Inhalation Exposure of Rats to Vapors of 1-Nitropropane at 100 ppm

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for LATdl
1-Nitropropane
or
Chlorinated
Solvents
Stabilizers

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Male and female Long-Evans rats were exposed by inhalation to vapors of 1-nitropropane at 100 ppm. The animals were exposed 7 hr per day, 5 days per week for periods up to 21½ months. Groups of rats were killed after 1, 3, 12, and 18 months of exposure and additional groups of exposed rats were removed from the exposure chamber after 3 and 12 months and held under nonexposure conditions for the remainder of the study. All animals remaining alive were killed 21½ months after the start of the study. The results of gross necropsies of the animals did not disclose any effects of exposure to 1-nitropropane on any of the organs or tissues. There were no histopathologic effects on the liver and, in particular, no induction of hepatocarcinomas. No effects were observed on final body weights or the weights of liver, kidney, or brain. There were no effects on serum chemistry or hematology.

INTRODUCTION

1-Nitropropane is one of a mixture of nitroparaffins produced from the vapor-phase nitration of propane and purified by distillation. It is used as a propellant fuel, gasoline additive, solvent, and in chemical synthesis.

The nitroparaffins are moderately toxic by oral intake, but the potential for toxic effects resulting from inhalation is of greater significance because of the possibility of exposures in industrial uses. Recent studies by inhalation have emphasized the effects on rats of subchronic exposures (Lewis *et al.*, 1979) and chronic exposures (Griffin *et al.*, 1980, 1981) to 2-nitropropane. These studies showed that if the concentration of 2-nitropropane is high enough (100 ppm or higher) severe hepatotoxicity results and continued exposure of the damaged liver to the chemical can lead to the formation of hepatocarcinomas. Neither hepatotoxicity nor the formation of hepatocarcinomas are a consequence of the exposure of rats to lower concentrations (25 ppm) of 2-nitropropane.

In the present report, the study of the potential effects of chronic exposures to nitroparaffins was extended to 1-nitropropane (1-NP). Rats were exposed to vapors of 1-NP at 100 ppm in a study which lasted for about 21½ months. The animals were exposed 7 hr per day, 5 days per week. Clinical laboratory examinations were conducted on groups of animals sacrificed at interim time intervals of 1, 3, 12, and 18 months, as well as at the terminal sacrifice.

METHODS AND MATERIALS

Animals. Five hundred BLU:(LE) BR Long-Evans rats, 250 males and 250 females, were obtained from the Blue Spruce Farms, Altamont, New York. The

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animals were divided into an exposure group consisting of 125 males and 125 females and a control group also consisting of 125 male and 125 female rats. The animals were individually housed in galvanized iron cages.

Test substance. The 1-nitropropane utilized in this study was identified as NIPAR S-10, LNB-62425, DR-5 obtained from International Minerals and Chemical Corporation.

Chamber operations. The test group was exposed to vapors of 1-nitropropane at 100 ppm for 7 hr per day, 5 days per week. To minimize oral exposure to 1-NP, which could occur through absorption of the compound in the diet, food and water were removed when the animals were placed in the exposure chamber. After the 7-hr exposure period, the cages were removed from the chamber and the water bottles and food cups were replaced in their respective cages. Food and water were similarly removed from the cages of the control animals during the exposure period. The control animals were housed in a room having environmental conditions similar to those in the exposure chamber.

The exposure chamber was 8 feet wide, 8 feet high, and 12 feet long, with controlled temperature and humidity, modified to permit introduction of the 1-NP vapors. The effluent from the vapor generator was injected into a section of the chamber containing circulating fans which mixed the effluent with air from the intake air duct. This fan circulated air within the entire exposure chamber itself and thereby maintained uniform air mixtures. An exhaust blower removed air from the chamber at a constant rate. It was operated at a velocity sufficient to provide 15 air changes per hour in the exposure chamber.

The vapor generator was maintained in a thermostated water bath at a temperature of 45°C. Vapors were generated by bubbling purified nitrogen through liquid 1-NP in an all-glass vessel. The vapor of 1-NP and nitrogen were then introduced into the mixing chamber prior to their transfer to the exposure chamber.

The concentration of 1-NP vapors in the exposure chamber was monitored by frequent sampling. Routinely at least three air samples were obtained daily: one at 30 min after the chamber was started, another after 3 hr, and the third after 5 hr. The air sample was removed from the chamber by means of an air-sampling pump operating with a limiting orifice to control flow rate of air through it. The air from the chamber was withdrawn through two glass impingers aligned in series, filled with ethyl acetate, and immersed in an ice bath. Prior to usage each sampling train, including the two ethyl acetate-filled impingers, the limiting orifice, and the sampling pump, was calibrated using a spirometer as the primary standard.

