Reference LANL 0464 62171

1/1/1982

SHORT-TERM ORAL TOXICITY OF 2,4,6-TRINITROTOLUENE IN MICE, RATS, AND DOGS

James V. Dilley, Charles A. Tyson, Ronald J. Spanggord, Daniel P. Sasmore, Gordon W. Newell, Jack C. Dacre

SRI International, Menlo Park, California, and U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland

The short-term oral toxicity of 2,4,6-trinitrotoluene (α-TNT) was determined in dogs, rats, and mice. Single-dose oral LD50s for α-TNT in corn oil were 1320 and 794 mg/kg in male and female rats, respectively, and 660 mg/kg in both male and female mice. For multiple-dose studies, dogs were dosed daily for up to 13 wk with α-TNT at 0, 0.2, 2.0, or 20 mg/kg by capsule; rats received 0, 0.002, 0.01, 0.05, or 0.25% and mice received 0, 0.001, 0.005, 0.025, or 0.125% α-TNT in their diets over the same period. All species receiving the highest doses exhibited anemia, with reduced erythrocytes, hemoglobin, and hematocrit. Alterations were observed in organ weights, including enlarged spleens (accompanied by hemosiderosis) and livers, and depressed body weight and/or body weight gain (temporary in dogs and mice). Alterations in clinical chemistry values included elevated cholesterol and depressed serum glutamic-pyruvic transaminase activity in dogs and rats; no effect on serum glutamic-oxaloacetic transaminase activity was observed. Some effects, such as SGPT depression in rats, appeared after 13 wk, suggesting a cumulative toxicity. Reduced testes size was observed in rats at the highest dose regardless of length of exposure. Most of the toxic effects were reversible, but testicular atrophy was not in rats allowed a 4-wk recovery period after treatment. Signs of anemia were present at intermediate dose levels. "No observable effects" levels for α-TNT were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg·d.

INTRODUCTION

2,4,6-Trinitrotoluene (α-TNT) has been the most commonly used explosive since World War I (Hathaway, 1977). Its toxicity to mammals has been documented (Drill, 1952; Dacre and Rosenblatt, 1974; Zakhari et al., 1978). In humans, TNT produces dermatitis, cyanosis, gastritis, anemia, liver function abnormalities, and possibly aplastic anemia (Himsworth and Glynn, 1942; Hassman, 1971; Hathaway, 1977). In dogs,

Research was supported by the U.S. Army Medical Research and Development Command under contract DAMD 17-76-C-6050. It was conducted in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Requests for reprints should be sent to C. A. Tyson, SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025.

Journal of Toxicology and Environmental Health, 9:565–585, 1982
Copyright © 1982 by Hemisphere Publishing Corporation
0098-4108/82/040565-21$2.25

9336

repeated administration of 5–33 mg/kg·d α-TNT orally (or in one case sc) for up to 15 wk produced a characteristic initial and rapid destruction of erythrocytes and diminished hemoglobin with accompanying anisocytosis and reticulocytosis and with icterus and methemoglobinemia in the peripheral circulation in the more severe cases (Voegtlin et al., 1921–1922). Increased phagocytosis was evident in spleen, liver, and bone marrow. In other studies at high dose levels (5 to 1500 mg/kg·d), neuromuscular effects (ataxia, asynergia, incoordination, occasional nystagmus, paresis of the hindquarters, weakness), irritation of the gastrointestinal tract, dark spleens, hemosiderosis, hyperplastic bone marrow, and premature death were also recorded (Donahue and Lawton, 1944; Von Oettingen et al., 1944).

Evidence of early gastric secretory disorder and an effect on pancreatic enzymes with long-term α-TNT treatment of dogs was reported (Kleiner, 1969, 1971b, 1972). Administration of 7 mg/kg twice a week for 9 mo sc in dogs produced renal pathological changes (Dynnik et al., 1977). Daily administration of α-TNT orally to dogs and monkeys at ≤ 1.0 mg/kg for 90 d, however, failed to produce toxic signs (Hart, 1974; Martin and Hart, 1974).

Several studies of the effects of repeated exposure of rats to α-TNT have been reported. White Wistar rats fed a low-protein, high-fat diet containing TNT at 150 mg/kg·d for up to 100 d showed compensatory anemia, bright red pigment in the urine, body weight suppression, hemosiderosis of the spleen, and liver lesions (fatty infiltration and necrosis) (Himsworth and Glynn, 1942). The effects were diminished or absent in rats fed protein- or carbohydrate-enriched diets. Young Wistar rats fed TNT at 0.3% in semisynthetic diets for periods of 60–200 d showed mild depression of hemoglobin and hematocrit, decreased rose bengal retention, and fatty degeneration of the liver (Smith et al., 1943). Rabbits, rats, and dogs with chronic α-TNT poisoning had increased urobilinogen in urine, but no change in serum bilirubin concentration or in osmotic resistance to red blood cells (Borbely and Selley, 1948). In contrast to dogs, rats given α-TNT orally at 30 mg/kg·d for 6 d exhibited progressively decreased phagocytosis (Pidemskii et al., 1968). Exposure of rats to 5 mg/kg·d for 90 d or to higher doses for shorter periods produced neuromuscular effects and changes in hematologic and immunologic indices, including inhibition of leukocyte phagocytosis (Mul'memko, 1966; Mul'memko et al., 1976). Studies in mice are apparently nonexistent.

Because humans may also be exposed to α-TNT in wastewaters from ammunition plants and the data base was inadequate for establishing water quality criteria for the wastewaters, additional studies were undertaken (Pearson et al., 1979). In this report we describe the results of single-dose oral LD50 studies with rats and mice and of 13 wk repeated-exposure studies with rats, mice, and dogs. The latter studies were intended (1) to define toxic signs and target organs or systems from repeated ingestion of α-TNT in these species and "effect, no-effect" levels more precisely than

possible from available animal data; (2) to establish a dose-response relation for the effects noted; and (3) to provide guidelines for establishing dose levels to use in longer studies. Additional groups were included to assess reversibility over 4 wk with no α-TNT treatment. A partial report of our findings has been presented (Dilley et al., 1978).

METHODS

Test Material

α-TNT was obtained from E. I. du Pont de Nemours (Wilmington, Del.). The compound was >99% pure, based on chemical and chromatographic analyses. The same lot was used throughout these studies; periodic analyses by high-pressure liquid chromatography (HPLC) showed no deterioration.

Animals and Housing

AKC-registered beagles, ~5–6 mo old, were obtained from Marshall Laboratory Animals (North Rose, N.Y.). Upon arrival, they were housed in pairs or singly in covered outdoor runs and quarantined for a minimum of 2 wk; any unhealthy animals were replaced before the study began.

Outbred Sprague-Dawley rats, approximately 6 wk old (130–170 g), and Swiss-Webster mice (15–20 g) were obtained from Simonsen Laboratories (Gilroy, Calif.). They were quarantined for 1 wk to ensure that only healthy animals were used. Rats were randomly assigned 2 or 3 to a cage for the 13-wk studies and 5 to a cage for the single-dose studies. Mice were housed 5 to a cage.

Rodents were kept in air-conditioned rooms (24 ± 3°C) with a relative humidity of 50 ± 10% and a photoperiod of 12 h. Plastic cages with wire tops and Ab-Sorb-Dri hardwood bedding were used. Food (Purina Rodent Laboratory Chow 5001) and water were given *ad libitum*.

