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Chronic Inhalation Exposure of Rats to Vapors of Nitroethane

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Male and female Long–Evans rats were exposed in inhalation chambers to vapors of nitroethane at concentrations of 100 or 200 ppm, 7 hr per day, 5 days per week for 2 years. During the study, general observations were made daily and body weights were obtained weekly for the first 6 months of the study and biweekly thereafter. Any rats that were found dead or sacrificed moribund during the 2-year exposure phase of the study were given a thorough gross examination and tissues were retained for microscopic examination. After 2 years of inhalation of nitroethane, all surviving rats were sacrificed and subjected to the same thorough gross examination. Blood samples were obtained from representative groups of animals for hematology and serum chemistry studies. All rats were examined histopathologically. Exposure of the rats to nitroethane had no pharmacologic effects nor were there any effects on mortality of rats of either sex at either level of exposure. Throughout most of the investigation, body weights of both sexes of both exposed groups were slightly less than those of respective controls, but lack of a well-defined dose–response relationship suggested the involvement of factors other than just exposure to nitroethane. There were no effects of exposure to nitroethane on hematology nor were there any biologically significant effects of exposure to nitroethane on clinical chemistry or on organ weights. No significant nonneoplastic or neoplastic pathology was found as a consequence of exposure of the rats to nitroethane. © 1988 Academic Press, Inc.

INTRODUCTION

Nitroethane (NE) is an industrial solvent produced as one component in a mixture of nitroparaffins from the vapor-phase nitration of propane and purified by distillation. Nitromethane, 1-nitropropane, and 2-nitropropane are also produced in the mixture. Although NE is a liquid at room temperature, the most likely route of exposure of industrial workers is by inhalation of vapors during various handling procedures. Studies by inhalation have emphasized the effects on laboratory animals, especially rats, of subchronic exposures (Lewis *et al.*, 1979) and chronic exposures (Griffin *et al.*, 1980, 1981, 1982). These studies showed that if the concentration of 2-nitropropane is sufficiently high (100 ppm or higher) severe hepatotoxicity results and continued exposure of the damaged liver to the chemical can lead to the formation of hepatocarcinomas. However, at lower concentrations (25 ppm) of 2-nitropropane, neither hepatotoxicity nor the formation of hepatocarcinomas ensue in rats even when the exposure is continued for the lifetime of the animals. Similarly, exposure of rats to 1-nitropropane at 100 ppm for the lifetime of the rats fails to elicit liver damage including chemically induced carcinogenesis. Our studies of the inhalation toxicity of this series of nitroparaffins have continued and have now been extended to include the lifetime exposure of rats to a third member of the series, nitroethane.

In the present report, the potential effects of chronic inhalation exposures to NE have been examined. Rats were subjected to a lifetime of exposures to one of two atmospheric concentrations of NE (100 or 200 ppm), each of which was well above

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usual levels of human industrial exposure. At the end of 2 years of these exposures, all surviving animals were sacrificed and a thorough clinical and anatomic pathological examination was performed on all animals in the study. Particular care was given to an examination of the effects of exposure to NE on the liver, a common target organ for toxic effects of some industrial solvents, and the known target organ of high-level exposure of 2-nitropropane.

METHODS AND MATERIALS

Experimental animals. The laboratory animals selected for this investigation were Long–Evans rats having the strain designation BLU:(LE)BR and were obtained from the Blue Spruce Farms, Altamont, New York. After arrival at the laboratory, the animals were quarantined and acclimated to the laboratory conditions.

Animal husbandry. During the investigation all animals were individually housed in stainless-steel wire mesh cages and were maintained in air-conditioned quarters except during the periods of exposure. A standard laboratory animal diet and water were freely available to the animals except during the daily exposure period. During exposure, food and water were removed from the cages of all animals including controls in order to reduce alimentary exposure to NE.

Experimental design. One hundred twenty-one (121) male and one hundred nineteen (119) female rats were randomly assigned to the following groups:

Group	Male	Female	NE concn. (ppm)
Control (I)	40	40	0
Exposed (II)	40	40	100
Exposed (III)	41	39	200

The intent was to assign 40 animals of each sex to each group, but after exposure was begun, it was determined that one animal assigned to group III–female was actually a male. The rats assigned to exposure groups (II and III) were exposed to appropriate concentrations of NE for 7 hr daily, 5 days each week, for 2 years.