After a suitable sampling period, the contents of the impingers were transferred to a volumetric flask and a known quantity of 2-nitropropane (2-NP) was added as an internal standard. After dilution to volume, an aliquot was injected into a gas chromatograph and compared with a standard containing the same concentration of 2-NP and a known concentration of 1-NP. Gas chromatographic conditions were as follows:

column: 6 ft glass packed with 10% Carbowax 20M on 80/100-mesh Chromosorb W-AW and maintained at 90°C;

injection port: 150°C,

flame ionization detector: 300°C.

Necropsy. Ten male and ten female rats from both the exposed and control

TABLE 1

WEEKLY MEAN CONCENTRATIONS OF 1-NITROPROPANE IN ATMOSPHERE OF EXPOSURE CHAMBER EXPRESSED AS ppm

Week	Concn	Week	Concn	Week	Concn	Week	Concn
1	104	21	104	41	102	61	100
2	97	22	101	42	101	62	103
3	104	23	103	43	99	63	103
4	94	24	103	44	99	64	102
5	105	25	105	45	98	65	102
6	94	26	103	46	98	66	103
7	92	27	100	47	101	67	103
8	106	28	99	48	95	68	101
9	103	29	100	49	101	69	100
10	101	30	102	50	99	70	99
11	102	31	110	51	100	71	100
12	99	32	100	52	99	72	99
13	100	33	102	53	102	73	102
14	102	34	103	54	104	74	101
15	108	35	101	55	100	75	99
16	101	36	96	56	99	76	98
17	98	37	98	57	100	77	99
18	100	38	102	58	98	78	101
19	101	39	100	59	97	79	101
20	100	40	102	60	96	80	99

Note. Average ±1 SD = 101 ± 3.

groups were killed after 1, 3, 12, and 18 months of exposure to 1-nitropropane. Also, at 3 and 12 months, 10 males and 10 females were removed from the exposed group and thereafter maintained under nonexposure conditions. All animals remaining alive were sacrificed 21½ months after exposure was initiated. A complete necropsy was performed on each animal killed at the end of each study period or whenever a rat became moribund or was found dead. All tissues and organs were examined during the necropsy, but special care was given to examination of the liver, the suspected target organ.

Microscopy. Representative sections from 26 organs, as well as samples from all gross pathology, were taken for microscopic preparations from each animal. The tissues were fixed in 10% formalin, processed on a Technicon auto tissue processor, embedded in paraffin, cut at 5 μm thickness, and stained with hematoxylin and eosin.

Clinical chemistry and hematology. At the time of necropsy blood samples via the aorta were obtained for clinical chemistry and hematology. Serum samples were used for determinations of the following: glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), isocitric dehydrogenase (ICDH), total bilirubin, total protein, creatinine, urea nitrogen (BUN), sodium, and potassium. Hematologic examination included measurements of the following: methemoglobin, hemoglobin, packed cell volume (hematocrit), red blood cell count, white blood cell count, and prothrombin time.

TABLE 2

BODY WEIGHTS OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	381 ± 32 (10)	367 ± 31 (10)	247 ± 40 (10)	219 ± 20 (10)
3 months exposed	509 ± 42 (10)	484 ± 48 (10)	300 ± 26 (10)	288 ± 19 (10)
12 months exposed	655 ± 65 (10)	580 ± 78 (10)	341 ± 42 (10)	333 ± 34 (10)
18 months exposed	674 ± 62 (10)	651 ± 101 (10)	428 ± 67 (10)	349 ± 28 (10)
21½ months exposed	671 ± 94 (60)	629 ± 74 (27)	397 ± 80 (59)	413 ± 70 (28)
3 months exposed/ 18½ months recovery[a]		755 ± 105 (4)		381 ± 42 (4)
12 months exposed/ 9½ months recovery[a]		636 ± 41 (6)		357 ± 66 (8)

Note. Values shown are means ±1 SD and are expressed as grams. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

RESULTS

Conditions of exposure. Weekly means of the concentration of vapors of 1-nitropropane in the atmosphere of the exposure chamber are presented in Table 1. The average of these weekly means was 101 ppm of 1-NP, which, at the ambient conditions of 1350 m of altitude and 21°C, is equivalent to 320 mg/m^3.