Experimental Design—Single-Dose Studies

For single-dose toxicity tests, the animals were fasted overnight (at least 16 h) before dosing. Four dose levels were used, and 10 males and 10 females received each dose. The test material was administered in corn oil via stainless steel gavage needles at a volume of 1 ml per 100 g of body weight (rats) or 0.1 ml per 10 g of body weight (mice). After dosing, the animals were allowed free access to food and water and were observed for toxic signs and mortality for up to 14 d. The LD50s and confidence intervals were calculated by the method of Thompson and Weil (Weil, 1952).

Experimental Design—Repeated-Exposure Studies

Beagles The beagles were divided among 3 treatment groups and 1 control group, with 5 males and 5 females each. α-TNT was mixed with

lactose diluent and administered to the dogs daily at 0, 0.2, 2.0, or 20 mg/kg body weight in 3.55-g gelatin capsules. Equal total weights of TNT and lactose (per kilogram of body weight) were given to each dog. Food (400 ± 5 g dry Wayne Bite-Size Kibble per dog) was given to the dogs for 2–3 h after compound administration; the dogs had been trained to feed on that schedule during quarantine. Water was provided *ad libitum*.

The treatment schedule was as follows: 1 male and 1 female from each group were treated for 4 wk and killed. A second male and female were treated for the same period and then held for an additional 4 wk without treatment to assess recovery. The remaining beagles were treated for 13 wk continuously, at the end of which 2 males and 2 females from each group were killed. The third male and female were allowed a 4-wk recovery period (no treatment) before they were killed.

Rats Each of 5 groups of rats contained 20 males and 20 females. The groups were fed 0, 0.002, 0.01, 0.05, or 0.25% α-TNT in their diets. In each group, 5 of each sex were treated for 4 wk, then killed; 5 of each sex were treated for 4 wk, allowed to recover for 4 wk, then killed; 5 of each sex were treated for 13 wk, then killed; and the last 5 of each sex were treated for 13 wk, allowed to recover for 4 wk, and then killed.

Mice The diets for mice contained 0, 0.001, 0.005, 0.025, and 0.125% α-TNT by weight. Group size, group composition, and treatment and recovery schedules were the same as those for rats.

Diet Preparation and Stability

Diets were prepared by mixing α-TNT with powdered Purina Laboratory Chow in a ball mill to form a stock mixture. The stock mixture was diluted with the Purina Chow to make the high-dose diet with a Hobart H-600-T rotary mixer, and this diet was further diluted with Chow to make the lower concentrations. Diets were prepared fresh every other week and refrigerated until use.

TNT Analyses

Animal feeds were analyzed for α-TNT to ensure proper concentration levels in the diet and stability of α-TNT during the feeding period. To 10 g feed was added 50 ml dichloromethane, and the suspension was stirred for 15 min with a magnetic stir-bar. The suspension was filtered through a Celite pad. The filtrate was passed through a short (50 X 25 mm) Florisil column that had been deactivated with 5% water. The column was washed with 25 ml dichloromethane, and the filtrates were combined and rotary-evaporated to dryness. The residue was dissolved in ethyl acetate and analyzed for α-TNT by reverse-phase HPLC under the following conditions: instrument, Spectra-Physics model 3500B liquid chromatograph; column, 30 X 1 cm C_{18}-μBondapak (Waters Associates, Milford, Mass.); solvent, methanol and water (60:40); flow rate, 1.6 ml/min;

detection, ultraviolet at 254 nm; and sensitivity, 0.1 ppm at 0.0025 aufs (absorbance units full scale) (10-μl loop injector).

Quantitation was performed by using benzophenone as the internal standard and peak areas were determined by digital integration with a Spectra-Physics Minigrator. Greater than 95% recovery of α-TNT was obtained from a sample prepared and stored at 4°C for 4 wk.

Laboratory Tests and Observations

All animals were observed at least once daily, and unusual signs were recorded. The animals were weighed once a week; food consumption was recorded 5 d/wk for dogs and weekly (at food changes) for rodents.

Blood samples were taken from the jugular vein of dogs the week before treatment was begun and after 4, 8, 13, and 17 wk. Blood samples from mice and rats were taken by direct cardiac puncture of the anesthetized animal at the time of sacrifice. Dogs and rats were fasted for a minimum of 16 h before these samples were obtained. The hematologic parameters determined were red blood cell (RBC) and total and differential white blood cell (WBC) counts, hematocrit (Hct), hemoglobin (Hb) content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Clinical chemistry tests performed on dog and rat sera included a full SMAC-20 analysis: glucose, creatinine, uric acid, Na^+, K^+, CO_2, Cl^-, Ca^{2+}, phosphorus, electrolyte balance, cholesterol, triglycerides, total bilirubin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase, Fe, total protein, albumin (A), globulin (G), and A/G ratio.

Urine samples collected from the bladders of dogs, rats, and mice at the time of sacrifice were observed or tested for the following: color, appearance, specific gravity, pH, albumin, sugar, WBC, RBC, epithelial cells, casts, bacteria, and crystals.

Necropsy and Pathology

All animals were weighed before sacrifice. Dogs were anesthetized by injection of Pentothal (sodium thiopental, Abbott Laboratories, North Chicago, Ill.) into the cephalic vein and then exsanguinated. Rodents were anesthetized with chloroform before blood was withdrawn at sacrifice. Immediately after sacrifice, the brain, heart, liver, kidneys, spleen, gonads (males only), and thyroid and adrenals (beagles only) were trimmed free from the surrounding tissues and weighed. These and the following additional tissues were examined grossly and fixed in 10% neutral buffered Formalin for histopathologic analysis: aorta, bone, bone marrow (smears only), cecum (rodents), colon, cholecyst (mice and dogs), duodenum, epididymis, esophagus, eye, ileum, jejunum, lung, lymph node, sciatic nerve, mammary glands (rodents), ovary, pancreas, parathyroid, pituitary,

prostate, salivary gland, seminal vesicles, skeletal muscle, skin (rodents), spinal cord, stomach, thymus (if identifiable), trachea, urocyst, uterus, and vagina.

Statistics

Data on body weight, weight gain, organ weight, hematology, and clinical chemistry were analyzed statistically for significant differences between treatment and control groups by a combination of Bartlett's chi-square test for unequal variances and *t*-tests for pooled and separate variances as appropriate (Scheffe, 1959). Significance was assessed at the 5 and 1% levels and by linear trend analysis (linear regression of response versus log dose). Further, Finney's ratio test was applied to the data to assess the magnitude of the difference between treatment and control group means, to compensate in part for the variability in the data (Finney, 1971). Food consumption data were analyzed statistically for differences at the 5% significance level using the Williams test (Williams, 1972). Only the *t*-test results are incorporated in the tables.

RESULTS

Single-Dose Oral LD50s

The single-dose oral LD50s and 95% confidence limits of α-TNT were 660 (524–831) and 660 (574–758) mg/kg in male and female mice and 1320 (955–1824) and 795 (602–1047) mg/kg in male and female rats, respectively. After dosing, the animals became inactive and developed tremors within 1 or 2 h, followed by mild convulsions and death within 4 h in some animals. Animals that survived the convulsions were still alive 14 d after exposure. Red urine was noted in cages of both species; at the highest doses this occurred within 60 min after dosing.

