131 ± 0.017**
Relative	0.89 ± 0.05	0.91 ± 0.03	0.86 ± 0.04	0.81 ± 0.02	0.72 ± 0.04**	0.63 ± 0.06**

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
FEMALE						
n	10	10	10	10	10	0
Necropsy body wt	204 ± 4	200 ± 3	195 ± 3	191 ± 3**	177 ± 2**	)
Heart						
Absolute	0.726 ± 0.017	0.708 ± 0.015	0.693 ± 0.009	0.671 ± 0.016*	0.628 ± 0.012**	)
Relative	3.55 ± 0.07	3.54 ± 0.06	3.56 ± 0.07	3.52 ± 0.07	3.55 ± 0.05	)
Right kidney						
Absolute	0.743 ± 0.016	0.724 ± 0.014[2]	0.724 ± 0.021	0.691 ± 0.017	0.690 ± 0.021	)
Relative	3.64 ± 0.07	3.66 ± 0.07[2]	3.71 ± 0.08	3.62 ± 0.04	3.89 ± 0.09*	)
Liver						
Absolute	6.487 ± 0.090	6.520 ± 0.161	6.601 ± 0.150	5.922 ± 0.134*	5.614 ± 0.129**	)
Relative	31.82 ± 0.67	32.66 ± 0.81	33.83 ± 0.53	31.03 ± 0.52	31.70 ± 0.53	)
Lungs						
Absolute	1.325 ± 0.039	1.188 ± 0.043	1.179 ± 0.049	1.212 ± 0.056	1.050 ± 0.030**	)
Relative	6.51 ± 0.23	5.96 ± 0.25	6.05 ± 0.26	6.35 ± 0.29	5.93 ± 0.16	)
Ovaries						
Absolute	0.106 ± 0.005	0.106 ± 0.003	0.117 ± 0.007	0.093 ± 0.003	0.095 ± 0.004	)
Relative	0.52 ± 0.03	0.53 ± 0.01	0.60 ± 0.04	0.49 ± 0.02	0.54 ± 0.02	)
Spleen						
Absolute	0.461 ± 0.016	0.434 ± 0.015	0.480 ± 0.009	0.462 ± 0.009	0.458 ± 0.012	)
Relative	2.26 ± 0.08	2.17 ± 0.06	2.46 ± 0.04	2.42 ± 0.05	2.59 ± 0.06**	)
Thymus						
Absolute	0.236 ± 0.008	0.219 ± 0.005	0.224 ± 0.008	0.227 ± 0.007	0.229 ± 0.012	)
Relative	1.15 ± 0.03	1.10 ± 0.02	1.15 ± 0.04	1.19 ± 0.04	1.29 ± 0.06	)

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

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	35.9 ± 0.6	34.7 ± 0.7	34.4 ± 0.6	33.8 ± 0.6*	31.5 ± 0.5**	29.1 ± 0.3**
Heart						
Absolute	0.164 ± 0.002	0.164 ± 0.004	0.167 ± 0.005	0.157 ± 0.003	0.150 ± 0.003*	0.150 ± 0.003*
Relative	4.58 ± 0.07	4.73 ± 0.09	4.88 ± 0.19	4.67 ± 0.08	4.78 ± 0.08	5.15 ± 0.11**
Right kidney						
Absolute	0.302 ± 0.008	0.305 ± 0.004	0.293 ± 0.007	0.290 ± 0.008	0.274 ± 0.009*	0.242 ± 0.004**
Relative	8.43 ± 0.19	8.82 ± 0.15	8.51 ± 0.22	8.59 ± 0.25	8.72 ± 0.27	8.34 ± 0.13
Liver						
Absolute	1.614 ± 0.050	1.621 ± 0.068	1.760 ± 0.036	1.611 ± 0.055	1.640 ± 0.047	1.377 ± 0.041**
Relative	44.98 ± 1.18	46.58 ± 1.30	51.19 ± 1.10**	47.73 ± 1.55	52.09 ± 0.85**	47.35 ± 1.33
Lungs						
Absolute	0.229 ± 0.007	0.234 ± 0.015	0.245 ± 0.012	0.245 ± 0.013	0.240 ± 0.013	0.250 ± 0.014
Relative	6.42 ± 0.26	6.74 ± 0.40	7.14 ± 0.39	7.27 ± 0.41	7.64 ± 0.42	8.62 ± 0.50**
Prostate gland						
Absolute	0.065 ± 0.007	0.061 ± 0.005	0.059 ± 0.006	0.073 ± 0.007	0.076 ± 0.008[2]	0.058 ± 0.005
Relative	1.81 ± 0.18	1.76 ± 0.16	1.70 ± 0.15	2.16 ± 0.21	2.38 ± 0.26[2]	1.98 ± 0.16
Seminal vesicles						
Absolute	0.361 ± 0.018	0.318 ± 0.024	0.340 ± 0.015	0.318 ± 0.016	0.321 ± 0.015	0.259 ± 0.018**
Relative	10.08 ± 0.55	9.19 ± 0.73	9.93 ± 0.55	9.42 ± 0.48	10.19 ± 0.44	8.91 ± 0.59
Spleen						
Absolute	0.075 ± 0.003[2]	0.074 ± 0.002	0.078 ± 0.001	0.075 ± 0.002	0.069 ± 0.002	0.069 ± 0.002
Relative	2.10 ± 0.06[2]	2.14 ± 0.05	2.26 ± 0.06	2.24 ± 0.06	2.20 ± 0.04	2.39 ± 0.07**
Right testis						
Absolute	0.127 ± 0.004	0.132 ± 0.002	0.128 ± 0.003	0.128 ± 0.003	0.124 ± 0.005	0.120 ± 0.003[2]
Relative	3.54 ± 0.09	3.81 ± 0.09	3.73 ± 0.11	3.78 ± 0.09	3.93 ± 0.13*	4.16 ± 0.12**[2]
Thymus						
Absolute	0.047 ± 0.003	0.045 ± 0.003	0.046 ± 0.004	0.051 ± 0.005	0.047 ± 0.006	0.046 ± 0.002
Relative	1.31 ± 0.07	1.29 ± 0.09	1.32 ± 0.10	1.50 ± 0.14	1.48 ± 0.17	1.58 ± 0.06