TABLE 3

LIVER WEIGHT OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	13.8 ± 6.5 (10)	13.1 ± 1.5 (10)	8.8 ± 1.2 (10)	8.0 ± 1.0 (10)
3 months exposed	16.7 ± 1.5 (10)	15.2 ± 2.2 (10)	10.0 ± 1.2 (10)	9.5 ± 0.9 (10)
12 months exposed	16.1 ± 3.0 (10)	13.8 ± 2.8 (10)	8.4 ± 1.2 (10)	8.7 ± 1.2 (10)
18 months exposed	19.5 ± 3.0 (10)	14.9 ± 3.3 (10)	12.3 ± 1.2 (10)	8.7 ± 1.3 (10)
21½ months exposed	15.7 ± 3.0 (60)	16.0 ± 2.6 (27)	10.4 ± 2.5 (59)	10.9 ± 2.5 (28)
3 months exposed/ 18½ months recovery[a]		16.7 ± 1.9 (4)		10.0 ± 1.7 (4)
12 months exposed/ 9½ months recovery[a]		15.5 ± 0.8 (6)		10.1 ± 1.4 (8)

Note. Values shown are means ±1 SD and are expressed as grams. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 4

KIDNEY WEIGHTS OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	2.86 ± 0.19 (10)	2.84 ± 0.42 (10)	1.90 ± 0.13 (10)	1.74 ± 0.20 (10)
3 months exposed	3.57 ± 0.25 (10)	3.38 ± 0.43 (10)	2.05 ± 0.30 (10)	2.05 ± 0.10 (10)
12 months exposed	3.69 ± 0.48 (10)	3.47 ± 0.43 (10)	2.17 ± 0.29 (10)	2.29 ± 0.24 (10)
18 months exposed	4.87 ± 1.81 (10)	4.00 ± 0.93 (10)	2.68 ± 0.20 (10)	2.47 ± 0.36 (10)
21½ months exposed	4.27 ± 0.85 (59)	4.83 ± 1.26 (24)	2.60 ± 0.38 (58)	2.94 ± 0.56 (27)
3 months exposed/ 18½ months recovery[a]		4.10 ± 0.53 (4)		2.36 ± 0.29 (4)
12 months exposed/ 9½ months recovery[a]		3.78 ± 0.29 (6)		2.50 ± 0.54 (4)

Note. Values shown are means ±1 SD and are expressed as grams. The number of animals is shown in parentheses.
[a] Compare with 21½-month controls.

Necropsy. Examination of tissues and organs at the time of necropsy did not disclose any effects of exposure to 1-NP. With particular regard to the liver, there were only very infrequent findings of apparent abnormalities and these findings were equally distributed among control and exposed groups of male and female rats. Grossly, there was no evidence of any change caused by 1-NP in either sex.

Microscopy. Sections of all tissues and any gross abnormalities of each rat were examined microscopically. In general, no microscopic changes attributable to the

TABLE 5

BRAIN WEIGHTS OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	2.01 ± 0.10 (10)	2.01 ± 0.07 (10)	1.85 ± 0.04 (10)	1.83 ± 0.14 (10)
3 months exposed	2.12 ± 0.10 (10)	2.17 ± 0.24 (10)	1.95 ± 0.07 (10)	1.95 ± 0.11 (10)
12 months exposed	2.27 ± 0.14 (10)	2.25 ± 0.07 (10)	2.15 ± 0.12 (10)	2.23 ± 0.66 (10)
18 months exposed	2.25 ± 0.17 (10)	2.23 ± 0.11 (10)	2.07 ± 0.10 (10)	2.02 ± 0.10 (10)
21½ months exposed	2.21 ± 0.31 (59)	2.27 ± 0.35 (26)	1.97 ± 0.29 (57)	2.00 ± 0.10 (28)
3 months exposed/ 18½ months recovery[a]		2.24 ± 0.08 (4)		1.94 ± 0.15 (4)
12 months exposed/ 9½ months recovery[a]		2.25 ± 0.11 (6)		1.93 ± 0.07 (8)

Note. Values shown are ±1 SD and are expressed as grams. The number of animals is shown in parentheses.
[a] Compare with 21½-month controls.

TABLE 6

LIVER WEIGHT RELATIVE TO BODY WEIGHT OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	3.60 ± 0.20 (10)	3.57 ± 0.30 (10)	3.59 ± 0.38 (10)	3.63 ± 0.32 (10)
3 months exposed	3.28 ± 0.30 (10)	3.14 ± 0.35 (10)	3.34 ± 0.20 (10)	3.30 ± 0.19 (10)
12 months exposed	2.44 ± 0.27 (10)	2.38 ± 0.38 (10)	2.46 ± 0.26 (10)	2.62 ± 0.25 (10)
18 months exposed	2.89 ± 0.41 (10)	2.42 ± 1.15 (10)	2.91 ± 0.39 (10)	2.51 ± 0.45 (10)
21½ months exposed	2.40 ± 0.48 (60)	2.57 ± 0.51 (27)	2.76 ± 0.49 (59)	2.77 ± 0.50 (28)
3 months exposed/ 18½ months recovery[a]		2.22 ± 0.12 (4)		2.66 ± 0.54 (4)
12 months exposed/ 9½ months recovery[a]		2.26 ± 0.16 (5)		2.75 ± 0.31 (8)