Repeated-Exposure Study—Dogs

Observations and Weights Loose mucoid stools, diarrhea, and an orange color in the urine were frequently noted in animals receiving α-TNT daily at 20 mg/kg. Neurologic signs were inactivity in males at this level for several days near the end of the treatment period and, on one occasion, nystagmus.

One male dog (A3-39) became moribund and emaciated and was killed during wk 12 after it suddenly stopped eating. Its kidneys, liver, and spleen were enlarged, weighing 76, 750, and 100 g, respectively—50–75% more than the weights of the same organs from control dogs killed after wk 13. At necropsy, swelling was noted in the left upper cerebral hemisphere of A3-39, the lungs were congested and dark, roundworms were found in the duodenum (these had been observed during wk 9 and occasionally in the stools of this dog at other times), and the lymph nodes

were hemorrhagic and reactive. There was marked anemia and agranulocytosis. Changes in clinical chemistry of the dog included an elevated cholesterol level of 318 mg% (controls, 130 ± 9 mg%) and a slightly increased alkaline phosphatase level of 205 mU/ml (controls, 95 ± 20 mU/ml).

All male and female groups lost 0.2–0.6 kg (average body weight) during wk 1, except females at the 20 mg/kg•d level. These females lost 1.4 kg (statistically significant, $p < 0.05$) and ate less (one-third as much as controls), suggesting aggravation of the weight loss by the high-dose treatment. Except for males and females at this level and females at the intermediate dose level, all groups recovered and were gaining weight by wk 4. The females given 2.0 mg/kg•d that lost weight through wk 4 and the female on the high dose that lost the most weight (2.0 kg) by wk 1, and was slowly gaining it back, recovered when withdrawn from the treatment.

All treatment groups were essentially stable in weight (range ≤0.6 kg) from wk 5 to wk 13. However, a slight downward trend in body weight for both sexes at the high dose was apparent after wk 9; this coincided with development of inactivity in the males. The male and female placed on recovery after 13 wk of treatment at this dose level continued to lose weight. These animals had loose stools and/or diarrhea occasionally throughout the recovery period. While the female appeared otherwise healthy, the male became thin and had occasional roundworms in his stools.

Elevation of liver and spleen weights and weight ratios at the 20 mg/kg•d level relative to control values was marked (Table 1). The female at this level after 4 wk of recovery also had a greatly enlarged spleen (77 g) and spleen-to-brain weight ratio (1.00). At 13 wk, adrenal weights and adrenal-to-brain weight ratios were highest in the dogs receiving the highest dose, but for males there was not a clear dose-response relation.

Blood and Urine Analyses At 4 wk both males and females at 20 mg/kg•d α-TNT had a moderate anemia, characterized by decreases in RBC, Hb, Hct, and MCHC and increased MCV (Table 2). Thereafter, the anemia improved slightly, with dramatic recovery in the females between 8 and 13 wk, except for MCV and MCHC values.

After 13 wk of treatment and 4 wk of recovery, the male dog showed lingering signs of anemia (low RBC, Hb, and Hct), leukocytosis, and high polymorphonuclear cell (PMN) levels—possibly a reflection of increased formation of new cells to compensate for the anemia. The condition of this dog had been deteriorating up to the time of sacrifice, and it was thin and had pigmented macrophages in its liver (possibly hemosiderosis). The female that had lost weight but otherwise appeared healthy also had high WBC levels characterized by marked granulocytosis. She had macrophages in her liver, hemosiderosis of the spleen, and vaginitis. The animals treated for 4 wk and then allowed to recover for 4 wk showed no signs related to ingestion of α-TNT.

TABLE 1. Organ Weight Analyses for Dogs Receiving α-TNT Capsules[a]

Parameter	Week	Group size	TNT administered to males (mg/kg·d)			TNT administered to females (mg/kg·d)		
			0	2.0	20	0	2.0	20
Body weight (kg)	4	1	11.3	10.4	9.4	9.8	9.2	8.0
	13	2	11.6	10.0	10.0[b]	8.0	7.9	8.4
Liver (g)	4	1	400	378	405	376	364	497
	13	2	486	380	602[b]	336	362	419
Spleen (g)	4	1	31	30	55	54	26	41
	13	2	48	30	74[b]	25	21	52
Adrenals (g)	13	2	1.8	1.2	2.7[b]	1.6	1.5	2.2
Liver/brain	4	1	4.8	4.6	5.6	4.5	4.4	7.8
	13	2	6.1	4.4	7.2[b]	4.4	4.3	5.4
Spleen/brain	4	1	0.37	0.36	0.75	0.64	0.32	0.64
	13	2	0.60	0.35	0.88[b]	0.32	0.25	0.65
Adrenals/brain	13	2	0.22	0.15	0.32[b]	0.20	0.17	0.28

[a]Values are group means for wk 13, single values for wk 4.
[b]Including male killed on wk 12.

Statistically significant changes in cholesterol (high), bilirubin (high), SGPT (low), and Fe (low) at the 20 mg/kg·d treatment level at 4 wk are shown in Table 2. These differences were also seen at 8 wk (data not shown) and at 13 wk (except for male Fe values). In contrast to SGPT, SGOT means were unchanged at this level: 43 and 46 mU/ml for males and females relative to control means of 40 and 39 mU/ml, respectively, at wk 13, for example. In addition, globulin levels of males given the high dose gradually increased with time, as did those of females for up to 8 wk, depressing A/G ratios. The treatment may have nonspecifically enhanced globulin synthesis. These differences were absent or not appreciable in recovery dogs.

No differences other than color were seen in urine samples from treated and control groups.

Histopathology Except for high-dose male A3-39, the dogs in the control and treatment groups appeared healthy and no gross alterations were observed at sacrifice. The high-dose female killed at 4 wk and the two females treated at this level and allowed to recover had congestion and/or hemosiderosis of the spleen, which, because of the pronounced anemia in these animals, were probably treatment-related. One dog treated at this level and allowed to recover had slight focal nephrocalcinosis in the kidneys, and another had parenchymal lymphocytes in the liver. The female exposed to α-TNT for 13 wk and allowed to recover had moderate solitary focus of alveolar histiocytosis of the lung, moderate focal

TABLE 2. Differences in Blood Measurements between Dogs Receiving α-TNT and Controls[a]