Test material. The test material was nitroethane, CAS 000079243, and was supplied by International Minerals and Chemical Corp. (later, ANGUS Chemical Co.). The lot number used for the investigation was 2J05-5B and the analysis, as supplied by the manufacturer, was nitroethane, 97.92%; nitromethane, 0.01%; 2-nitropropane, 2.07%.

Chamber operations. Vapors of NE were generated by bubbling purified nitrogen through liquid NE in an all-glass vessel maintained in a thermostated water bath at a temperature of 45°C. Sufficient liquid NE to maintain a constant liquid level in the generator was added automatically.

The exposure chambers were 5 ft wide, 5 ft high, and 5 ft deep with tapering conical sections above and below. Chamber contents were exhausted from the lower section. The effluent from the NE vapor generators was conducted to a vortex part of the upper conical section where it was mixed with filtered air-conditioned air. The chambers were operated in the open dynamic mode.

The concentration of NE within the chamber was monitored using a MIRAN IA infrared gas analyzer. Concentrations were monitored at least three and usually four times each day.

Observations. The animals were observed daily for general appearance and for signs of pharmacologic, behavioral, or other toxic effects of exposure to NE. Mori-

bund animals were sacrificed and these and any animals found dead were subjected to the pathological examination described below.

The animals were weighted weekly during the first 6 months of the study and at 2-week intervals thereafter.

At the time of the terminal sacrifice, blood samples were obtained from 10 male and 10 female rats for clinical laboratory studies of hematology and serum chemistry. Hematologic parameters examined included erythrocyte count (RBC), leucocyte count (WBC), mean corpuscular volume (MCV), packed cell volume (HCT), and hemoglobin (HGB). Serum chemistry parameters included glutamic-oxaloacetic transaminase, also known as aspartite aminotransferase (SGOT); glutamic-pyruvic transaminase, also known as alanine aminotransferase (SGPT); total bilirubin (BILI); total protein (PROT); blood urea nitrogen (BUN); creatine (CREAT); sodium (NA); and potassium (K).

Pathology. Full necropsies were performed on all animals found dead or sacrificed moribund and on all remaining animals surviving 2 years. Each of the animals was given a thorough gross examination and the following organs were weighed: brain (including cerebellum and medulla), liver, kidneys, lungs, and heart.

The following organs and tissues were examined, removed, and fixed in 10% buffered formalin for processing and preparation of slides for microscopic examination: liver, heart, lung, artery or aorta, lymph nodes, thymus, spleen, salivary gland, pancreas, kidneys, urinary bladder, mammary glands, trachea, thyroid, esophagus, stomach, colon, intestine, adrenals, eye, pituitary, brain (including cerebrum, cerebellum, and medulla), muscle, nerve, bone, bone marrow, skin and subcutis, spinal cord. For males: testes, prostate, epididymas, seminal vesicles. For females: ovaries, uterus, cervix, oviduct.

Statistical analyses. The daily concentration of NE in each chamber was calculated as the arithmetic mean of the individual concentration measurements. Concentrations for each week of the study were calculated as the mean of the daily concentrations. Means and standard deviations of the weekly (or biweekly) body weights of each sex and group were calculated. Comparisons were made between the control and the exposed groups using Student's t test. Organ weights were expressed as both absolute weight (grams) and weight relative to the whole body weight (percentage of body weight). Means and standard deviations of each of these parameters and the parameters of clinical chemistry and hematology were calculated. Variances were tested for homogeneity using Bartlett's test, and in cases where the variances proved to be homogenous, an analysis of variance (ANOVA) was performed. If the ANOVA indicated statistical significance between means at $P = 0.05$, Duncan's multiple range test was used to determine which pairs of means were significantly different.