Note. Values shown are means ±1 SD and are expressed as percentages. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

inhalation of 1-NP were observed in any of the rats. At this time, only the microscopic examination of the livers will be reported and the other findings will be reported in detail later. There was no evidence of chemical injury to the livers of rats exposed to 100 ppm of 1-NP even when the exposure was extended to 21½

TABLE 7

LIVER WEIGHT RELATIVE TO BRAIN WEIGHT OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	683 ± 67 (10)	649 ± 63 (10)	476 ± 63 (10)	437 ± 55 (10)
3 months exposed	786 ± 64 (10)	703 ± 103 (10)	510 ± 48 (10)	489 ± 51 (10)
12 months exposed	709 ± 134 (10)	613 ± 122 (10)	391 ± 58 (10)	411 ± 91 (10)
18 months exposed	868 ± 125 (10)	668 ± 141 (10)	440 ± 79 (10)	432 ± 63 (10)
21½ months exposed	705 ± 113 (59)	711 ± 137 (26)	539 ± 88 (58)	567 ± 96 (28)
3 months exposed/ 18½ months recovery[a]		744 ± 58 (4)		523 ± 115 (4)
12 months exposed/ 9½ months recovery[a]		690 ± 33 (6)		522 ± 69 (8)

Note. Values shown are means ±1 SD and are expressed as percentages. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 8

SERUM GOT IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	211 ± 41 (10)	205 ± 56 (10)	146 ± 35 (10)	122 ± 42 (9)
3 months exposed	98 ± 16 (10)	69 ± 14 (10)	89 ± 11 (10)	73 ± 10 (10)
12 months exposed	103 ± 28 (9)	115 ± 92 (10)	78 ± 25 (10)	49 ± 13 (9)
18 months exposed	115 ± 35 (10)	102 ± 23 (10)	88 ± 19 (10)	105 ± 34 (10)
21½ months exposed	107 ± 31 (11)	94 ± 26 (10)	104 ± 27 (11)	120 ± 28 (9)
3 months exposed/ 18½ months recovery[a]		74 ± 36 (4)		95 ± 26 (4)
12 months exposed/ 9½ months recovery[a]		81 ± 22 (6)		85 ± 21 (8)

Note. Values shown are means ±1 SD and are expressed as IU/liter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

months. No hepatocarcinomas were found in any animals in the study of 1-NP at 100 ppm.

There were few incidences of liver vacuolization and a number of parenchymal abscesses among animals that were found dead or sacrificed moribund during the

TABLE 9

SERUM GPT IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	19 ± 3 (10)	16 ± 3 (10)	19 ± 10 (10)	15 ± 3 (10)
3 motnhs exposed	46 ± 11 (10)	52 ± 16 (10)	31 ± 5 (10)	33 ± 10 (10)
12 months exposed	36 ± 18 (9)	26 ± 12 (9)	27 ± 14 (10)	22 ± 16 (9)
18 months exposed	38 ± 19 (10)	24 ± 4 (10)	27 ± 6 (10)	25 ± 8 (10)
21½ months exposed	23 ± 9 (11)	20 ± 7 (10)	23 ± 8 (11)	25 ± 6 (9)
3 months exposed/ 18½ months recovery[a]		23 ± 11 (4)		24 ± 10 (4)
12 months exposed/ 9½ months recovery[a]		23 ± 9 (6)		29 ± 17 (8)

Note. Values shown are means ±1 SD and are expressed as IU/liter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 10

SERUM ICDH IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	14 ± 5 (10)	8 ± 3 (10)	14 ± 4 (10)	12 ± 5 (10)
3 months exposed	46 ± 13 (10)	61 ± 38 (10)	23 ± 11 (10)	12 ± 5 (8)
12 months exposed	42 ± 41 (9)	22 ± 14 (9)	10 ± 5 (9)	9 ± 7 (8)
18 months exposed	18 ± 5 (7)	12 ± 3 (10)	17 ± 12 (10)	13 ± 8 (10)
21½ months exposed	20 ± 15 (11)	14 ± 4 (10)	16 ± 6 (11)	25 ± 20 (8)
3 months exposed/ 18½ months recovery[a]		15 ± 14 (4)		15 ± 3 (3)
12 months exposed/ 9½ months recovery[a]		11 ± 5 (6)		11 ± 5 (7)

Note. Values shown are means ±1 SD and are expressed as IU/liter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

study. Affected animals were in both control and exposed groups. These findings were apparently due to an intercurrent systemic infection among these animals and they were not associated with exposure to 1-NP.