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.3 ± 0.7	28.5 ± 0.6	28.4 ± 0.6	27.4 ± 0.5*	26.0 ± 0.3**	22.9 ± 0.2**
Heart						
Absolute	0.140 ± 0.004	0.144 ± 0.004	0.157 ± 0.006*	0.144 ± 0.004	0.150 ± 0.005	0.121 ± 0.002*
Relative	4.78 ± 0.12	5.07 ± 0.18	5.52 ± 0.15**	5.28 ± 0.14	5.80 ± 0.21**	5.27 ± 0.10
Right kidney						
Absolute	0.203 ± 0.004	0.201 ± 0.005	0.206 ± 0.003	0.202 ± 0.005	0.185 ± 0.002**	0.172 ± 0.002**
Relative	6.95 ± 0.13	7.06 ± 0.17	7.25 ± 0.11	7.39 ± 0.15	7.12 ± 0.06	7.54 ± 0.12*
Liver						
Absolute	1.480 ± 0.030	1.471 ± 0.038	1.560 ± 0.038	1.511 ± 0.031	1.397 ± 0.035	1.178 ± 0.022**
Relative	50.59 ± 0.85	51.61 ± 1.20	54.97 ± 1.45*	55.23 ± 1.06*	53.72 ± 1.02	51.47 ± 0.75
Lungs						
Absolute	0.232 ± 0.014	0.235 ± 0.014	0.254 ± 0.019	0.225 ± 0.011	0.228 ± 0.016	0.211 ± 0.015
Relative	8.01 ± 0.62	8.27 ± 0.49	8.89 ± 0.59	8.29 ± 0.55	8.81 ± 0.66	9.17 ± 0.58
Ovaries						
Absolute	0.032 ± 0.003	0.030 ± 0.003	0.029 ± 0.003	0.032 ± 0.003	0.025 ± 0.002	0.025 ± 0.003
Relative	1.10 ± 0.11	1.04 ± 0.13	1.04 ± 0.10	1.19 ± 0.10	0.96 ± 0.08	1.09 ± 0.15
Spleen						
Absolute	0.099 ± 0.003	0.092 ± 0.003	0.104 ± 0.008	0.106 ± 0.009	0.089 ± 0.003	0.074 ± 0.003*[2]
Relative	3.40 ± 0.12	3.25 ± 0.14	3.69 ± 0.30	3.90 ± 0.37	3.43 ± 0.12	3.24 ± 0.10[2]
Thymus						
Absolute	0.064 ± 0.003	0.068 ± 0.003	0.059 ± 0.004	0.057 ± 0.003	0.054 ± 0.001	0.058 ± 0.003
Relative	2.17 ± 0.08	2.37 ± 0.11	2.07 ± 0.14	2.07 ± 0.12	2.07 ± 0.05	2.55 ± 0.11

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

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

APPENDIX B

Hematology and Clinical Chemistry Results

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE						
n						
Day 5	10	10	9	10	10	10
Day 21	10	10	9	9	10	10
Week 13	9	7	8	9	10	4
Hematocrit (automated) (%)						
Day 5	40.4 ± 0.6	41.2 ± 0.5	40.2 ± 0.7	39.8 ± 0.4	42.0 ± 0.7	44.3 ± 0.6**
Day 21	43.0 ± 0.4	44.5 ± 0.9	43.5 ± 0.6	42.9 ± 0.5	44.5 ± 0.7	43.8 ± 0.7
Week 13	44.3 ± 0.5	43.3 ± 0.4	43.3 ± 0.5	43.6 ± 0.7	44.5 ± 0.6	37.6 ± 3.7
Hematocrit (manual) (%)						
Day 5	46.3 ± 0.9	46.7 ± 0.7	45.6 ± 0.5[2]	45.1 ± 0.5[3]	47.7 ± 1.3	50.0 ± 0.8*
Day 21	47.9 ± 0.8	49.4 ± 0.7	48.3 ± 0.7	47.1 ± 0.6	49.1 ± 0.7	47.8 ± 0.9
Week 13	49.3 ± 0.4[4]	48.9 ± 0.5	47.9 ± 0.6	48.4 ± 0.8	48.8 ± 0.4	43.3 ± 4.2
Hemoglobin (g/dL)						
Day 5	14.3 ± 0.1	14.7 ± 0.2	14.2 ± 0.2	14.2 ± 0.1	15.0 ± 0.3	15.8 ± 0.2**
Day 21	15.5 ± 0.1	15.8 ± 0.3	15.6 ± 0.2	15.4 ± 0.2	15.8 ± 0.2	15.5 ± 0.3
Week 13	15.1 ± 0.1	14.7 ± 0.1	14.9 ± 0.2	14.9 ± 0.2	15.3 ± 0.1	13.3 ± 1.3
Erythrocytes (10^6/µL)						
Day 5	6.74 ± 0.10	6.84 ± 0.09	6.61 ± 0.12	6.62 ± 0.05	6.99 ± 0.13	7.38 ± 0.09**
Day 21	7.54 ± 0.07	7.76 ± 0.16	7.46 ± 0.10	7.43 ± 0.06	7.81 ± 0.14	7.78 ± 0.17
Week 13	8.36 ± 0.09	8.18 ± 0.10	8.14 ± 0.12	8.11 ± 0.11*	8.20 ± 0.11	6.27 ± 0.85**
Reticulocytes (10^6/µL)						
Day 5	0.36 ± 0.02	0.34 ± 0.02	0.31 ± 0.02	0.33 ± 0.03	0.30 ± 0.02	0.22 ± 0.01**
Day 21	0.18 ± 0.01	0.22 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.10 ± 0.01**
Week 13	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.53 ± 0.18
Nucleated erythrocytes (10^3/µL)						
Day 5	0.12 ± 0.03	0.14 ± 0.03	0.14 ± 0.03	0.11 ± 0.04	0.09 ± 0.03	0.00 ± 0.00**
Day 21	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.04	0.03 ± 0.01	0.07 ± 0.03	0.02 ± 0.01
Week 13	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.05 ± 0.03	0.03 ± 0.01	0.08 ± 0.08
Mean cell volume (fL)						
Day 5	60.0 ± 0.3	60.2 ± 0.3	60.8 ± 0.3	60.1 ± 0.3	60.2 ± 0.3	60.1 ± 0.4
Day 21	57.1 ± 0.6	57.4 ± 0.2	58.3 ± 0.3	57.7 ± 0.3	57.0 ± 0.2	56.4 ± 0.4
Week 13	52.9 ± 0.3	53.0 ± 0.3	53.2 ± 0.3	53.8 ± 0.3	54.3 ± 0.1**	61.0 ± 3.1**
Mean cell hemoglobin (pg)						
Day 5	21.3 ± 0.2	21.5 ± 0.2	21.5 ± 0.1	21.4 ± 0.1	21.5 ± 0.1	21.4 ± 0.2
Day 21	20.6 ± 0.2	20.4 ± 0.1	21.0 ± 0.2	20.7 ± 0.2	20.3 ± 0.2	20.0 ± 0.2
Week 13	18.1 ± 0.1	18.2 ± 0.1	18.3 ± 0.1	18.4 ± 0.1*	18.6 ± 0.2**	21.6 ± 1.1**
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.5 ± 0.3	35.7 ± 0.2	35.4 ± 0.2	35.7 ± 0.2	35.8 ± 0.1	35.7 ± 0.2
Day 21	36.1 ± 0.2	35.5 ± 0.2	36.0 ± 0.2	35.9 ± 0.2	35.6 ± 0.2	35.5 ± 0.1
Week 13	34.2 ± 0.2	34.3 ± 0.1	34.4 ± 0.2	34.2 ± 0.2	34.4 ± 0.2	35.4 ± 0.1*
Platelets (10^3/µL)						
Day 5	978.1 ± 28.3	987.0 ± 28.1	994.6 ± 24.6	1,040.3 ± 31.5	967.6 ± 46.9	1,066.8 ± 32.2
Day 21	779.5 ± 17.2	819.9 ± 18.7	812.2 ± 19.0	768.2 ± 19.8	747.4 ± 15.9	659.3 ± 27.3**
Week 13	658.1 ± 18.2	652.4 ± 9.5	653.9 ± 13.0	650.6 ± 26.8	624.1 ± 12.6	964.3 ± 178
Leukocytes (10^3/µL)						
Day 5	7.27 ± 0.38	9.14 ± 0.48	7.67 ± 0.43	8.56 ± 0.45	7.26 ± 0.54	7.53 ± 0.60
Day 21	9.08 ± 0.21	9.56 ± 0.50	9.68 ± 0.84	8.94 ± 0.78	8.52 ± 0.37	8.02 ± 0.46
Week 13	9.71 ± 0.44	8.11 ± 0.59	9.28 ± 0.49	10.40 ± 0.70	8.14 ± 0.51	11.83 ± 1.77