RESULTS

Conditions of exposure. The mean concentrations of NE in each chamber and for each week of the 2-year duration of the experiment are presented in Table 1. The grand average of the weekly means of the chamber operated at a nominal level of 100 ppm (Group II) was 99.8 ppm, and of the chamber operated at 200 ppm (Group III) was 199.2 ppm. The altitude of the site of the experiment (Alamogordo, NM) is 1350 m, and at 25°C, a concentration of 99.8 ppm is equivalent to 263 mg of NE per cubic meter of air. Similarly, a concentration of 199.2 ppm is equivalent to 525 mg of NE per cubic meter of air.

TABLE 1

WEEKLY MEAN CONCENTRATIONS OF NITROETHANE IN ATMOSPHERE OF EXPOSURE CHAMBERS EXPRESSED AS PARTS PER MILLION

Week	Group II	Group III	Week	Group II	Group III
1	101	198	53	98	200
2	99	199	54	98	200
3	99	198	55	100	198
4	99	198	56	100	205
5	99	200	57	100	200
6	98	198	58	100	199
7	99	198	59	100	198
8	100	199	60	99	199
9	100	198	61	99	198
10	99	197	62	100	200
11	99	197	63	101	198
12	99	198	64	99	198
13	98	195	65	100	198
14	98	198	66	99	199
15	100	200	67	101	201
16	100	201	68	101	199
17	102	201	69	99	199
18	100	198	70	99	199
19	101	199	71	100	200
20	100	199	72	100	199
21	99	193	73	100	199
22	99	198	74	100	199
23	99	199	75	100	200
24	100	199	76	100	200
25	99	197	77	100	198
26	97	196	78	100	201
27	98	195	79	99	200
28	99	198	80	100	199
29	98	195	81	100	198
30	98	198	82	98	199
31	99	197	83	99	199
32	100	199	84	100	200
33	99	198	85	99	198
34	98	200	86	99	198
35	98	198	87	99	198
36	99	199	88	99	199
37	99	200	89	99	200
38	100	199	90	100	199
39	100	201	91	100	199
40	100	199	92	100	200
41	100	199	93	99	198
42	98	198	94	100	198
43	98	199	95	100	199
44	101	199	96	99	199
45	100	199	97	100	199
46	100	200	98	100	199
47	99	197	99	100	200
48	98	195	100	100	199
49	100	200	101	100	199
50	99	199	102	99	200
51	100	199	103	100	199
52	98	199	104	101	200
			105	99	199

TABLE 2

SURVIVAL OF LONG–EVANS RATS EXPOSED TO NITROETHANE FOR 2 YEARS

	Group	Concn. of NE	Initial number	Number surviving	Percentage surviving
Males	I	0	40	20	50.0
	II	100 ppm	40	19	47.5
	III	200 ppm	41	24	58.5
Females	I	0	40	17	42.5
	II	100 ppm	40	17	42.5
	III	200 ppm	39	25	64.1

General observations. During the 2 years of exposure to NE, the rats tolerated the exposures well and did not display pharmacologic or other overt effects of exposure to the nitroparaffin. The number of animals surviving the full 2 years of exposure to NE was approximately the same among the various control and exposed groups, although the largest number (and percentage) of surviving animals was among both male and female rats exposed to NE at 200 ppm. Survival is shown in Table 2.

Body weights. Mean body weights of the animals at 4-week intervals during the study are shown in Table 3. Generally, after exposure was initiated, the mean body weights of exposed groups of rats were less than the mean body weights of the control groups, although the difference was small. At the selected probability (P) value of 0.05, the t test indicated statistical significance throughout the study between Group I (control) and Group II (exposed—100 ppm) males, although, surprisingly, statistical significance between Group I and Group III (exposed—200 ppm) males occurred only during Weeks 6–15 and occasionally thereafter. Among the females, statistically significant differences were observed throughout the entire study between Group I and Group III but only occasionally between Group I and Group II animals. The lack of a well-defined relationship between body weight and exposure concentration of NE, at least among the males, suggests that factors other than exposure to NE may have been involved. Although every attempt was made to duplicate conditions between exposed and control groups, including removal of food during exposure periods, the control animals were not housed in an exposure chamber during the exposure periods. This small difference in treatment of the groups may have influenced the body weights.