Body and organ weights. At the time of necropsy final body weights and weights of liver, kidney, and brain were obtained. Group means of these weights are pre-

TABLE 11

SERUM TOTAL BILIRUBIN IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	0.4 ± 0.1 (10)	0.3 ± 0.1 (10)	0.2 ± 0.1 (10)	0.2 ± 0.1 (10)
3 months exposed	0.3 ± 0.3 (10)	0.2 ± 0.1 (10)	0.3 ± 0.1 (10)	0.3 ± 0.1 (10)
12 months exposed	0.7 ± 0.4 (9)	0.5 ± 0.1 (10)	0.6 ± 0.1 (10)	0.5 ± 0.1 (9)
18 months exposed	0.6 ± 0.2 (10)	0.4 ± 0.1 (10)	0.5 ± 0.2 (10)	0.5 ± 0.3 (10)
21½ months exposed	0.7 ± 0.1 (11)	0.7 ± 0.2 (10)	0.9 ± 0.8 (11)	0.5 ± 0.2 (10)
3 months exposed/ 18½ months recovery[a]		0.6 ± 0.2 (10)		0.9 ± 0.3 (4)
12 months exposed/ 9½ months recovery[a]		0.7 ± 0.1 (6)		0.9 ± 0.3 (8)

Note. Values shown are means ±1 SD and are expressed as mg/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 12

SERUM TOTAL PROTEIN IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	5.1 ± 0.2 (10)	5.0 ± 0.2 (10)	6.5 ± 0.3 (10)	6.4 ± 0.3 (10)
3 months exposed	7.6 ± 0.9 (10)	7.4 ± 0.5 (10)	6.3 ± 0.5 (10)	6.0 ± 0.6 (9)
12 months exposed	6.3 ± 0.2 (9)	6.0 ± 0.3 (10)	7.2 ± 0.6 (10)	7.3 ± 0.4 (9)
18 months exposed	5.8 ± 0.7 (10)	5.7 ± 0.3 (10)	6.5 ± 0.4 (10)	6.5 ± 0.3 (10)
21½ months exposed	5.7 ± 0.4 (10)	5.6 ± 0.4 (10)	6.7 ± 0.5 (11)	6.6 ± 0.9 (10)
3 months exposed/ 18½ months recovery[a]		5.6 ± 0.3 (4)		7.1 ± 0.5 (4)
12 months exposed/ 9½ months recovery[a]		6.1 ± 0.3 (6)		7.3 ± 0.6 (8)

Note. Values shown are means ±1 SD and are expressed as g/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

sented in Tables 2–5, respectively. It should be noted that in these tables and those that follow presenting the results of serum chemistry and hematology, the "number of animals" shown in parentheses represents the number of animals for which data was utilized to calculate the statistical values. In some cases this was the same as the number of animals in the subgroup, but in other cases, e.g., when samples could

TABLE 13

SERUM CREATININE IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	0.6 ± 0.1 (10)	0.6 ± 0.1 (10)	0.7 ± 0.1 (10)	0.7 ± 0.1 (10)
3 months exposed	0.7 ± 0.2 (10)	0.6 ± 0.1 (10)	0.6 ± 0.1 (10)	0.6 ± 0.2 (10)
12 months exposed	0.9 ± 0.2 (9)	0.9 ± 0.1 (10)	0.8 ± 0.2 (10)	0.7 ± 0.1 (9)
18 months exposed	1.1 ± 0.3 (10)	1.1 ± 0.8 (10)	0.9 ± 0.3 (10)	0.9 ± 0.1 (10)
21½ months exposed	1.0 ± 0.2 (11)	1.6 ± 1.2 (10)	1.1 ± 0.2 (11)	1.1 ± 0.2 (10)
3 months exposed/ 18½ months recovery[a]		0.8 ± 0.3 (4)		0.9 ± 0.1 (4)
12 months exposed/ 9½ months recovery[a]		1.1 ± 0.5 (6)		0.9 ± 0.2 (8)

Note. Values shown are means ±1 SD and are expressed as mg/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 14

SERUM UREA NITROGEN (BUN) IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	23 ± 3 (10)	18 ± 2 (10)	23 ± 2 (10)	22 ± 5 (10)
3 months exposed	25 ± 2 (10)	21 ± 4 (10)	18 ± 4 (10)	14 ± 2 (9)
12 months exposed	29 ± 6 (9)	23 ± 4 (10)	24 ± 5 (10)	18 ± 2 (9)
18 months exposed	30 ± 13 (10)	20 ± 2 (9)	21 ± 7 (10)	21 ± 4 (10)
21½ months exposed	27 ± 10 (11)	29 ± 12 (8)	28 ± 8 (11)	23 ± 3 (10)
3 months exposed/ 18½ months recovery[a]		23 ± 8 (4)		20 ± 2 (4)
12 months exposed/ 9½ months recovery[a]		22 ± 4 (6)		21 ± 4 (8)

Note. Values shown are means ±1 SD and are expressed as mg/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

not be obtained from all animals, the number is less. One set of animals was excluded from the tables. These were the animals which were found dead or sacrificed moribund during the study and which were of widely differing ages and for which clinical laboratory data usually were not available.