Parameter	Week	TNT administered to males (mg/kg·d)			TNT administered to females (mg/kg·d)		
		0	2.0	20	0	2.0	20
RBC ($\times 10^6$)	4	6.2 ± 0.2	5.9 ± 0.1	5.0 ± 0.2	6.5 ± 0.3	5.8 ± 0.2	4.8 ± 0.1
	13	6.8 ± 0.3	6.4 ± 0.3	5.0 ± 0.6	6.1 ± 0.2	6.0 ± 0.3	5.8 ± 0.4
Hb (g%)	4	14.7 ± 0.5	14.0 ± 0.3	12.1 ± 0.44[b]	15.7 ± 0.6	14.2 ± 0.5	11.8 ± 0.2[b]
	13	15.6 ± 0.8	14.9 ± 0.6	12.0 ± 1.6	14.3 ± 0.6	13.8 ± 0.6	14.0 ± 0.9
Hct (%)	4	42 ± 1.5	40 ± 0.7	36 ± 1.2	44 ± 1.7	40 ± 1.6	35 ± 0.9[b]
	13	45 ± 2.4	44 ± 1.8	37 ± 4.9	41 ± 1.5	41 ± 1.3	42 ± 3.1
MCV (μm^3)	4	67 ± 0.5	68 ± 0.2	72 ± 0.8	68 ± 0.5	68 ± 0.4	73 ± 0.9
	13	66 ± 1.2	68 ± 0.9	72 ± 1.2	67 ± 0.0	68 ± 1.2	73 ± 0.7
MCHC(%)	4	36 ± 0.2	35 ± 0.3	34 ± 0.2[b]	36 ± 0.3	36 ± 0.2	33 ± 0.2[b]
	13	34 ± 0.2	34 ± 0.1	33 ± 0.2[b]	34 ± 0.1	34 ± 0.4	33 ± 0.2[c]
Bilirubin (mg%)	4	0.10 ± 0.00	0.12 ± 0.02	0.16 ± 0.02	0.10 ± 0.0	0.12 ± 0.02	0.18 ± 0.02[c]
	13	0.13 ± 0.03	0.17 ± 0.03	0.23 ± 0.03	0.13 ± 0.03	0.20 ± 0.00	0.30 ± 0.00[b]
Cholesterol (mg%)	4	131 ± 12	136 ± 11	181 ± 11[c]	133 ± 13	183 ± 11	208 ± 14[b]
	13	130 ± 9	127 ± 24	185 ± 67	155 ± 19	162 ± 16	167 ± 14
SGPT (mU/ml)	4	36 ± 4	30 ± 4	14 ± 4[b]	32 ± 3	32 ± 7	8.6 ± 1.9[b]
	13	34 ± 5	28 ± 3	14 ± 6	32 ± 5	27 ± 2	7.7 ± 1.2[b]
Iron (μg%)	4	273 ± 8	144 ± 20[b,d]	173 ± 28[c]	226 ± 15	161 ± 10	171 ± 23
	13	262 ± 16	215 ± 18	232 ± 67	255 ± 12	215 ± 7	194 ± 37
A/G	4	3.3 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	3.3 ± 0.1	3.5 ± 0.2	2.8 ± 0.3
	13	3.8 ± 0.5	4.5 ± 0.7	2.1 ± 0.5	5.3 ± 0.1	3.8 ± 0.2	3.9 ± 0.1

[a]Values are means ± SE for groups of 5 and 3 animals at 4 and 13 wk, respectively. Values at 13 wk include male at 20 mg/kg·d killed on wk 12.

[b]Significant at $p < 0.01$.

[c]Significant at $p < 0.05$.

[d]Mean ± SE for males given 0.2 mg/kg·d = 256 ± 20, not statistically significant.

hemosiderosis of the spleen, and slight diffuse vaginitis, which may have been attributable to incomplete recovery from the effects of the more prolonged exposure to α-TNT.

The high-dose male and one of two females had pigmented macrophages in the liver and enlarged livers at 13 wk. Several other observations restricted to the high-dose male killed in wk 12 of treatment were: hyperplasia of the bone marrow, extramedullary hematopoiesis, hyperplasia of the prostate, and intestinal parasites [nematode(s) in the duodenum]. All other lesions in males or females at this sacrifice time were so distributed among the groups, and the groups were so small, that we cannot attribute them to α-TNT.

Repeated Exposure Study—Rats

Observations and Weight No outward signs of toxicity were apparent in the rats. Urine from both sexes at the highest treatment levels (0.05 and 0.25%) was red on d 2, and this continued until sacrifice. In males and females subjected to treatment at 0.01%, red urine appeared on d 50 and the color persisted until sacrifice. When the rats were allowed a recovery period, the red color disappeared from the urine after about 2 wk.

Average daily food consumption at the two highest dose levels during the treatment period was suppressed in an apparently dose-related manner: 27.2, 23.2, and 19.2 g per male and 16.7, 15.6, and 12.5 g per female for 0, 0.05, and 0.25% α-TNT in the feed, respectively. The depression was statistically significant ($p < 0.05$) by the Williams test at almost every recording at the highest dose level and occasionally at the next highest level. Rats at these two levels normally showed increased food consumption immediately after discontinuation of α-TNT in the diet, particularly those subjected to 13 wk of treatment. Differences in mean food consumption for other groups were not referable to the treatment. Based on these data, the calculated average daily intake of α-TNT was 1.40, 6.97, 34.7, and 160 mg/kg (body weight) for males and 1.45, 7.41, 36.4, and 164 mg/kg for females at 0.0025, 0.01, 0.05, and 0.25% α-TNT, respectively.

The most dramatic effects on body weight gain were seen during the first 2 wk (Table 3). Differences in initial body weights contributed partly to the differences between the female treated and control groups. When treated rats were allowed to recover, an immediate surge in body weight gain occurred in affected groups; rats treated for 4 wk substantially recovered in body weight, but those given α-TNT for 13 wk required a longer period.

Organ weight analysis showed statistically significant differences in spleen and testes weights and organ-to-brain weight ratios in rats at the highest dose level (Table 4). Marginal increases in these parameters for spleen relative to controls were also noted for males at the 0.05% level at

TABLE 3. Body Weights of Rats Given α-TNT for 13 wk (Mean ± SE)

Group	Week	Group size	TNT in diet—males (%)				TNT in diet—females (%)			
			0	0.01	0.05	0.25	0	0.01	0.05	0.25
Killed after treatment	0	20	168 ± 2	168 ± 2	169 ± 2	170 ± 2	158 ± 1	153 ± 1	148 ± 2[a]	147 ± 2[a]
	1	20	215 ± 2	211 ± 5	212 ± 4	182 ± 4[a]	175 ± 2	175 ± 2	170 ± 2	147 ± 2[a]
	2	20	269 ± 3	261 ± 4	261 ± 4	217 ± 5[a]	196 ± 2	193 ± 2	183 ± 3[a]	161 ± 2[a]
	4	20	342 ± 5	338 ± 4	330 ± 4	280 ± 5[a]	219 ± 2	217 ± 3	204 ± 3[a]	181 ± 3[a]
	8	10	433 ± 10[b]	420 ± 8	415 ±10	334 ± 7[a]	246 ± 3[b]	252 ± 8	231 ± 5	201 ± 6[a]
	13	10	500 ± 15	474 ± 9	466 ± 12	369 ± 10[a]	265 ± 5	264 ± 8	240 ± 4	210 ± 6[a]
Allowed to recover	13	5	500 ± 15[c]	490 ± 12	486 ± 20	377 ± 11[d]	265 ± 5[c]	271 ± 12	246 ± 6	218 ± 7[a]
	14	5	521 ± 15	494 ± 13	507 ± 20	411 ± 13[d]	274 ± 4	278 ± 12	251 ± 7	230 ± 8[d]
	17	5	520 ± 15	504 ± 13	514 ± 24	435 ± 15	272 ± 4	269 ± 11	255 ± 8	238 ± 11

[a]Significant at $p < 0.01$.
[b]Group size = 15 (includes 8-wk control rats).
[c]Group size = 10 (includes 13-wk control rats).
[d]Significant at $p < 0.05$.