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE (continued)						
Segmented neutrophils (10^3/µL)						
Day 5	0.79 ± 0.06	1.05 ± 0.11	0.74 ± 0.03	0.87 ± 0.09	0.81 ± 0.15	0.66 ± 0.08
Day 21	1.04 ± 0.11	1.15 ± 0.13	1.19 ± 0.15	1.16 ± 0.15	1.24 ± 0.13	1.29 ± 0.14
Week 13	1.58 ± 0.24	1.00 ± 0.14	1.15 ± 0.13	1.86 ± 0.42	1.11 ± 0.17	3.00 ± 0.60
Lymphocytes (10^3/µL)						
Day 5	6.45 ± 0.34	8.08 ± 0.40	6.86 ± 0.41	7.62 ± 0.38	6.41 ± 0.48	6.83 ± 0.56
Day 21	8.00 ± 0.22	8.32 ± 0.49	8.43 ± 0.70	7.76 ± 0.73	7.21 ± 0.32	6.64 ± 0.42*
Week 13	7.98 ± 0.36	7.00 ± 0.55	8.01 ± 0.45	8.40 ± 0.38	7.01 ± 0.48	8.75 ± 1.44
Monocytes (10^3/µL)						
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.04	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02
Day 21	0.02 ± 0.02	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.03	0.06 ± 0.02
Week 13	0.08 ± 0.04	0.10 ± 0.06	0.08 ± 0.05	0.10 ± 0.05	0.01 ± 0.01	0.05 ± 0.05
Eosinophils (10^3/µL)						
Day 5	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
Day 21	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.04 ± 0.02
Week 13	0.07 ± 0.04	0.03 ± 0.02	0.05 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.03
Methemoglobin (g/dL)						
Day 5	0.04 ± 0.01	0.08 ± 0.03	0.05 ± 0.02	0.07 ± 0.02	0.02 ± 0.01	0.05 ± 0.02
Day 21	0.07 ± 0.01	0.10 ± 0.02	0.08 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
Week 13	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	0.08 ± 0.02
FEMALE						
n						
Day 5	10	10	10	10	10	10
Day 21	9	9	8	9	8	9
Week 13	8	10	9	10	9	0
Hematocrit (automated) (%)						
Day 5	43.3 ± 0.5	43.3 ± 0.6	43.2 ± 0.6	43.9 ± 0.6	44.9 ± 0.5	47.1 ± 0.6**
Day 21	45.0 ± 0.6	45.4 ± 0.4	45.8 ± 0.7	46.5 ± 0.7	45.3 ± 0.5	44.8 ± 0.7
Week 13	44.8 ± 0.5	44.4 ± 0.4	44.4 ± 0.4	44.7 ± 0.3	43.5 ± 0.4	)
Hematocrit (manual) (%)						
Day 5	44.8 ± 0.8[3]	45.8 ± 0.5	45.6 ± 0.5	46.2 ± 0.9	46.2 ± 0.5	48.1 ± 0.4**
Day 21	48.7 ± 0.8[2]	48.1 ± 0.6	50.2 ± 0.8	50.1 ± 0.6	49.2 ± 0.5	46.8 ± 0.6
Week 13	48.9 ± 0.5	49.1 ± 0.5	49.1 ± 0.4	48.7 ± 0.3	47.8 ± 0.5	)
Hemoglobin (g/dL)						
Day 5	14.9 ± 0.2	15.0 ± 0.2	15.1 ± 0.2	15.2 ± 0.2	15.8 ± 0.2**	16.5 ± 0.2**
Day 21	15.7 ± 0.2	15.8 ± 0.1	15.8 ± 0.2	16.0 ± 0.2	15.8 ± 0.2	15.7 ± 0.2
Week 13	15.4 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	)
Erythrocytes (10^6/µL)						
Day 5	7.10 ± 0.08	7.13 ± 0.10	7.11 ± 0.10	7.27 ± 0.12	7.52 ± 0.08*	7.86 ± 0.12**
Day 21	7.47 ± 0.14	7.51 ± 0.08	7.61 ± 0.11	7.77 ± 0.12	7.81 ± 0.10	7.98 ± 0.11**
Week 13	7.79 ± 0.08	7.80 ± 0.06	7.74 ± 0.06	7.73 ± 0.05	7.59 ± 0.08	)
Reticulocytes (10^6/µL)						
Day 5	0.28 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.21 ± 0.01*	0.18 ± 0.01**	0.16 ± 0.01**
Day 21	0.12 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	0.10 ± 0.01
Week 13	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	)