Hematology. At the termination of the investigation, blood samples were obtained from selected animals for studies of hematology. These data are summarized in Table 4. At the selected probability level of 0.05, there was no effect of exposure of male or female rats to 100 or 200 ppm of NE on erythrocyte count, packed cell volume, mean corpuscular volume, or hemoglobin. Heterogeneous variances precluded analysis of variance calculations of male leucocyte count data if all values were included, but an inspection of the data showed that one outlying value in Group I caused the heterogeneity. If this value is eliminated, the ANOVA calculation is permitted and no significant difference between any pairs of means is indicated. Similarly, a single outlying value in a Group III animal prevented ANOVA calculation of the female leucocyte count data, but if eliminated, allowed the ANOVA calculation which showed no statistically different means.

TABLE 3

MEANS OF BODY WEIGHTS (GRAMS) OF ALL RATS SURVIVING IN EACH GROUP AT 4-WEEK INTERVALS DURING THE STUDY

Week of study	Males			Females		
	I	II	III	I	II	III
0	191	188	195	164	167	161
4	344	319	330	225	224	217
8	415	384	393	250	249	240
12	464	423	437	265	259	256
16	490	457	467	274	269	259
20	517	490	498	286	283	273
24	537	508	522	294	291	280
31	586	546	553	311	306	291
35	592	559	565	323	310	294
39	600	563	576	326	314	297
43	610	577	587	335	324	306
47	634	592	598	349	336	316
51	660	614	631	367	347	326
55	670	631	648	375	358	330
59	660	614	641	365	344	320
64	692	645	674	393	373	342
68	703	644	669	402	378	347
72	712	644	676	411	389	357
76	708	646	679	412	385	358
80	662	644	664	395	385	355
84	669	606	626	402	375	352
88	688	643	649	414	385	367
92	686	642	650	418	379	374
96	700	654	644	424	390	387
100	703	657	656	424	397	381
104	686	645	653	439	386	382

TABLE 4

HEMATOLOGY OF RATS EXPOSED TO NITROETHANE AT 100 OR 200 ppm FOR 2 YEARS

Group	RBC $\times 10^6$	HCT (%)	MCV (fl)	HGB (g %)	WBC $\times 10^3$
			Males		
I	7.74 ± 0.96	37.4 ± 5.7	48.0 ± 2.6	15.1 ± 1.6	13.1 ± 12.7
II	6.84 ± 1.55	33.1 ± 6.5	49.1 ± 4.7	13.4 ± 2.8	11.1 ± 2.2
III	6.86 ± 1.16	33.3 ± 5.0	48.7 ± 2.6	13.6 ± 2.0	10.3 ± 4.9
			Females		
I	7.54 ± 0.73	37.4 ± 2.4	50.0 ± 3.4	15.3 ± 1.2	6.2 ± 1.3
II	7.58 ± 0.80	37.3 ± 2.7	49.4 ± 2.7	15.2 ± 1.5	6.7 ± 1.3
III	7.52 ± 0.36	36.7 ± 2.1	49.0 ± 1.8	15.2 ± 0.7	8.3 ± 2.8

Note. Values shown are means ± one standard deviation.

TABLE 5

SERUM CHEMISTRY OF RATS EXPOSED TO NITROETHANE AT 100 OR 200 ppm FOR 2 YEARS

Group	SGOT (U/liter)	SGPT (U/liter)	T. bilirubin (mg/dl)	T. protein (g/dl)
		Males		
I	44.9 ± 11.6	18.3 ± 4.7	0.48 ± 0.11	6.45 ± 0.43
II	44.9 ± 9.3	19.9 ± 4.4	0.43 ± 0.12	6.55 ± 0.69
III	49.4 ± 12.8	20.8 ± 5.8	0.43 ± 0.10	6.76 ± 0.81
		Females		
I	50.7 ± 18.8	22.2 ± 7.8	0.66 ± 0.22	7.55 ± 0.50
II	50.6 ± 16.0	23.7 ± 6.9	0.59 ± 0.25	8.01 ± 0.59
III	57.5 ± 38.8	27.0 ± 15.1	0.55 ± 0.26	8.18 ± 0.58
	BUN (mg/dl)	Creatinine (mg/dl)	Sodium (meq/liter)	Potassium (meq/liter)
		Males		
I	16.1 ± 5.0	1.04 ± 0.30	147.7 ± 1.5	5.92 ± 0.79
II	27.6 ± 26.9	1.32 ± 0.93	147.5 ± 2.2	5.74 ± 0.70
III	16.2 ± 2.7	0.89 ± 0.11	147.6 ± 19	5.57 ± 0.48
		Females		
I	14.1 ± 2.1	0.94 ± 0.09	146.5 ± 1.9	5.65 ± 0.70
II	15.1 ± 2.6	0.95 ± 0.13	146.5 ± 2.2	5.74 ± 0.59
III	18.7 ± 4.2	1.07 ± 0.18	145.6 ± 2.1	5.65 ± 0.61