TABLE 4. Organ Weights and Weight Ratios for Rats Given α-TNT for 13 wk[a]

Parameter	Week	TNT in diet—males (%)			TNT in diet—females (%)		
		0	0.05	0.25	0	0.05	0.25
Body weight (g)	4	324 ± 8	352 ± 2	312 ± 8	229 ± 4	213 ± 8	185 ± 8[b]
	13	478 ± 22	446 ± 9	361 ± 16[b]	252 ± 4	234 ± 5	202 ± 9[b]
	17	520 ± 15	514 ± 24	435 ± 15	272 ± 4	255 ± 8	238 ± 10
Spleen weight (g)	4	0.73 ± 0.08	0.89 ± 0.07	1.5 ± 0.06[b]	0.61 ± 0.04	0.60 ± 0.04	1.1 ± 0.04[b]
	13	0.73 ± 0.04	0.90 ± 0.03[c,d]	2.0 ± 0.20[c]	0.47 ± 0.03	0.56 ± 0.04	1.4 ± 0.18[c]
	17	0.93 ± 0.06	0.90 ± 0.02	1.1 ± 0.07	0.58 ± 0.03	0.55 ± 0.01	1.0 ± 0.11[c]
Testes weight (g)	4	5.0 ± 0.1	4.6 ± 0.2	1.7 ± 0.2[b]			
	13	3.4 ± 0.1	3.5 ± 0.1	1.1 ± 0.1[b]			
	17	3.6 ± 0.3	3.8 ± 0.3	1.6 ± 0.1[b]			
Spleen/brain	4	0.34 ± 0.03	0.43 ± 0.03	0.84 ± 0.03[b]	0.31 ± 0.02	0.32 ± 0.02	0.58 ± 0.01[b]
	13	0.34 ± 0.01	0.40 ± 0.02[c,e]	0.93 ± 0.10[c]	0.25 ± 0.01	0.28 ± 0.02	0.71 ± 0.09[c]
	17	0.41 ± 0.02	0.38 ± 0.01	0.45 ± 0.03	0.28 ± 0.02	0.26 ± 0.01	0.43 ± 0.05[c]
Testes/brain	4	2.3 ± 0.1	2.2 ± 0.1	0.8 ± 0.1[b]			
	13	1.6 ± 0.1	1.6 ± 0.1	0.5 ± 0.0[b]			
	17	1.6 ± 0.1	1.6 ± 0.1	0.6 ± 0.1[b]			

[a]Values are means ± SE for groups of 5 animals.
[b]Significant at $p < 0.01$.
[c]Significant at $p < 0.05$.
[d]Mean ± SE for males fed 0.01% TNT diet = 0.79 ± 0.05, not statistically significant.
[e]Mean ± SE for males fed 0.01% TNT diet = 0.36 ± 0.03, not statistically significant.

13 wk. Linear trend analysis indicated that the difference was probably related to treatment. These differences were also statistically significant for testes weights and spleen weights for male and female recovery groups, respectively. Other differences seen occasionally were liver weights and liver-to-brain weight ratios (increase) and kidney weights and kidney-to-brain weight ratios (decrease) for high-dose treatment and recovery groups, but none of the changes was outside the normal range.

Blood Analyses Low RBC, Hb, and Hct and high MCV in particular in the rats at the highest dose levels were more pronounced at 13 wk than at 4 wk (Table 5). Leukocytosis was evident in rats at the highest dose level, characterized at the 13-wk sacrifice by marked lymphocytosis. Some hematologic parameters were elevated, though not consistently, in rats allowed recovery after α-TNT treatment, suggesting that some type of overcompensation for the α-TNT-induced hemolytic anemia was still present 4 wk later.

Cholesterol was elevated (usually $p < 0.01$) in rats at the highest dose level at 4 and 13 wk (Table 6). SGPT was lower than normal and Fe was also lower and statistically cited in some cases at this level after 13 wk; in females Fe was also lower at the two intermediate dose levels. Again, corollary changes in SGOT were not observed. Uric acid was approximately 80% higher at 13 wk in males and females receiving the highest dose and in females at the intermediate dose level, but the values were within the normal range (data not shown). None of the changes above was seen in rats allowed a 4-wk recovery period.

Histopathology All 5 males and 5 females at the highest dose that were killed immediately after treatment had hemosiderosis of the spleen. The males also had testicular atrophy accompanied by hyperplasia of the interstitial cells and, after 13 wk, atrophy of the epididymis. Effects on the testes (atrophy) and hemosiderosis of the spleen were also observed in several rats from both groups allowed to recover for 4 wk, and atrophy of the epididymis was observed in the 5 males at this level killed at 17 wk. These lesions either did not occur or were far less frequent in other groups. Many rats had evidence of chronic respiratory disease, but all groups (including controls) were affected to a similar extent.

Repeated-Exposure Studies—Mice

Observations and Weight Red urine was observed within the first week of treatment at the two highest doses. The color disappeared 8–10 d after discontinuation of the α-TNT treatment. No overt signs of toxicity occurred that were clearly associated with the treatment. Food consumption and body weight decreased initially in the groups at the highest dose levels and remained slightly low for females fed the 0.125% α-TNT diet (3.9 g per mouse versus 4.6 g per mouse for control females). Average daily intake of α-TNT was calculated as 1.56, 7.46, 35.7, and 193 mg/kg

TABLE 5. Hematologic Values for Rats Given α-TNT for 13 wk[a]