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
FEMALE (continued)						
Nucleated erythrocytes (10^3/µL)						
Day 5	0.13 ± 0.03	0.07 ± 0.02	0.17 ± 0.07	0.05 ± 0.02	0.02 ± 0.01**	0.03 ± 0.02**
Day 21	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.03	0.01 ± 0.01
Week 13	0.06 ± 0.02	0.03 ± 0.02	0.14 ± 0.04	0.05 ± 0.02	0.13 ± 0.04	)
Mean cell volume (fL)						
Day 5	61.0 ± 0.2	60.8 ± 0.3	60.8 ± 0.4	60.4 ± 0.3	59.7 ± 0.3**	59.9 ± 0.2**
Day 21	60.3 ± 0.4	60.4 ± 0.2	60.3 ± 0.4	59.8 ± 0.1	58.0 ± 0.4**	56.1 ± 0.2**
Week 13	57.5 ± 0.1	56.9 ± 0.2	57.4 ± 0.2	57.8 ± 0.1	57.4 ± 0.1	)
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.1	21.1 ± 0.1	21.3 ± 0.1	21.0 ± 0.1	21.0 ± 0.1	20.9 ± 0.2
Day 21	21.1 ± 0.2	21.0 ± 0.1	20.8 ± 0.1	20.6 ± 0.2	20.3 ± 0.1**	19.7 ± 0.1**
Week 13	19.7 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.8 ± 0.1	)
Mean cell hemoglobin concentration (g/dL)						
Day 5	34.4 ± 0.2	34.7 ± 0.2	35.0 ± 0.1**	34.6 ± 0.1	35.3 ± 0.2**	35.0 ± 0.2**
Day 21	35.0 ± 0.2	34.8 ± 0.2	34.4 ± 0.2	34.4 ± 0.4	35.0 ± 0.2	35.2 ± 0.2
Week 13	34.4 ± 0.2	34.7 ± 0.2	34.4 ± 0.2	34.1 ± 0.2	34.6 ± 0.2	)
Platelets (10^3/µL)						
Day 5	986.4 ± 40.8	957.9 ± 21.4	983.1 ± 30.4	1,000.6 ± 21.1	1,069.6 ± 31.1	1,043.9 ± 39.4
Day 21	737.9 ± 24.6	754.8 ± 12.7	773.4 ± 14.2	736.3 ± 24.5	759.3 ± 22.5	603.1 ± 22.0**
Week 13	645.5 ± 9.7	646.8 ± 14.8	667.4 ± 11.9	673.7 ± 8.6*	762.2 ± 48.8**	)
Leukocytes (10^3/µL)						
Day 5	11.61 ± 0.43	9.75 ± 0.44	9.95 ± 0.64	10.58 ± 0.47	10.27 ± 0.50	11.05 ± 0.85
Day 21	9.39 ± 0.32	9.28 ± 0.49	10.11 ± 0.49	9.71 ± 0.59	9.50 ± 0.34	8.23 ± 0.65
Week 13	6.39 ± 0.48	7.70 ± 0.30*	7.80 ± 0.51*	8.22 ± 0.44**	9.01 ± 0.31**	)
Segmented neutrophils (10^3/µL)						
Day 5	1.40 ± 0.15	0.94 ± 0.09	1.05 ± 0.20	0.91 ± 0.16	0.87 ± 0.10	1.20 ± 0.23
Day 21	0.90 ± 0.12	1.14 ± 0.11	0.90 ± 0.17	1.26 ± 0.17	1.29 ± 0.20	1.24 ± 0.10
Week 13	1.19 ± 0.18	1.45 ± 0.12	1.32 ± 0.21	1.31 ± 0.12	1.02 ± 0.15	)
Lymphocytes (10^3/µL)						
Day 5	10.13 ± 0.37	8.76 ± 0.39	8.86 ± 0.56	9.66 ± 0.42	9.35 ± 0.45	9.78 ± 0.79
Day 21	8.39 ± 0.36	8.03 ± 0.39	9.06 ± 0.34	8.28 ± 0.49	8.03 ± 0.39	6.96 ± 0.59
Week 13	5.14 ± 0.40	6.22 ± 0.21*	6.34 ± 0.37*	6.86 ± 0.38**	7.88 ± 0.29**	)
Monocytes (10^3/µL)						
Day 5	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Day 21	0.06 ± 0.03	0.07 ± 0.03	0.08 ± 0.04	0.10 ± 0.02	0.14 ± 0.04	0.02 ± 0.02
Week 13	0.04 ± 0.04	0.05 ± 0.03	0.11 ± 0.04	0.04 ± 0.03	0.07 ± 0.03	)
Eosinophils (10^3/µL)						
Day 5	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
Day 21	0.06 ± 0.02	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.01 ± 0.01
Week 13	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	)
Methemoglobin (g/dL)						
Day 5	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Day 21	0.03 ± 0.01	0.08 ± 0.03	0.04 ± 0.02	0.07 ± 0.02	0.05 ± 0.04	0.06 ± 0.03[2]
Week 13	0.07 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.11 ± 0.03	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=8.
[3] n=9.
[4] n=10.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	10	4
Urea nitrogen (mg/dL)						
Day 5	21.6 ± 0.5	22.4 ± 0.3	21.3 ± 0.3	22.5 ± 0.4	22.2 ± 0.4	23.5 ± 0.8*
Day 21	22.8 ± 0.5	23.0 ± 0.4	22.6 ± 0.5	23.6 ± 0.5	22.3 ± 0.8	21.8 ± 1.4
Week 13	21.2 ± 0.6	21.5 ± 0.3	21.4 ± 0.3	21.7 ± 0.8	22.3 ± 0.5	22.8 ± 1.3
Creatinine (mg/dL)						
Day 5	0.68 ± 0.02	0.62 ± 0.03	0.68 ± 0.01	0.63 ± 0.03	0.65 ± 0.02	0.58 ± 0.01**
Day 21	0.80 ± 0.03	0.75 ± 0.02	0.78 ± 0.03	0.76 ± 0.02	0.75 ± 0.02	0.65 ± 0.03**
Week 13	0.69 ± 0.02	0.68 ± 0.02	0.65 ± 0.02	0.64 ± 0.02	0.62 ± 0.03*	0.60 ± 0.04*
Total protein (g/dL)						
Day 5	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Day 21	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.1**	5.6 ± 0.1**
Week 13	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.1*	5.8 ± 0.2**
Albumin (g/dL)						
Day 5	4.1 ± 0.0	3.9 ± 0.0*	4.0 ± 0.0	3.9 ± 0.0**	4.0 ± 0.1	4.1 ± 0.1
Day 21	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	3.8 ± 0.0**
Week 13	4.1 ± 0.0	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.0	3.7 ± 0.1
Total bilirubin (mg/dL)						
Day 5	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.4 ± 0.0**
Day 21	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	0.7 ± 0.1	0.5 ± 0.1**
Week 13	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
Direct bilirubin (mg/dL)						
Day 5	0.41 ± 0.04	0.44 ± 0.03	0.35 ± 0.03	0.38 ± 0.04	0.36 ± 0.05	0.24 ± 0.03**
Day 21	0.67 ± 0.07	0.67 ± 0.05	0.70 ± 0.06	0.65 ± 0.03	0.47 ± 0.06*	0.33 ± 0.08**[2]
Week 13	0.37 ± 0.04	0.47 ± 0.04	0.40 ± 0.03	0.43 ± 0.04	0.37 ± 0.04	0.23 ± 0.03[3]
Cholesterol (mg/dL)						
Day 5	100 ± 4	100 ± 2	100 ± 3	91 ± 2*	91 ± 2	92 ± 3
Day 21	93 ± 2	90 ± 2	87 ± 2	88 ± 2	88 ± 2	94 ± 5
Week 13	88 ± 2	87 ± 3	91 ± 2	88 ± 2	78 ± 1**	70 ± 2**
Triglycerides (mg/dL)						
Day 5	197 ± 11	227 ± 10	197 ± 7	210 ± 9	201 ± 12	105 ± 13**
Day 21	316 ± 21	308 ± 19	294 ± 20	301 ± 17	239 ± 26*	133 ± 25**
Week 13	237 ± 17	239 ± 17	241 ± 21	262 ± 24	210 ± 18	72 ± 9*
Alanine aminotransferase (IU/L)						
Day 5	35 ± 1	34 ± 1	32 ± 1	33 ± 1	35 ± 2	39 ± 2
Day 21	41 ± 2	37 ± 1	37 ± 1	42 ± 2	38 ± 2	41 ± 3
Week 13	42 ± 1	41 ± 2	40 ± 3	38 ± 1	39 ± 1	44 ± 6
Alkaline phosphatase (IU/L)						
Day 5	535 ± 17	640 ± 10	710 ± 11**	693 ± 12**	729 ± 11**	517 ± 19
Day 21	391 ± 7	452 ± 6	506 ± 12**[4]	528 ± 24**	537 ± 21**[4]	376 ± 18
Week 13	199 ± 7	258 ± 8**	282 ± 7**	291 ± 9**	359 ± 11**	349 ± 31**
Creatine kinase (IU/L)						
Day 5	338 ± 76	323 ± 105	261 ± 67	246 ± 42	250 ± 61	310 ± 63
Day 21	103 ± 9[4]	107 ± 15	104 ± 14[4]	110 ± 13	123 ± 15	117 ± 23[2]
Week 13	153 ± 26	128 ± 26[4]	131 ± 26	133 ± 14	146 ± 25	332 ± 113