Note. Values shown are means ± one standard deviation.

Serum chemistry. Clinical chemistry determinations on blood samples obtained at the termination of the investigation are shown in Table 5. There was no effect of exposure of male or female rats to either 100 or 200 ppm of NE on glutamic-oxaloacetic transminase, glutamic-pyruvic transaminase, total bilirubin, sodium, or potassium. In the case of SGOT and SGPT calculations of ANOVA among females, a single outlying value was eliminated from Group III. There was no significant difference between means of total protein among male rats, but at $P = 0.05$, there was a slight but significant elevation of total protein among Group III (200 ppm) females compared to Group I (controls). Elevations of blood urea nitrogen of two male rats in Group II (100 ppm) prevented ANOVA calculations, but there was no difference between Group I (controls) and Group III (200 ppm). There was a statistically significant difference in BUN between Group I (control) females and Group III (200 ppm) females, but not between Group I and Group II (100 ppm). Two males in Group II with elevated creatinine levels prevented ANOVA calculation but an examination of the means does not suggest an effect of NE. There was no significant difference between means of creatinine levels among females.

Organ weights. During pathological examination of the animals, major organs were weighed. Mean organ weights expressed as the absolute weight and as a fraction (percentage) of the total body weight are presented in Table 6. An examination of the data summarized in the table does not suggest any biologically significant effect of

TABLE 6

ORGAN WEIGHTS OF RATS SURVIVING TO THE TERMINAL SACRIFICE AND WHICH WERE EXPOSED TO NITROETHANE AT 100 OR 200 ppm FOR 2 YEARS

	Males		Females	
Group	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
		Liver		
I	14.13 ± 2.42	2.16 ± 0.35	11.11 ± 4.50	2.74 ± 101
II	15.01 ± 3.86	2.51 ± 0.68	10.02 ± 1.70	2.84 ± 0.63
III	14.61 ± 2.41	2.38 ± 0.23	9.39 ± 1.30	2.68 ± 0.37
		Kidney		
I	4.54 ± 0.90	0.70 ± 0.13	2.88 ± 0.45	0.72 ± 0.13
II	4.57 ± 0.63	0.77 ± 0.18	2.97 ± 0.32	0.85 ± 0.20
III	4.60 ± 0.90	0.76 ± 0.18	2.83 ± 0.32	0.81 ± 0.13
		Brain		
I	2.35 ± 0.14	0.37 ± 0.06	2.11 ± 0.11	0.53 ± 0.07
II	2.24 ± 0.08	0.38 ± 0.06	2.05 ± 0.14	0.58 ± 0.09
III	2.27 ± 0.14	0.38 ± 0.05	2.07 ± 0.15	0.59 ± 0.07
		Heart		
I	1.82 ± 0.22	0.28 ± 0.03	1.38 ± 0.29	0.34 ± 0.07
II	1.85 ± 0.36	0.32 ± 0.11	1.32 ± 0.13	0.38 ± 0.07
III	1.89 ± 0.36	0.31 ± 0.05	1.42 ± 0.27	0.41 ± 0.11
		Lung		
I	3.36 ± 2.93	0.52 ± 0.08	2.17 ± 0.47	0.54 ± 0.13
II	2.59 ± 0.29	0.43 ± 0.07	2.17 ± 0.27	0.62 ± 0.15
III	2.78 ± 0.36	0.46 ± 0.07	2.20 ± 0.30	0.63 ± 0.11

Note. Values are means ± one standard deviation and are presented as absolute weights and percentage of body weight.