TABLE 15

SERUM SODIUM IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	146 ± 1 (10)	144 ± 2 (10)	146 ± 3 (10)	146 ± 2 (10)
3 months exposed	140 ± 1 (10)	139 ± 2 (10)	138 ± 2 (9)	137 ± 2 (9)
12 months exposed	137 ± 2 (7)	137 ± 2 (10)	135 ± 2 (10)	133 ± 4 (7)
18 months exposed	142 ± 2 (10)	142 ± 1 (9)	140 ± 2 (10)	140 ± 2 (10)
21½ months exposed	144 ± 2 (11)	143 ± 1 (10)	142 ± 2 (11)	142 ± 1 (10)
3 months exposed/ 18½ months recovery[a]		142 ± 1 (4)		142 ± 1 (4)
12 months exposed/ 9½ months recovery[a]		144 ± 4 (6)		142 ± 3 (8)

Note. Values shown are means ±1 SD and are expressed as meq/liter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 16

SERUM POTASSIUM IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	4.6 ± 0.3 (10)	4.5 ± 0.2 (10)	4.1 ± 0.3 (10)	4.1 ± 0.2 (10)
3 months exposed	5.4 ± 0.6 (10)	5.1 ± 0.6 (10)	4.4 ± 0.4 (9)	4.3 ± 0.5 (9)
12 months exposed	4.9 ± 0.3 (7)	5.3 ± 0.5 (10)	4.6 ± 0.3 (10)	4.3 ± 0.4 (7)
18 months exposed	5.8 ± 1.7 (10)	5.3 ± 1.0 (10)	4.5 ± 0.7 (10)	4.6 ± 0.3 (10)
21½ months exposed	5.4 ± 0.5 (11)	5.4 ± 0.9 (10)	4.9 ± 0.4 (11)	5.1 ± 0.7 (10)
3 months exposed/ 18½ months recovery[a]		4.8 ± 0.2 (4)		5.4 ± 1.9 (4)
12 months exposed/ 9½ months recovery[a]		5.0 ± 0.6 (6)		4.7 ± 0.6 (8)

Note. Values shown are means ±1 SD and are expressed as meq/liter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

Growth appeared normal for both sexes and only inconsistent differences were seen in body weights between control and exposed groups. The slightly lower body weights of males exposed 12 months is considered to be an anomaly in view of the remaining data. The slightly increased weights of males exposed 3 months and recovered 18½ months is also considered anomalous and likely due to the small sample size.

TABLE 17

METHEMOGLOBIN IN BLOOD OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	25 ± 20 (9)	32 ± 25 (10)	13 ± 8 (10)	29 ± 20 (7)
3 months exposed	24 ± 14 (9)	30 ± 22 (10)	38 ± 15 (10)	49 ± 23 (7)
12 months exposed	16 ± 10 (9)	22 ± 19 (10)	17 ± 15 (10)	22 ± 11 (10)
18 months exposed	36 ± 8 (9)	49 ± 7 (10)	36 ± 13 (10)	29 ± 12 (12)
21½ months exposed	120 ± 73 (10)	70 ± 37 (10)	74 ± 72 (9)	46 ± 45 (10)
3 months exposed/ 18½ months recovery[a]		29 ± 12 (4)		19 ± 12 (3)
12 months exposed/ 9½ months recovery[a]		43 ± 14 (6)		50 ± 62 (8)

Note. Values shown are means ±1 SD and are expressed as mg/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 18

HEMOGLOBIN IN BLOOD OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	14.0 ± 0.4 (8)	14.0 ± 1.0 (10)	14.2 ± 0.5 (10)	14.3 ± 1.0 (10)
3 months exposed	16.2 ± 1.0 (10)	15.5 ± 0.9 (10)	14.2 ± 0.9 (10)	13.4 ± 1.3 (8)
12 months exposed	15.4 ± 0.6 (9)	15.0 ± 0.9 (10)	15.6 ± 0.5 (10)	15.6 ± 0.7 (10)
18 months exposed	15.6 ± 3.4 (9)	16.8 ± 1.6 (10)	16.4 ± 0.7 (10)	16.4 ± 2.1 (10)
21½ months exposed	16.7 ± 0.6 (10)	16.0 ± 1.7 (10)	18.1 ± 2.1 (10)	16.5 ± 2.5 (10)
3 months exposed/ 18½ months recovery[a]		14.3 ± 4.8 (4)		16.5 (3)
12 months exposed/ 9½ months recovery[a]		18.5 ± 2.7 (6)		17.4 ± 1.3 (7)

Note. Values shown are means ±1 SD and are expressed as g/deciliter. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

There was no effect of exposure of the rats on the weights of liver, kidney, or brain. At 18 months there was a greater mean liver weight among the control males. This is considered to have no significance with regard to exposure to 1-NP. No effects were observed on kidney or brain.