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
MALE (continued)						
Sorbitol dehydrogenase (IU/L)						
Day 5	27 ± 2[4]	25 ± 1	25 ± 1	28 ± 2	27 ± 2	22 ± 1[2]
Day 21	32 ± 3	25 ± 1	29 ± 1	28 ± 2	29 ± 1	22 ± 1[4]**
Week 13	27 ± 1	28 ± 2	29 ± 3	26 ± 1	26 ± 1	24 ± 4
Bile acids (µmol/L)						
Day 5	4.9 ± 1.5[4]	5.0 ± 1.1	6.5 ± 1.2	6.6 ± 1.4	23.6 ± 2.8**[4]	37.7 ± 4.0**
Day 21	5.4 ± 2.3[5]	1.3 ± 0.3[3]	6.4 ± 1.9[5]	7.6 ± 3.9[5]	17.8 ± 4.5*[2]	75.2 ± 15.4**[4]
Week 13	5.1 ± 0.8	12.0 ± 1.9**[2]	13.8 ± 2.4**	11.1 ± 2.5*[6]	22.8 ± 3.0**	36.0 ± 1.7**[3]
FEMALE						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 5	22.2 ± 0.5	22.6 ± 0.8	22.7 ± 0.4	21.1 ± 0.8	24.2 ± 0.6	21.0 ± 0.6
Day 21	22.1 ± 0.4	22.0 ± 0.6	21.9 ± 0.8	23.4 ± 0.6	23.9 ± 0.6	25.8 ± 1.0**
Week 13	22.0 ± 0.7	22.9 ± 0.5	24.8 ± 0.8*	23.5 ± 0.8	22.9 ± 0.4	)
Creatinine (mg/dL)						
Day 5	0.63 ± 0.02	0.61 ± 0.01	0.58 ± 0.03	0.59 ± 0.03	0.57 ± 0.02	0.58 ± 0.03
Day 21	0.62 ± 0.02	0.62 ± 0.02	0.64 ± 0.02	0.61 ± 0.02	0.57 ± 0.02	0.43 ± 0.03**
Week 13	0.63 ± 0.03	0.63 ± 0.02	0.62 ± 0.01	0.66 ± 0.02	0.56 ± 0.03	)
Total protein (g/dL)						
Day 5	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1*	5.3 ± 0.1**
Day 21	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.6 ± 0.1**	5.2 ± 0.1**
Week 13	6.7 ± 0.1	6.7 ± 0.1	6.3 ± 0.1**	5.9 ± 0.1**	5.5 ± 0.0**	)
Albumin (g/dL)						
Day 5	3.8 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.0	3.7 ± 0.0	3.7 ± 0.1
Day 21	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	3.8 ± 0.0**	3.7 ± 0.1**
Week 13	4.4 ± 0.1	4.4 ± 0.1	4.1 ± 0.0**	3.9 ± 0.0**	3.7 ± 0.0**	)
Total bilirubin (mg/dL)						
Day 5	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.0**	0.4 ± 0.0**
Day 21	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
Week 13	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	)
Direct bilirubin (mg/dL)						
Day 5	0.23 ± 0.02	0.31 ± 0.03	0.28 ± 0.04[4]	0.30 ± 0.06[2]	0.22 ± 0.02[5]	0.20 ± 0.00[7]
Day 21	0.32 ± 0.03	0.27 ± 0.03	0.25 ± 0.03[2]	0.31 ± 0.04[4]	0.23 ± 0.02[8]	0.26 ± 0.06[5]
Week 13	0.26 ± 0.02[4]	0.27 ± 0.03[4]	0.25 ± 0.02	0.28 ± 0.04	0.21 ± 0.01[4]	)
Cholesterol (mg/dL)						
Day 5	107 ± 2	104 ± 3	104 ± 3	98 ± 3*	92 ± 2**	100 ± 2**
Day 21	112 ± 3	106 ± 2	103 ± 2*	110 ± 3	107 ± 4	85 ± 4**
Week 13	110 ± 4	109 ± 2	100 ± 3	88 ± 2**	86 ± 3**	)