NE on weights of the major organs. Although heterogeneous variances restricted ANOVA calculations of most liver data, there was no evidence of an effect on liver weights, and it can be said with certainty that there was no enlargement or increase in liver weight resulting from inhalation of NE. There was no statistically significant effect on absolute or relative kidney weights of male rats or of absolute kidney weights of female rats. With regard to relative kidney weights of the females, at $P = 0.05$ there was a statistically significant difference between Group I (controls) and Group II (100 ppm exposed) but not between other groups. However, the difference between the two means is very small; there was no correlation with exposure level, and the finding is considered spurious. Among males, absolute brain weights but not relative brain weights displayed a statistically significant difference between Group I (controls) and Group II (100 ppm exposed). Among females there were no significant differences in absolute weights, but between Group I (controls) and both exposed groups, there were statistically significant differences at $P = 0.05$. Since brain weights are very constant within similar groups of animals, the relative brain weight differences among females

may be a reflection of the previously noted differences in total body weight. There were no significant differences in absolute or relative weights of heart or lungs among animals of either sex.

Pathology. At the completion of 2 years of exposure to NE, all surviving rats were sacrificed and a complete necropsy was performed on each. Complete necropsies were also performed on all rats that were either found dead or sacrificed moribund. The tissues were examined microscopically with emphasis placed on the determination of neoplastic changes. A summary of these findings is presented in Table 7.

An examination of the data indicated that the expected incidence of age-related degenerative diseases was seen in approximately equal frequencies in all groups. The incidences of nodular hyperplasia and adenomas of the pituitary gland associated with the endocrine target organ response were similar in all groups.

The total number of tumors in the control group was 46 in males and 46 in females. In treated Group II there were 44 tumors in males and 56 tumors in females, and in treated Group III there were 33 tumors in males and 45 tumors in females. These results indicated that in all treated groups, except Group II females, there were fewer tumors than in the control groups. This increase in tumors in the females of Group II appeared to be related to the increased frequency of pituitary adenoma–endocrine complex related tumors.

In the control rats there were foci of hyperplasia in the liver manifested by compact, small hepatocytes frequently with amphoteric cytoplasm. In other control animals, there were foci of nodular hyperplasia in which increased numbers of hepatocytes, frequently with larger hyperchromatic nuclei and prominent nucleoli, were found. Both of these types of focal lesions were multicentric and microscopic and therefore did not present a gross mass lesion. In the control male rats, there were eight (8) instances of focal hyperplasia and four (4) instances of focal nodular hyperplasia of the liver. In the control female rats there was one (1) instance of focal hyperplasia and one (1) instance of nodular hyperplasia (with gross) of the liver.

In Group II there was one (1) male instance of nodular hyperplasia of the liver and no malignancies.

In Group III there were two (2) male rats with nodular hyperplasia of the liver and one (1) hepatocarcinoma in a female rat.

These data do not indicate any significant hepatic pathologic difference between the control and exposed (NE) rat groups, but they do indicate a normal spontaneous risk of hepatic nodules in aged rats.

DISCUSSION

In this study of the inhalation of NE by rats, the animals were exposed to either 100 or 200 ppm of NE for 7 hr daily, 5 days per week for 2 years. This exposure regimen approximates that encountered in industrial settings, and the 2-year period represents essentially a lifetime of such exposures. Using the published respiratory minute volume of 0.073 liter/min for rats weighing 113 g (Altman and Dittmer, 1964), it can be calculated that the rats were exposed to about 71 or 141 mg/kg of NE daily for the exposure levels of 100 or 200 ppm, respectively. That is not to say that these amounts of NE were absorbed by the animals. The amount absorbed by the animals would also depend on other factors such as the partition coefficient of NE between blood and air and the size of the animals. Nevertheless, these relatively