TABLE 19

PACKED CELL VOLUME (HEMATOCRIT) OF BLOOD OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	43 ± 2 (9)	42 ± 2 (10)	42 ± 1 (10)	42 ± 2 (10)
3 months exposed	45 ± 1 (10)	45 ± 1 (10)	42 ± 2 (10)	42 ± 3 (8)
12 months exposed	46 ± 1 (9)	45 ± 3 (10)	45 ± 1 (10)	45 ± 2 (10)
18 months exposed	44 ± 3 (9)	44 ± 3 (10)	43 ± 2 (10)	43 ± 5 (10)
21½ months exposed	44 ± 2 (10)	42 ± 5 (10)	43 ± 2 (10)	44 ± 8 (10)
3 months exposed/ 18½ months recovery[a]		45 ± 1 (4)		43 (3)
12 months exposed/ 9½ months recovery[a]		46 ± 1 (5)		46 ± 3 (7)

Note. Values shown are means ±1 SD and are expressed as percentages. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

TABLE 20

RED BLOOD CELL COUNTS OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	4.9 ± 0.7 (9)	4.5 ± 0.5 (10)	3.3 ± 0.5 (10)	3.5 ± 0.9 (10)
3 months exposed	6.3 ± 0.5 (10)	6.2 ± 0.4 (10)	5.3 ± 0.3 (10)	6.3 ± 1.2 (8)
12 months exposed	7.3 ± 0.4 (9)	7.7 ± 0.6 (10)	6.9 ± 0.3 (10)	7.3 ± 0.4 (10)
18 months exposed	5.7 ± 0.7 (9)	6.1 ± 1.9 (10)	7.5 ± 0.7 (10)	7.9 ± 1.4 (10)
21½ months exposed	5.5 ± 0.9 (10)	5.6 ± 0.8 (10)	5.8 ± 1.0 (10)	5.6 ± 1.4 (10)
3 months exposed/ 18½ months recovery[a]		5.2 ± 0.2 (4)		4.0 (3)
12 months exposed/ 9½ months recovery[a]		5.3 ± 0.9 (6)		4.4 ± 1.3 (7)

Note. Values shown are means ±1 SD and are expressed as cells per cubic millimeter × 10^6. The number of animals is shown in parentheses.
[a] Compare with 21½-month controls.

In order to examine further the possibility of an effect on the liver weights of exposed rats, the liver weights, relative to body weights and relative to brain weights, were calculated and the results are shown in Tables 6 and 7. Because of the uniformity of brain weights, the comparisons of ratios of liver weights brain weights

TABLE 21

WHITE BLOOD CELL COUNTS OF RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	4.0 ± 0.4 (9)	6.0 ± 0.8 (10)	3.6 ± 0.6 (10)	3.6 ± 0.8 (10)
3 months exposed	5.1 ± 1.0 (10)	5.8 ± 1.2 (10)	4.1 ± 1.0 (10)	3.1 ± 0.9 (8)
12 months exposed	3.7 ± 0.8 (9)	4.0 ± 0.9 (10)	3.1 ± 1.0 (10)	2.9 ± 0.9 (10)
18 months exposed	3.0 ± 1.6 (9)	3.7 ± 1.4 (10)	3.1 ± 2.8 (10)	3.7 ± 2.1 (10)
21½ months exposed	3.6 ± 1.8 (10)	5.2 ± 3.0 (10)	2.2 ± 0.6 (10)	2.1 ± 0.6 (10)
3 months exposed/ 18½ months recovery[a]		3.7 ± 1.0 (4)		2.8 (3)
12 months exposed/ 9½ months recovery[a]		3.5 ± 1.7 (6)		1.8 ± 1.0 (7)

Note. Values shown are means ±1 SD and are expressed as cells per cubic millimeter × 10^3. The number of animals is shown in parentheses.
[a] Compare with 21½-month controls.