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine (continued)

	0 ppm	250 ppm	500 ppm	750 ppm	1,500 ppm	3,000 ppm
FEMALE (continued)						
Triglycerides (mg/dL)						
Day 5	136 ± 7	159 ± 12	135 ± 10	138 ± 22	70 ± 6**	70 ± 10**
Day 21	168 ± 12	136 ± 12*	124 ± 13*	145 ± 18	103 ± 11**	69 ± 7**
Week 13	104 ± 11	113 ± 10	106 ± 11	100 ± 18	74 ± 7	)
Alanine aminotransferase (IU/L)						
Day 5	32 ± 1	33 ± 1	32 ± 1	31 ± 1	36 ± 1*	43 ± 2**
Day 21	31 ± 1	29 ± 1	30 ± 1	30 ± 1	38 ± 2**	66 ± 3**
Week 13	38 ± 3	35 ± 2	33 ± 1	35 ± 1	39 ± 1	)
Alkaline phosphatase (IU/L)						
Day 5	373 ± 5	477 ± 13	469 ± 7	469 ± 13	383 ± 13	308 ± 10
Day 21	305 ± 6	357 ± 12	356 ± 5*	385 ± 11**	406 ± 16**	313 ± 18
Week 13	164 ± 6	185 ± 4**	249 ± 5**	251 ± 11**	254 ± 11**	)
Creatine kinase (IU/L)						
Day 5	135 ± 18[4]	158 ± 25	227 ± 33	163 ± 29	205 ± 30[2]	280 ± 55*
Day 21	226 ± 68	132 ± 15	104 ± 19	148 ± 42[4]	132 ± 24	183 ± 46[6]
Week 13	110 ± 14	89 ± 11[4]	122 ± 18	113 ± 14	133 ± 20	)
Sorbitol dehydrogenase (IU/L)						
Day 5	21 ± 1	20 ± 1	21 ± 1	19 ± 1	20 ± 1	22 ± 1
Day 21	23 ± 1	22 ± 1	20 ± 1	20 ± 1*	20 ± 1*	19 ± 1**
Week 13	24 ± 1	23 ± 1	24 ± 1	22 ± 1	19 ± 1**	)
Bile acids (µmol/L)						
Day 5	14.0 ± 3.1	9.7 ± 2.4	12.5 ± 1.2	19.4 ± 3.4	38.5 ± 3.6**	31.7 ± 3.5**
Day 21	11.3 ± 2.9	9.3 ± 2.8[4]	25.4 ± 2.3**	13.6 ± 3.0	50.6 ± 3.8**	214.4 ± 27.0**
Week 13	14.6 ± 2.1	12.3 ± 2.0	18.3 ± 2.8	17.8 ± 3.0	51.9 ± 4.1**	)

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

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

Study Parameters	0 ppm	500 ppm	750 ppm	1,500 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	374 ± 6	358 ± 5	347 ± 4**	300 ± 7**
Left epididymis	0.480 ± 0.010	0.482 ± 0.009	0.496 ± 0.010	0.464 ± 0.008
Left cauda epididymis	0.196 ± 0.005	0.199 ± 0.005	0.198 ± 0.006	0.186 ± 0.004
Left testis	1.55 ± 0.02	1.56 ± 0.02	1.51 ± 0.03	1.48 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.09 ± 0.68	10.46 ± 0.29	10.91 ± 0.44	10.84 ± 0.41
Spermatid heads (10^7/testis)	17.14 ± 0.98	16.31 ± 0.40	16.50 ± 0.63	16.00 ± 0.54
Spermatid count (mean/10^{-4}mL suspension)	85.70 ± 4.90	81.55 ± 1.98	82.48 ± 3.13	80.00 ± 2.71
Epididymal spermatozoal measurements				
Motility (%)	94.76 ± 1.42	92.30 ± 1.76	87.34 ± 3.75	83.69 ± 2.77**
Concentration (10^6/g caudal epididymal tissue)	331.5 ± 33.2	259.0 ± 29.6[2]	290.5 ± 26.3	598.2 ± 139