TABLE 7

INCIDENCE AND DISTRIBUTION OF TUMORS IN RATS EXPOSED TO NITROETHANE

Location and type	Group I		Group II		Group III	
	Males (40)	Females (40)	Males (40)	Females (40)	Males (41)	Females (39)
Skin and subcutis						
Adenoma		1				
Papilloma	2		1	1	1	1
Polyp	1					
Undifferentiated carcinoma				1		
Adnexal carcinoma				1	1	
Fibroma	1				1	1
Angioma	1			1		
Lipoma	1					
Fibromyxoma			1			
Undifferentiated sarcoma		1		2		
Fibrosarcoma			1	1	2	1
Angiosarcoma						1
Metastasis, primary mesenchymal			1		1	
Fibromyxosarcoma						1
Mammary gland						
Adenoma				1		
Fibroadenoma		5	1	3		3
Cystadenoma	2	2		2	1	1
Multiple fibroadenoma			3		1	
Adenocarcinoma		1				
Metastasis, primary mesenchymal			1			1
Spleen						
Undifferentiated sarcoma						1
Leukemia		1				
Metastasis, primary mesenchymal		1	1		1	
Lymph node						
Metastasis, primary epithelial			1	4		
Angioma	1					
Undifferentiated sarcoma						1
Lymphosarcoma			2			
Metastasis, primary mesenchymal		1	1	1	1	
Thymus						
Undifferentiated sarcoma						1
Metastasis, primary mesenchymal		1	1		1	
Bone marrow						
Metastasis, primary epithelial				1		
Undifferentiated sarcoma						1
Leukemia		1	1		1	
Metastasis, primary mesenchymal		1		1		
Muscle						
Metastasis, primary epithelial				1		
Myosarcoma		1				
Metastasis, primary mesenchymal		1	2	1	1	2
Adipose tissue						
Metastasis, primary epithelial				2		
Metastasis, primary mesenchymal			2		1	

TABLE 7—*Continued*

Location and type	Group I		Group II		Group III	
	Males (40)	Females (40)	Males (40)	Females (40)	Males (41)	Females (39)
Kidney						
Adenocarcinoma				1		
Metastasis, primary epithelial				3		
Angioma			1			
Lipoma		1				
Metastasis, primary mesenchymal		2	1	1		
Urinary bladder						
Papilloma			1			
Polyp			1			
Transitional cell carcinoma			1		1	
Metastasis, primary epithelial				2		
Metastasis, primary mesenchymal		1		1		
Pituitary gland						
Adenoma	22	27	16	26	16	23
Adrenal cortex						
Metastasis, primary mesenchymal		1				
Adrenal medulla						
Pheochromocytoma	7	1	3		3	1
Thyroid gland						
Adenoma			1			
Adenoma c cell			2		1	1
Cystadenoma			1		1	
Adenocarcinoma	1		1	1	2	
Metastasis, primary epithelial				1		
Adenocarcinoma c cell			2		1	
Islet						
Adenoma	4		3	2	1	2
Trachea						
Metastasis, primary mesenchymal			1			
Lung						
Metastasis, primary epithelial		1		4		
Metastasis, primary mesenchymal		2	1	1	1	
Heart						
Metastasis, primary epithelial				1		
Aorta						
Metastasis, primary epithelial				1		
Epididymis						
Metastasis, primary mesenchymal			1		1	
Uterus						
Polyp		1				
Adenocarcinoma				1		2
Metastasis, primary epithelial				2		
Metastasis, primary mesenchymal		1				
Leiomyosarcoma				2		
Cervix						
Adenocarcinoma				1		
Metastasis, primary epithelial				1		
Undifferentiated sarcoma				1		
Metastasis, primary mesenchymal		1				

TABLE 7—*Continued*

Location and type	Group I		Group II		Group III	
	Males (40)	Females (40)	Males (40)	Females (40)	Males (41)	Females (39)
Oviduct						
Polyp		1				
Adenocarcinoma		1		1		2
Metastasis, primary epithelial				1		
Leiomyosarcoma				1		
Ovary						
Metastasis, primary epithelial						1
Salivary gland						
Adenoma			1			
Undifferentiated carcinoma				1		
Esophagus						
Metastasis, primary epithelial				1		
Metastasis, primary mesenchymal		1	1			
Stomach						
Metastasis, primary mesenchymal			1			
Small intestine						
Lymphosarcoma			1			
Metastasis, primary mesenchymal			2		1	
Colon						
Myosarcoma			1			
Metastasis, primary mesenchymal			2			
Liver						
Hepatocellular carcinoma						1
Metastasis, primary epithelial				4		
Metastasis, primary mesenchymal		2	3	1	1	1
Bile ductules						
Metastasis, primary mesenchymal		1	1	1		
Pancreas						
Adenocarcinoma				1		
Metastasis, primary epithelial				3		
Metastasis, primary mesenchymal		2	2			
Eye						
Melanoma		1				
Brain						
Astrocytoma	2		1	1		
Metastasis, primary mesenchymal				1	1	
Meninges						
Metastasis, primary mesenchymal		1				
Nerve						
Metastasis, primary mesenchymal			1			
Unidentified						
Undifferentiated sarcoma	1					