Parameter	Week	TNT in diet—males (%)				TNT in diet—females (%)			
		0	0.01	0.05	0.25	0	0.01	0.05	0.25
RBC (X 10^6)	4	6.7 ± 0.1	7.3 ± 0.2	7.1 ± 0.1	5.8 ± 0.4	6.8 ± 0.3	7.7 ± 0.2	6.9 ± 0.2	5.2 ± 0.2
	13	8.5 ± 0.1	8.3 ± 0.1	7.9 ± 0.1	6.2 ± 0.2	7.8 ± 0.0	7.6 ± 0.1	7.3 ± 0.1	5.5 ± 0.2
	17	8.3 ± 0.1	8.4 ± 0.2	8.7 ± 0.1	8.5 ± 0.2	7.9 ± 0.1	7.7 ± 0.2	8.2 ± 0.2	6.8 ± 0.9
Hb (g%)	4	13.4 ± 0.2	14.1 ± 0.2	13.4 ± 0.1	12.0 ± 0.8	13.0 ± 0.5	14.1 ± 0.4	12.8 ± 0.3	11.2 ± 0.3
	13	14.9 ± 0.2	14.9 ± 0.2	13.5 ± 0.3[b]	12.7 ± 0.1[c]	14.8 ± 0.2	14.0 ± 0.2[b,d]	13.6 ± 0.3[b]	11.7 ± 0.3[c]
	17	14.5 ± 0.2	14.9 ± 0.3	15.6 ± 0.2	16.1 ± 0.5[b]	14.5 ± 0.2	14.6 ± 0.3	14.8 ± 0.2	13.8 ± 1.8
Hct (%)	4	39 ± 0.1	40 ± 0.7	38 ± 0.2[b]	36 ± 2.3	39 ± 1.7	41 ± 0.9	38 ± 0.7	34 ± 1.2
	13	42 ± 0.3	42 ± 0.5	38 ± 0.8[c]	37 ± 0.4[c]	43 ± 0.5	41 ± 0.5[b,d]	40 ± 0.7[b]	35 ± 0.8[c]
	17	41 ± 0.6	42 ± 0.9	44 ± 0.4	48 ± 1.0[c]	41 ± 0.7	42 ± 0.7	42 ± 0.5	41 ± 5.3
MCV (μm^3)	4	58 ± 0.9	54 ± 0.7	54 ± 0.5	62 ± 1.2	57 ± 0.2	54 ± 0.6	55 ± 0.7	60 ± 1.2
	13	49 ± 0.5	50 ± 0.2	48 ± 0.6	58 ± 1.8	55 ± 0.7	53 ± 0.7	54 ± 0.8	62 ± 1.6
	17	49 ± 0.9	49 ± 0.8	50 ± 0.4	56 ± 0.4	52 ± 1.0	54 ± 0.2	51 ± 1.1	59 ± 0.9
WBC (X 10^3)	4	8.6 ± 2.1	7.1 ± 1.4	8.8 ± 1.3	21.8 ± 5.0	9.2 ± 1.6	9.3 ± 0.8	9.1 ± 0.9	8.6 ± 1.0
	13	7.4 ± 0.6	10.7 ± 1.3	9.5 ± 1.0	13.5 ± 1.0	7.7 ± 0.6	5.3 ± 0.5[b,d]	7.8 ± 0.7	17.4 ± 2.4
	17	8.3 ± 1.3	8.2 ± 1.3	11.1 ± 1.8	13.0 ± 1.5	5.2 ± 0.6	6.0 ± 0.3	7.3 ± 0.6	6.1 ± 1.1
PMN (%)	4	17 ± 4	16 ± 3	14 ± 1	15 ± 2	11 ± 1	13 ± 1	14 ± 2	14 ± 2
	13	12 ± 1	15 ± 4	13 ± 2	8 ± 1	16 ± 2	17 ± 4	11 ± 2	8 ± 2
	17	19 ± 1	17 ± 2	20 ± 2	36 ± 8	18 ± 4	26 ± 6	14 ± 2	21 ± 3
Lymphocytes (%)	4	80 ± 4	81 ± 4	85 ± 1	81 ± 2	87 ± 2	85 ± 2	83 ± 2	85 ± 2
	13	84 ± 2	82 ± 4	85 ± 2	90 ± 1	81 ± 2	80 ± 5	86 ± 1	91 ± 2
	17	77 ± 0	79 ± 3	75 ± 2	61 ± 8	81 ± 5	71 ± 5	83 ± 3	76 ± 4

[a]Values are means ± SE for groups of 5 animals.
[b]Significant at $p < 0.05$.
[c]Significant at $p < 0.01$.
[d]Means ± SE for Hb, Hct, and WBC, respectively, of females fed 0.002% TNT diet = 14.5 ± 0.7, 41 ± 1.9, and 7.5 ± 0.9 (not statistically significant).

TABLE 6. Clinical Chemistry Determinations for Rats Given α-TNT for 13 wk[a]

Parameter	Week	TNT in diet—males (%)				TNT in diet—females (%)			
		0	0.01	0.05	0.25	0	0.01	0.05	0.25
Cholesterol (mg%)	4	47 ± 1	47 ± 2	47 ± 2	56 ± 2[b]	60 ± 4	65 ± 1	68 ± 5	88 ± 3[c]
	13	42 ± 2	40 ± 4	47 ± 2	69 ± 3[c]	67 ± 5	74 ± 5	80 ± 4	110 ± 6[c]
	17	48 ± 4	48 ± 4	46 ± 2	55 ± 3	71 ± 6	67 ± 5	72 ± 5	44 ± 18
SGPT (mU/ml)	4	41 ± 2	46 ± 1	41 ± 2	37 ± 3	36 ± 1	36 ± 3	30 ± 2	22 ± 1
	13	37 ± 1	37 ± 4	26 ± 3	15 ± 2[c]	57 ± 28	25 ± 3	28 ± 3	13 ± 3
	17	40 ± 5	32 ± 4	37 ± 2	40 ± 7	33 ± 3	33 ± 2	32 ± 7	44 ± 10
Iron (μg%)	4	194 ± 19	207 ± 16	226 ± 7	189 ± 11	270 ± 17	327 ± 49	303 ± 29	189 ± 16
	13	201 ± 12	123 ± 4[c,d]	133 ± 6[c]	131 ± 8[c]	367 ± 24	280 ± 35	243 ± 43	223 ± 18
	17	182 ± 13	185 ± 12	148 ± 6	150 ± 14	345 ± 37	314 ± 14	276 ± 20	356 ± 31

[a]Values are means ± SE for groups of 5 animals.
[b]Significant at $p < 0.05$.
[c]Significant at $p < 0.01$.
[d]Mean ± SE for males fed 0.002% TNT diet = 171 ± 15, not statistically significant.

(body weight) for males and 1.57, 8.06, 37.8, and 188 mg/kg for females fed the 0.00125, 0.0050, 0.025, and 0.125% diets, respectively.

Significant body weight depression was noted in both sexes at the 0.125% level during wk 1 but not consistently thereafter. Female mice in the control group grew at a lower than normal rate from wk 4 on; the reasons were not clear from observations of the animals or at their sacrifice. Females at the two lowest treatment levels grew at normal rates throughout the study, based on past experience with this strain.

The only treatment-related effect on organ weight was the enlargement of spleens common to all groups at the 0.125% dose level, even after 4 wk of recovery (Table 7). In some cases, the difference from control values was statistically significant. Although a dose-response relation was not always demonstrable, the fact that spleen weights were always largest in the 0.125% groups suggests a relation to treatment. Livers of male mice given α-TNT for 13 wk and allowed 4 wk of recovery were also significantly larger at sacrifice, and there was microscopic evidence of necrosis in liver tissue from two of these mice.

Hematology Although few statistically significant differences were observed in hematologic values at 4 wk, some parameters—lower RBC, Hct, corresponding changes in mean corpuscular values (e.g., MCH), and changes in the percentages of PMNs and lymphocytes—were different from control means at the 0.125% level, and these differences appeared to be dose-related (Table 7). No distinct signs of anemia were present in other groups, but the changes in male PMNs and lymphocytes were intensified at wk 13.

Histopathology No microscopic lesions related to treatment were identified in mice killed after 4 wk of α-TNT exposure, with or without a 4-wk recovery period. After 13 wk of treatment, hemosiderosis of the spleen was observed only in 3 of 5 males and all 5 females at the highest dose level. The incidence was increased in males at the 0.125% level, and 1 male and 4 females allowed 4 wk of recovery after 13 wk of treatment at the 0.025% level also exhibited this effect.

DISCUSSION

In the studies of dogs, we verified the early appearance of pronounced anemia, evidence of adaptation to it as the study progressed, and reversibility of the condition when dogs were allowed to recover, as described by Voegtlin et al. (1921–1922). Associated with this condition were decreased serum Fe, increased bilirubin, hemosiderosis of the spleen, liver lesions caused indirectly by the hemolysis and disruption of the hematopoietic system, and peripheral blood destruction with phagocytosis in the spleen and liver. Neurological effects (inactivity, occasional nystagmus) were milder than those reported by Voegtlin et al. (1921–1922), but this is probably due to the lower doses we used.