[1] Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid measurements, and sperm concentration are not significant by Dunn's test.
[2] n=9.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test (necropsy body weight only) or Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

Study Parameters	0 ppm	500 ppm	750 ppm	1,500 ppm
n	10	10	10	9
Necropsy body weight (g)	204 ± 4	195 ± 3	191 ± 3**	177 ± 2**[2]
Estrous cycle length (days)	4.95 ± 0.05	5.00 ± 0.00	6.00 ± 0.33**	5.67 ± 0.44[3]
Estrous stages (% of cycle)				
Diestrus	38.2	38.2	44.5	41.8
Proestrus	14.5	19.1	16.4	15.5
Estrus	30.0	24.5	24.5	22.7
Metestrus	17.3	18.2	14.5	20.0

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

Study Parameters	0 ppm	250 ppm	750 ppm	3,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	35.9 ± 0.6	34.7 ± 0.7	33.8 ± 0.6*	29.1 ± 0.3**
Left epididymis	0.055 ± 0.002	0.061 ± 0.001	0.056 ± 0.002	0.054 ± 0.002
Left cauda epididymis	0.022 ± 0.001	0.024 ± 0.001	0.022 ± 0.001	0.021 ± 0.001
Left testis	0.123 ± 0.004	0.125 ± 0.003	0.125 ± 0.003	0.117 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	17.10 ± 0.78	17.33 ± 0.94	18.67 ± 0.81	20.52 ± 1.04*
Spermatid heads (10^7/testis)	2.09 ± 0.09	2.17 ± 0.12	2.31 ± 0.07	2.37 ± 0.08
Spermatid count (mean/10^{-4}mL suspension)	65.28 ± 2.71	67.78 ± 3.83	72.20 ± 2.24	74.18 ± 2.45
Epididymal spermatozoal measurements				
Motility (%)	84.84 ± 3.43[2]	82.86 ± 4.99	78.86 ± 8.08	51.56 ± 11.77*
Concentration (10^6/g caudal epididymal tissue)	1107 ± 234	791 ± 186	904 ± 271	676 ± 201

[1] Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid heads per testis, spermatid count, and sperm concentration are not significant by Dunn's test.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine[1]

Study Parameters	0 ppm	250 ppm	750 ppm	3,000 ppm
n	10	10	10	10
Necropsy body weight (g)	29.3 ± 0.7	28.5 ± 0.6	27.4 ± 0.5*	22.9 ± 0.2**
Estrous cycle length (days)	4.30 ± 0.13	4.45 ± 0.16	4.10 ± 0.07	5.15 ± 0.27*
Estrous stages (% of cycle)				
Diestrus	33.3	26.7	30.0	28.3
Proestrus	20.8	21.7	20.0	20.0
Estrus	26.7	35.8	29.2	39.2
Metestrus	19.2	15.8	20.8	12.5

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of 1,3-Diphenylguanidine in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% hamster S9 Trial 4
TA98	0	16 ± 2.0	14 ± 1.2	33 ± 3.5	24 ± 2.0	31 ± 5.7	22 ± 3.2
	1					31 ± 0.9	29 ± 0.6
	3					29 ± 4.7	31 ± 3.8
	10					34 ± 3.6	39 ± 4.6
	33					38 ± 2.6	51 ± 5.8
	100	19 ± 0.3	16 ± 1.5	68 ± 2.6	63 ± 7.8	47 ± 3.0	46 ± 3.8
	333	15 ± 1.0	16 ± 1.2	81 ± 4.0	51 ± 5.3		
	1,000	19 ± 3.3	14 ± 3.6	75 ± 7.5	55 ± 4.3		
	3,333	19 ± 4.6	18 ± 3.5	74 ± 6.8	51 ± 5.2		
	6,666		18 ± 0.6				
	10,000	6 ± 2.8[3]		78 ± 10.4[4]	58 ± 5.6[4]		
Trial summary		Negative	Negative	Positive	Equivocal	Negative	Positive
Positive control[5]		298 ± 9.2	480 ± 38.2	1,325 ± 87.4	1,012 ± 54.6	1,300 ± 95.3	1,254 ± 53.9

TA98 (continued)

Dose (µg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
0	29 ± 2.0	22 ± 2.0	32 ± 8.4
1			
3			
10			
33			
100	31 ± 0.9	19 ± 0.7	
333	32 ± 2.5	22 ± 3.7	19 ± 3.2
1,000	34 ± 5.0	21 ± 1.7	19 ± 0.7
3,333	42 ± 3.2	31 ± 4.3	29 ± 6.4
6,666			29 ± 5.5
10,000	44 ± 1.9[4]	31 ± 5.2[4]	30 ± 3.6[4]
Trial summary	Negative	Negative	Negative
Positive control	396 ± 14.7	411 ± 13.7	479 ± 43.6

TABLE D1 Mutagenicity of 1,3-Diphenylguanidine in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% hamster S9 Trial 4
TA100	0	146 ± 25.8	96 ± 2.8	110 ± 0.9	120 ± 7.0	114 ± 4.1	95 ± 8.2
	1					96 ± 8.7	147 ± 3.2
	3					94 ± 2.8	157 ± 13.6
	10					125 ± 6.7	170 ± 3.8
	33					150 ± 6.4	231 ± 9.6
	100	120 ± 13.8	93 ± 7.4	271 ± 10.2	215 ± 31.3	213 ± 15.3	243 ± 15.5
	333	88 ± 2.6	96 ± 6.2	269 ± 22.3	201 ± 18.2		
	1,000	104 ± 7.2	105 ± 17.0	265 ± 16.3	213 ± 23.6		
	3,333	122 ± 12.9	94 ± 5.3	260 ± 2.0	177 ± 14.1		
	6,666		88 ± 5.2				
	10,000	65 ± 25.1 [3]		196 ± 11.5 [4]	162 ± 20.1 [4]		
Trial summary		Negative	Negative	Equivocal	Equivocal	Weakly Positive	Positive
Positive control		378 ± 12.7	348 ± 10.4	1,408 ± 32.1	1,067 ± 33.1	1,641 ± 35.6	1,411 ± 31.3

TA100 (continued)