high levels of exposure had remarkably little effect on the animals even though the exposures continued for nearly the entire life span of the animals. Mortality was not affected by exposure to NE. There appeared to be an effect (slight reduction) on the body weight of the exposed animals, but lack of a clear dose–response relationship made this finding difficult to interpret.

There were no changes in hematologic patterns in the animals exposed to NE nor were there any clearly defined effects on serum chemistry. Of particular interest was a complete lack of an elevation of the serum transaminases. The transaminases frequently become elevated when chemically induced liver injury occurs.

The lack of a chemically injurious effect of NE on liver tissue was also demonstrated by failure of NE at either exposure level to induce liver enlargement. In fact, the only observed changes in organ weights, especially relative organ weights, may have been related more to lower whole-body weights in exposed groups than to an elevation of the organ weight.

The thorough gross and microscopic pathologic examination of tissues and organs, with care and emphasis placed on examination of the liver, provided further evidence of a lack of effect of NE on the animals. The histopathologic examination disclosed the usual age-associated degenerative diseases and the endocrine target organ response to pituitary hyperplasia, also age-related. Nodular hyperplasia of the liver was rare, but it occurred in all groups.

In reports of prior investigations of the carcinogenicity of nitroparaffins (Griffin *et al.*, 1980, 1981), we have demonstrated that hepatocellular carcinoma ensues only when rats are exposed by inhalation to 2-nitropropane at concentrations of 100 ppm or higher but not at 25 ppm in lifetime exposures. On the other hand, 1-nitropropane fails to induce carcinomas even at concentrations of 100 ppm (Griffin *et al.*, 1982). NE can now be added to the list of nitroparaffins which do not induce hepatocellular carcinomas even at concentrations of 200 ppm. The Nutrition Foundation in the United States recently published (1983) a report of an international expert advisory committee entitled "Relevance of Rodent Liver Hepatoma to Human Carcinogenic Risk." This report pointed out that rat and mouse liver react differently to chemicals regarding the formation of focal histologic changes to hepatocellular carcinoma. They stated that the term "hepatoma" should be used to describe nodular formations and not to call these and other proliferative lesions in the mouse liver cancer or precancerous. These hepatomas rarely, if ever, invade tissues, metastasize, or interfere with the life span of the mouse. Most pathologists have considered them to be benign histologically and called them adenomas or nodules of hypertrophy and hyperplasia.

The argument that only mice and rats and perhaps hamsters should be used in determining carcinogenic potential in a bioassay, because the life span is relatively short and large numbers of animals are available for statistics, is being questioned today. Animals used in carcinogen bioassays should receive the chemical in the manner for which it is intended to be administered and the material should be absorbed, metabolized, excreted or stored, and have a pharmaco- or toxicokinetic pattern similar to man. The sites at which toxicity and tumors occur should be similar to those found in man.

CONCLUSIONS

The following conclusions can be made from this study of inhalation exposure of male and female rats to nitroethane at 100 or 200 ppm for 2 years:

1. There were no pharmacologic effects from exposure to NE at either atmospheric concentration.

2. There was no effect on mortality on either sex at either exposure level.

3. Body weights of both sexes of both groups of exposed rats were slightly less than those of respective controls. Lack of a well-defined dose–response relationship suggested the involvement of factors other than just exposure to NE.

4. There was no effect of exposure of rats of either sex to either level of NE on hematology.

5. Although there were some small differences in certain serum chemistry parameters, and among some individual animals, there were no biologically significant group differences between exposed and control rats of either sex.

6. There were no biologically significant effects of exposure to NE on organ weights.

7. There was no significant difference in the nonneoplastic or neoplastic pathology related to exposure to NE.

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