TABLE 7. Differences from Control Values in Mice Given α-TNT for 13 wk[a]

Parameter	Week	TNT in diet—males (%)			TNT in diet—females (%)		
		0	0.025	0.125	0	0.025	0.125
Body weight (g)	4	26 ± 2	31 ± 2	29 ± 3	26 ± 1	28 ± 1	26 ± 2
	13	33 ± 1	31 ± 3	32 ± 2	25 ± 2	30 ± 0	30 ± 1
	17	37 ± 1	35 ± 1	38 ± 1	28 ± 3	31 ± 1	32 ± 1
Spleen (g)	4	0.07 ± 0.01	0.09 ± 0.01	0.18 ± 0.04	0.11 ± 0.01	0.12 ± 0.02	0.14 ± 0.01
	13	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.14 ± 0.00[b]
	17	0.11 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.15 ± 0.01[c]
Spleen/brain	4	0.16 ± 0.02	0.20 ± 0.01	0.37 ± 0.09	0.23 ± 0.02	0.23 ± 0.03	0.27 ± 0.02
	13	0.19 ± 0.01	0.21 ± 0.02	0.26 ± 0.03	0.13 ± 0.01	0.19 ± 0.02	0.27 ± 0.01[b]
	17	0.20 ± 0.02	0.20 ± 0.02	0.30 ± 0.03	0.16 ± 0.02	0.17 ± 0.01	0.28 ± 0.02[c]
RBC ($\times 10^6$)	4	8.5 ± 0.2	8.4 ± 0.2	7.3 ± 0.2	9.5 ± 0.5	7.9 ± 0.2	7.3 ± 0.4
	13	9.8 ± 0.2	9.2 ± 0.5	8.5 ± 0.4	10.3 ± 0.5	9.6 ± 0.5	9.4 ± 0.5
Hct (%)	4	42 ± 1.1	42 ± 1.2	38 ± 1.3	47 ± 2.4	40 ± 1.3	38 ± 1.7[c]
	13	47 ± 0.5	42 ± 7.1	42 ± 2.1	48 ± 2.8	51 ± 4.9	46 ± 2.0
MCH (%)	4	16 ± 0.1	17 ± 0.1[c,d]	18 ± 0.5[c]	16 ± 0.1	17 ± 0.5	18 ± 0.4
	13	17 ± 0.2	16 ± 0.5	18 ± 0.2	16 ± 0.3	17 ± 0.5	17 ± 0.2
WBC ($\times 10^3$)	4	4.1 ± 1.1	10.0 ± 2.0	4.4 ± 0.8	4.5 ± 0.6	3.6 ± 0.6	3.6 ± 0.6
	13	7.4 ± 0.5	5.8 ± 0.5	7.7 ± 1.8	6.7 ± 0.9	8.1 ± 0.9	6.0 ± 1.0
PMN (%)	4	12 ± 0	11 ± 1	21 ± 5	11 ± 7	17 ± 4	22 ± 4
	13	20 ± 4	30 ± 6	49 ± 13	19 ± 3	21 ± 6	20 ± 2
Lymphocytes (%)	4	85 ± 3	83 ± 1	76 ± 5	87 ± 6	78 ± 5	73 ± 4
	13	77 ± 4	68 ± 7	49 ± 14	79 ± 3	78 ± 6	78 ± 2

[a]Values are means ± SE for groups of 5 animals.
[b]Significant at $p < 0.01$.
[c]Significant at $p < 0.05$.
[d]Mean ± SE for mice fed 0.005% TNT diet = 16 ± 0.1, not statistically significant.

Toxic jaundice, reported to occur in humans, is difficult to reproduce in experimental animals (Himsworth and Glynn, 1942), and histological signs of injury to liver cells were absent in the treated dogs in this study. The clinical chemistry, however, showed mild hypercholesterolemia in the treated dogs, which must be of hepatic origin since serum glucose, alkaline phosphatase, and triglycerides were unaltered. This is consistent with reports of increased cholesterol in bile and impaired cholesterol metabolism in liver (Pidemskii, 1964; Kleiner, 1971a). Further, SGPT, an indicator considered specific for liver, is altered at the high dose level. SGOT levels were not similarly affected. The SGPT depression did not appear to be due to selective inhibition of the enzyme by α-TNT, since we found no inhibition in experiments *in vitro* with either enzyme and millimolar concentrations of the chemical. α-TNT or its metabolites are reportedly inhibitors of protein synthesis (Pidemskii, 1964; Stewart et al., 1945). The hypercholesterolemia and selective effects on SGPT have apparently not been reported previously in experimental studies of α-TNT poisoning.

Several effects observed in the dogs were also seen in the rodents. Each species showed depressed body weight and/or body weight gain and reduced food consumption (temporary with dogs and mice), mild to moderate anemia, and alterations in organ weights, including enlarged spleens (accompanied by hemosiderosis) and livers (marginal). Rats, like dogs, had increased cholesterol and lower Fe and SGPT levels. These changes affirm the involvement of the hematopoietic system and the liver as principal targets for α-TNT toxicity in spite of the absence of histological evidence of structural changes (Hathaway, 1977).

The anemia produced by α-TNT ingestion in these three species closely resembles that reported in the literature for TNT and described as hemolytic in origin (Voegtlin et al., 1921–1922; Himsworth and Glynn, 1942). Erythrocytes, Hb, and Hct values were depressed. Usually, the bone marrow was normal to hyperplastic, and some degree of extramedullary hematopoiesis was occasionally evident. Red cells were generally normochromic and normocytic. The male dog killed early was unusual in that it had not only a reduced number of erythrocytes but also a reduced number of granulocytes, making speculation concerning the mechanism difficult. Two of the five rats given TNT in a fat diet also had hypoplastic bone marrow (Himsworth and Glynn, 1942).

Some findings not common to the three species were testicular atrophy in rats, lymphocytosis in rats, accompanied by increased uric acid levels (possibly indicating renal dysfunction), red pigment in rodent urine, slightly larger kidneys (in one severely ill dog), and elevated serum bilirubin in dogs. Testicular atrophy was most pronounced in rats; it required a high α-TNT level and was not reversed in 4 wk. The effect has not been reported previously in experimental studies with α-TNT. Polymorphonucleosis has been seen in rats fed a high-fat diet containing

TNT (Himsworth and Glynn, 1942); the difference from the present values may be due to differences in diet or strain. Red pigment in rat urine has been attributed to TNT metabolites, which were not present in the urine of dogs (Smith et al., 1943). The elevated bilirubin in dogs is probably related to the fact that anemia was initially most severe in this species.

Our estimated "no observable effects" levels for α-TNT in the test species were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg·d. This suggests that the dog is the most sensitive of the three species. Others have concluded that the dog is more sensitive than the rat (Smith et al., 1943; Dacre and Rosenblatt, 1974).

It is interesting that SGPT activity was greatly suppressed in both dogs and rats at the highest dose level. In dogs the effect was observed after 4 wk—earlier than in rats. The fact that the effect was not observed in rats at the 0.05% level (roughly comparable to the high dose administered to dogs) probably reflects differences in the concentration of the metabolite responsible for depressed SGPT activity with time in the two species, with the dogs being slightly more vulnerable.