TABLE 22

PROTHROMBIN TIME IN RATS EXPOSED TO 1-NITROPROPANE AT 100 ppm

	Males		Females	
	Controls	Exposed	Controls	Exposed
1 month exposed	16.3 ± 2.4 (9)	15.6 ± 0.9 (10)	11.6 ± 1.3 (8)	11.7 ± 1.2 (9)
3 months exposed	15.4 ± 2.6 (4)	15.2 ± 2.4 (10)	13.4 ± 2.3 (9)	16.0 (3)
12 months exposed	12.1 ± 1.6 (9)	12.8 ± 2.1 (10)	11.3 ± 0.7 (10)	12.8 ± 2.0 (10)
18 months exposed	20.1 ± 6.3 (9)	17.1 ± 5.3 (10)	15.0 ± 0.9 (9)	17.0 ± 5.4 (9)
21½-months exposed	13.7 ± 2.0 (10)	12.9 ± 1.9 (9)	15.0 ± 2.0 (10)	12.8 ± 12.6 (10)
3 months exposed/ 18½ months recovery[a]		12.9 ± 0.9 (4)		13.5 ± 2.1 (5)
12 months exposed/ 9½ months recovery[a]		16.1 ± 5.0 (5)		15.0 (3)

Note. Values shown are means ±1 SD and are expressed as seconds. The number of animals is shown in parentheses.

[a] Compare with 21½-month controls.

yields a sensitive measure of changes in liver weights during experimental conditions. There was no effect demonstrated by these two relative measures of liver weights of rats exposed to 1-NP. (Note: the anomalously high liver weights in control males at 18 months is also reflected in the relative weights.) This lack of effect on liver weights is particularly significant, since liver weights are known to be increased in rats exposed to 2-nitropropane.

Serum chemistry. The results of examinations of serum chemistry are shown in Tables 8–16. None of these measurements disclosed any evidence of an effect of exposure of the rats to 1-NP.

Hematology. The results of hematology studies are shown in Tables 16–22. None of these studies showed an effect of exposure to 1-NP.

Comparison of results obtained on the two groups of animals exposed for either three months or twelve months, and then removed for recovery, with results from other groups did not reveal any effects of exposure to 1-NP.

DISCUSSION

In a previous publication (Griffin *et al.*, 1980) a review was made of the necessary time course and concentration effect of 2-nitropropane required to induce severe damage to hepatic parenchyma as an essential step in the induction of hepatic carcinoma in the rat. By contrast, 1-nitropropane, even at a high concentration and even when the exposure is continued to near the lifetime of the experimental animal, does not cause chemical injury to the liver and does not induce hepatocarcinoma. At a concentration of 100 ppm, hepatocarcinomas are induced in animals exposed to 2-NP, but not 1-NP. One is hard pressed even to find any evidence of exposure of the rats to 1-NP. Body weights and organ weights, including liver weights, were

unchanged by exposure to 1-NP. There were no effects on clinical pathology and no histopathologic effects on the liver.

Damage to the liver parenchymal cells is an essential precursor to the induction of hepatocarcinoma in the rat with 2-NP. Hepatocellular carcinomas induced by 2-NP only occur when the degree of exposure is sufficient to cause severe hepatotoxicity. We believe that the cellular proliferative response is due to intense hyperregeneration following the severe damage to the hepatic cells. Under these conditions, with 2-NP, it is not possible for the cellular regeneration processes to overcome the toxic effect of the chemicals to the liver cells. Some of the new emerging regenerated hepatic cells become autonomous, leading to neoplasia.

We are presently studying other doses of 2-NP in great detail. Twenty-five ppm 2-NP produced no toxicity to the parenchymal cell of the liver and therefore no cellular proliferative response and, consequently, no carcinomas. On the other hand, at 100 ppm and 200 ppm 2-NP hepatocarcinomas and metastates were observed.

The fact that with 1-NP no liver parenchymal cell toxicity and no hepatocellular carcinomas were observed gives credence to the theory that we have stated in the past concerning the relationship between exposure and degree of liver cell damage and carcinogenic potential.

CONCLUSIONS

The following conclusions can be made from this study of inhalation exposure of rats to 1-nitropropane at 100 ppm.

1. No gross or microscopic effects on tissues or organs were observed.
2. No histopathologic changes or induction of hepatocarcinomas were observed in any of the exposed rats.
3. No effect on total body weights or on the weights of liver, kidney, or brain were recorded.
4. There was no effect on serum chemistry or hematology.
5. There was no effect on the appearance and behavior of the rats.

REFERENCES

LEWIS, T. R., ULRICH, C. E., AND BUSEY, W. M. (1979). Subchronic inhalation toxicity of nitromethane and 2-nitropropane. *J. Environ. Pathol. Toxicol.* **2**, 233–249.

GRIFFIN, T. B., COULSTON, F., AND STEIN, A. A. (1980). Chronic inhalation exposure of rats to vapors of 2-nitropropane at 25 ppm. *Ecotoxicol. Environ. Safety* **4**, 267–281.

GRIFFIN, T. B., STEIN, A. A., AND COULSTON, F. (1981). Histologic study of tissues and organs from rats exposed to vapors of 2-nitropropane at 25 ppm. *Ecotoxicol. Environ. Safety* **4**, 194–201.