	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
0	117 ± 17.1	110 ± 7.3	124 ± 5.5
1			
3			
10			
33			
100	103 ± 3.8	109 ± 0.0	
333	98 ± 4.1	111 ± 8.1	98 ± 9.3
1,000	103 ± 8.5	109 ± 3.2	81 ± 3.8
3,333	137 ± 5.9	154 ± 0.9	90 ± 9.6
6,666			96 ± 3.7
10,000	137 ± 6.6 [4]	150 ± 8.0 [4]	117 ± 13.9 [4]
Trial summary	Negative	Equivocal	Negative
Positive control	546 ± 21.2	464 ± 5.2	664 ± 19.7

TABLE D1 Mutagenicity of 1,3-Diphenylguanidine in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA1535	0	27 ± 1.2	23 ± 4.0	31 ± 4.2	7 ± 1.2	7 ± 0.3	12 ± 0.3	9 ± 1.5
	1							
	3							
	10							
	33			19 ± 2.6				
	100	35 ± 3.7	31 ± 2.9		10 ± 4.4	9 ± 1.8	8 ± 2.0	9 ± 1.7
	333	37 ± 2.9	33 ± 3.8	25 ± 0.9	8 ± 2.1	9 ± 2.4	7 ± 1.2	7 ± 1.2
	1,000	34 ± 4.5	33 ± 4.3	29 ± 5.0	13 ± 2.5	7 ± 0.6	6 ± 0.3	8 ± 3.0
	3,333	18 ± 6.7	45 ± 9.5	19 ± 9.6	15 ± 1.5	9 ± 4.5	9 ± 3.2	5 ± 0.7
	6,666		47 ± 8.5	11 ± 5.9				
	10,000	0 ± 0.0[3]			31 ± 1.2[3]	12 ± 3.7[4]	18 ± 5.8[4]	10 ± 2.3[4]
Trial summary		Negative	Weakly Positive	Negative	Equivocal	Negative	Negative	Negative
Positive control		392 ± 7.5	366 ± 31.1	355 ± 40.6	435 ± 25.0	452 ± 6.0	168 ± 3.8	257 ± 29.8

TABLE D1 Mutagenicity of 1,3-Diphenylguanidine in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% hamster S9 Trial 4
TA1537	0	5 ± 1.5	3 ± 0.7	6 ± 1.2	5 ± 1.0	4 ± 1.7	5 ± 1.2
	1						
	3						
	10						
	33						
	100	6 ± 3.2		7 ± 2.3	7 ± 1.2		
	333	6 ± 0.6	7 ± 1.3	13 ± 2.9	5 ± 0.6	5 ± 0.9	9 ± 2.3
	1,000	6 ± 0.0	5 ± 0.3	7 ± 1.2	6 ± 1.3	8 ± 0.3	10 ± 3.8
	3,333	5 ± 1.5	7 ± 1.2	8 ± 2.3	10 ± 2.5	9 ± 2.3	10 ± 2.7
	6,666		4 ± 0.3			9 ± 2.7	10 ± 2.1
	10,000	29 ± 13.8[3]	Toxic	10 ± 2.0[4]	15 ± 5.9[4]	43 ± 22.8[4]	9 ± 2.0[4]
Trial summary		Equivocal	Negative	Negative	Equivocal	Equivocal	Negative
Positive control		135 ± 32.9	241 ± 23.8	465 ± 40.4	422 ± 28.3	396 ± 9.5	72 ± 3.8

TA1537 (continued)

Dose (µg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3	+10% rat S9 Trial 4
0	7 ± 0.3	6 ± 1.5	11 ± 1.0	4 ± 0.3
1				
3				
10				
33				
100	8 ± 2.0	4 ± 0.7		
333	7 ± 0.6	6 ± 0.9	5 ± 1.5	9 ± 2.0
1,000	8 ± 0.6	4 ± 0.7	5 ± 1.5	7 ± 1.2
3,333	10 ± 1.8	5 ± 1.2	10 ± 1.5	12 ± 1.3
6,666			7 ± 0.3	13 ± 2.4
10,000	14 ± 1.2[4]	65 ± 14.7[4]	10 ± 3.0[4]	15 ± 0.0[4]
Trial summary	Negative	Equivocal	Negative	Equivocal
Positive control	168 ± 8.5	160 ± 22.2	163 ± 7.4	385 ± 11.6

[1] Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Mortelmans *et al.* (1986); 0 µg/plate is the solvent control.

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

[3] Slight toxicity.

[4] Precipitate on plate.

[5] The positive controls in the absence of metabolic activation were 4-nitro-*o*-phenylenediamine (TA98), sodium azide (TA100 and TA1535), and 9-aminoacridine (TA1537). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE D2 Frequency of Micronucleated Erythrocytes in Peripheral Blood of Male and Female Mice Administered 1,3-Diphenylguanidine in Dosed Feed for 13 Weeks[1]

	Dose (ppm)	Micronucleated NCEs/1,000 NCEs[2]	Number examined
MALE	0	0.38 ± 0.13	4
	250	0.70 ± 0.20	5
	500	1.00 ± 0.27	5
	750	1.20 ± 0.12	5
	1,500	0.70 ± 0.20	5
	3,000	1.30 ± 0.12	5
		P=0.058	
FEMALE	0	0.30 ± 0.12	5
	250	1.00 ± 0.16	5
	500	0.80 ± 0.20	5
	750	1.40 ± 0.19*	5
	1,500	1.20 ± 0.12	5
	3,000	1.30 ± 0.20	5
		P=0.037[3]	

[1] A detailed description of the protocol is found in MacGregor *et al*. (1990). Data are presented as mean ± standard error.
[2] NCEs = normochromatic erythrocytes. Two thousand normochromatic erythrocytes were scored per animal.
[3] Differences from the controls were significant by trend test (ILS, 1990).
* Positive (P=0.005) by pairwise comparison to the control group with a trend test.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N,N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352
30	Dibutyl Phthalate	Feed	95-3353
31	Isoprene	Inhalation	95-3354

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
PRINTED AS OF SEPTEMBER 1995 (continued)

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387
39	Cadmium Oxide	Inhalation	95-3388
40	β-Bromo-β-nitrostyrene	Gavage	94-3389