Some alterations, particularly those associated with liver function (e.g., decreased SGPT activity, lymphocytosis, increased serum cholesterol, and decreased Fe levels in rats), appeared to become more pronounced as the treatment progressed. This indicates that some effects of repeated α-TNT administration are cumulative. Delayed effects on the liver have been noted in other studies of TNT poisoning (Drill, 1952).

The effects of short-term exposure to α-TNT (up to 4 wk) appear to be almost totally reversible. At the end of a 4-wk recovery period, the body weights of rats at the high dose level were still slightly lower (not significantly) than those of controls, and there were lingering signs of overcompensation to the anemia in females receiving 0.25% α-TNT (these signs were also observed after 13 wk of treatment in males). Longer exposure to α-TNT (13 wk) requires a longer recovery period for reversibility. Thus at 0.05% α-TNT, liver-to-body weight ratios in females remained altered (in an apparently dose-related manner) at the end of the recovery period. At 0.25% α-TNT, the signs of residual toxicity were clearer. At the end of the 4-wk recovery period, the body weights of females remained significantly depressed, they still had signs of anemia, and their spleens and livers remained enlarged. After the recovery period, the testes and kidney weights of males were lower than those of controls, and there was slight granulocytosis in the males. These factors—the cumulative nature of α-TNT toxicity and more long-lasting effects after longer exposure periods for some parameters—are design considerations for longer term studies to assess hazards to humans.

REFERENCES

Borbely, F. and Selley, C. 1948. Anemia due to trinitrotoluene poisoning. *Orv. Hetil.* 89:92–94.
Dacre, J. C. and Rosenblatt, D. H. 1974. Mammalian toxicology and toxicity to aquatic organisms

of four important types of waterborne munitions pollutants—an extensive literature review. U.S. Army Medical Bioengineering Research and Development Laboratory Tech. Rep. 7403, Ft. Detrick, Md. AD778725.

Dilley, J. V., Tyson, C. A., Sasmore, D. P., Spanggord, R. J., Newell, G. W., and Dacre, J. C. 1978. Subacute oral toxicity of TNT and a TNT/RDX mixture to dogs and rodents. *Toxicol. Appl. Pharmacol.* 45:256.

Donahue, D. D. and Lawton, A. H. 1944. Appraisal of the toxic dose of TNT with continued oral administration to dogs. *U.S. Public Health Bull.* 285:37–39.

Drill, V. A. 1952. Hepatotoxic agents: Mechanism of action and dietary interrelationship. *Pharmacol. Rev.* 4:1–42.

Dynnik, V. I., Ostrovskaya, I. S., Prilipskii, Y. V., and Baturina, T. S. 1977. Effects of some chemical and physical factors on the state of the kidneys. *Urol. Nefrol.* 4:19–23; *Chem. Abstr.* 87:194981k.

Finney, D. J. 1971. *Probit Analysis.* Cambridge: Cambridge Univ. Press.

Hart, E. R. 1974. Subacute toxicity of RDX and TNT in dogs. Office of Naval Research contract N00014-73-C-0162. Litton Bionetics, Inc., Kensington, MD. AD A035717.

Hassman, P. 1971. Trinitrotoluene. *Prac. Lek.* 23:285–294.

Hathaway, J. A. 1977. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. *J. Occup. Med.* 19:341–345.

Himsworth, H. P. and Glynn, L. E. 1942. Experimental trinitrotoluene poisoning: The effect of diet. *Clin. Sci.* 4:421–447.

Kleiner, A. I. 1969. Change in the content of ammonia, phosphates and lactic acid levels in the gastric juice of dogs during chronic trinitrotoluene poisoning. *Farmakol. Toksikol. (Moscow)* 32:578–579.

Kleiner, A. I. 1971a. External secretory function of the liver in chronic trinitrotoluene intoxication. *Vragh. Delo.* 12:121–122.

Kleiner, A. I. 1971b. External secretory function of the pancreas in dogs with chronic trinitrotoluene poisoning. *Gig. Tr. Prof. Zabol.* 15:47–48.

Kleiner, A. I. 1972. The effect of trinitrotoluene on the main functions of the stomach of dogs in a chronic experiment. *Farmakol. Toksikol. (Moscow)* 35:445–448.

Martin, D. P. and Hart, E. R. 1974. Subacute toxicity of RDX and TNT in monkeys. Office of Naval Research contract N00014-73-C-0162. Litton Bionetics, Inc., Kensington, MD. AD A044650.

Mul'menko, A. M. 1966. Effect of vitamins B1 and PP on the nervous system during chronic poisoning with trinitrotoluene. *Tr. Permsk. Gos. Med. Inst.* 72:66–73.

Mul'menko, A. M., Pidemskii, E. L., and Chuckichev, E. M. 1976. Possible use of the phagocytic reaction of leukocytes for the early diagnosis of trinitrotoluene poisoning. *Izuch. Biol. Deistviya Prod. Org. Sint. Prir. Soedin,* 107–115.

Pearson, J. G. Glennon, J. P., Barkley, J. J., and Highfill, J. W. 1979. An approach to the toxicological evaluation of a complex industrial wastewater. In *Aquatic Toxicology,* ASTM STP 667, eds. L. L. Marking and R. A. Kimerle, pp. 284–301. Philadelphia: American Society for Testing and Materials.

Pidemskii, E. L. 1964. Effect of vitamin B complex on some liver functions in acute trinitrotoluene poisoning. *Farmakol. Toksikol. (Moscow)* 27:89–91.

Pidemskii, E. L., Chuckichev, E. M., and Mul'menko, A. M. 1978. Niacin action on phagocytosis in chronic trinitrotoluene poisoning. *Farmakol. Toksikol. (Moscow)* 31:365–366.

Scheffe, A. 1959. *The Analysis of Variance,* pp. 20–21. New York: Wiley.

Smith, M. I., Westfall, B. B., and Stohlman, E. F. 1943. Experimental trinitrotoluene poisoning with attempts at detoxification. *J. Ind. Hyg. Toxicol.* 25:391–395.

Stewart, A., Witts, L. J., Higgins, G., and O'Brien, J. R. P. 1945. Some early effects of exposure to trinitrotoluene. *Br. J. Ind. Med.* 2:74–82.

Voegtlin, C., Hooper, C. W., and Johnson, J. M. 1921–1922. Trinitrotoluene poisoning—its nature, diagnosis, and prevention. *J. Ind. Hyg.* 3:239–254, 280–292.

n Oettingen, W. F., Donahue, D. D., Snyder, R. K., Horecker, B. L., Monaco, A. R., Lawton, A. H., Sweeney, T. R., and Neal, P. A. 1944. Experimental studies on the toxicity and potential dangers of trinitrotoluene (TNT). *U.S. Public Health Bull.* 285:1-78.

il, C. W. 1952. Tables for convenient calculation of median-effective dose (LD50 or ED50) and instructions in their use. *Biometrics* 8:249-263.

liams, D. A. 1972. The comparison of several dose levels with zero dose control. *Biometrics* 28:519-531.

khari, S., Villaume, J. E., and Craig, P. N. 1978. Occupational health and safety aspects of 2,4,6-trinitrotoluene (TNT). Final report, USAMRDC contract DAMD 17-77-C-7020, Franklin Institute Research Laboratories, Philadelphia.

Received May 15, 1981
Accepted September 19, 1981