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# Dose Response Studies of Carcinogenesis in Rats by Nitrosodiethylamine[1]

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## ABSTRACT

A dose-response study was conducted in Fischer rats with nitrosodiethylamine, which was administered in regulated amounts as a solution in drinking water. Groups of 20 female rats each received a different treatment; one group consisted of 12 animals; one of the groups was untreated. The concentrations of the solutions fed ranged from 113 to 0.45 mg/liter at six successive concentrations differing by a factor of 2.5. The treatment times were 17 weeks at the highest concentration; 22 weeks with 45 mg/liter; and 30 weeks with 18, 7, 2.8, 1.1 and 0.45 mg/liter. The two lowest dose levels were also given for 60 weeks, and the 0.45-mg/liter dose was given for 104 weeks. Animals were allowed to die naturally with tumors, and the time to death with tumors was an index of the potency of treatment. In the top four treatment groups, the potency measured in this way was proportional to the total dose of carcinogen administered. At all other doses, survival time was much less dependent on the dose administered, whether or not tumors were induced by the treatment. The principal tumors found were in the upper gastrointestinal tract, mainly the esophagus, at all doses. In the two highest dose groups, there was a high incidence of liver tumors also. There were few liver tumors in the lower dose groups, but there was a dose-related incidence of tumors of the upper gastrointestinal tract. It was remarkable that a nitrosodiethylamine concentration of 0.45 mg/liter (0.45 ppm) administered for 104 weeks induced tumors of the upper gastrointestinal tract in 70% of the treated rats.

## INTRODUCTION

The first extensive dose-response study of a carcinogenic nitrosamine was conducted with NDEA[3] (2) and showed that this compound was a very potent carcinogen in rats, a fact confirmed in many succeeding experiments. That study was the basis for the formulation by Druckrey of an equation relating time to formation of tumor to dose of carcinogen. In that experiment, the nitrosamine was dissolved in water at various concentrations and fed to rats *ad libitum* until death. While this is an acceptable treatment protocol, the built-in variation in dose of the nitrosamine which each animal of a group received, because feeding was *ad libitum* until death, suggested to us that another such study would be useful in which nitrosamine concentration would be constant and in which each animal received a similar total dose. Other dose-response studies have been conducted with nitrosopyrrolidine (10) and nitrosopiperidine (3). Our many previous experiments with nitrosamines have shown that administration of controlled volumes of aqueous solutions as drinking water for defined periods, followed by natural death of the animals from tumors, results in a very uniform response, which it should be possible to quantify. Such an experiment was, therefore, undertaken using a series of concentrations of NDEA differing from one another by a factor of 2.5. At the higher doses, treatment was planned for 30 weeks' (but was foreshortened because of early deaths), whereas at the lower dose rates the treatment time was extended in some groups. The concentrations of NDEA in the solutions varied from 113 to 0.45 mg/liter, and the cumulative dose received by the rats varied from 192 mg in one group to 1.35 mg in another group.

## MATERIALS AND METHODS

NDEA was prepared as described previously (8).

The animals were female Fischer F-344 rats of the colony of the Frederick Cancer Research Center and were maintained in a barrier facility. The animals were 6 to 8 weeks old at the beginning of the treatment. Groups of 20 were assigned to each treatment (except for 1 group of 12) and were housed 4 to a plastic cage with a wire mesh bottom. There were 20 untreated animals as controls. All of the animals were fed Rockland rat diet *ad libitum*. The treatments consisted of giving to each cage of rats 80 ml of a solution of NDEA as drinking water on 5 days of each week, a total of 400 ml/cage/week. On the remaining 2 days of each week, tap water was given *ad libitum*, allowing animals to make up any water deficit that they had incurred. Almost all of the solutions were consumed, permitting fairly precise quantification of average dose per animal.

The treatment solutions were prepared by appropriate dilution with neutral deionized water of stock solutions of NDEA in ethanol. These sterile stock solutions were diluted within the barrier. To avoid confusion, all of the solutions were prepared and diluted individually, rather than diluting one master solution serially.

The concentrations of the solutions varied from 113 to 0.45 mg/liter. The solutions of 113 and 45 mg/liter were administered for, respectively, 17 and 22 weeks, because some animals had died with tumors. The 18-, 7-, 2.8-, 1.1-, and 0.45-mg/liter solutions were all given for 30 weeks. In addition 1.1 mg/liter was given to another group of rats for 60 weeks, and 0.45 mg/liter was given to an additional group for 60 and 104 weeks. These treatments and the total dose received by each group of rats are stated in Table 1 and Chart 1.

At the end of the treatment period, the animals were allowed to die naturally, apart from a few that were killed when moribund or at Week 130 of the experiment. Each animal was completely necropsied, and all lesions and major organs were fixed for histological examination.

### *Statistical Methods*

The survival patterns for the several test groups are compared both graphically and analytically. The graphs of the survival curves are based on the Kaplan-Meier methodology (7) which adjusts for censored observations. The individual survival curves are compared, pairwise, using the "log-rank" statistic of Peto and Peto (9). The analyses were produced with the computer program developed and described by Thomas *et al.* (15).

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[3] The abbreviation used is: NDEA, nitrosodiethylamine.

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Table 1

*Nitrosodiethylamine*

Group treatments were as follows. Group 0: NDEA (0 mg/liter), 0 week treatment, 0 mg total dose per animal, 20 rats. Group 1: NDEA (113 mg/liter), 17 weeks treatment, 192 mg total dose per animal, 20 rats. Group 2: NDEA (45 mg/liter), 22 weeks treatment, 99 mg total dose per animal, 20 rats. Group 3: NDEA (18 mg/liter), 30 weeks treatment, 54 mg total dose per animal, 12 rats. Group 4: NDEA (7 mg/liter), 30 weeks treatment, 21 mg total dose per animal, 20 rats. Group 5: NDEA (2.8 mg/liter), 30 weeks treatment, 8.4 mg total dose per animal, 19 rats. Group 6: NDEA (1.1 mg/liter), 30 weeks treatment, 3.3 mg total dose per animal, 20 rats. Group 7: NDEA (1.1 mg/liter), 60 weeks treatment, 6.6 mg total dose per animal, 20 rats. Group 8: NDEA (0.45 mg/liter), 30 weeks treatment, 1.35 mg total dose per animal, 20 rats. Group 9: NDEA (0.45 mg/liter), 60 weeks treatment, 2.7 mg total dose per animal, 20 rats. Group 10: NDEA (0.45 mg/liter), 104 weeks treatment, 4.7 mg total dose per animal, 20 rats.

| | Group 0 | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Abdomen** | | | | | | | | | | | |
| Neurosarcoma | 1 | | | | | | | | | | |
| **Adrenal** | | | | | | | | | | | |
| Cortical adenoma | 1 | | | | | | | | | | |
| Cortical carcinoma | 3 | | | | | | | 1 | 1 | | 1 |
| Cortical hyperplastic nodule | | | | | | | 1 | 3 | | 1 | 1 |
| Benign pheochromocytoma | 1 | | | | | | | 2 | 1 | 1 | |
| Malignant pheochromocytoma | 2 | | | | | 1 | 2 | | | | |
| **Bone marrow** | | | | | | | | | | | |
| Leukemia, early | | 1 | | | | | | | | | |
| **Cervix** | | | | | | | | | | | |
| Neurosarcoma | | | | | | | | | | | 1 |
| **Clitoral gland** | | | | | | | | | | | |
| Adenoma | | | | | | 1 | | | | | |
| Carcinoma | | | | | | | | | | 1 | |
| Squamous cell carcinoma | 1 | | | | | | | | 1 | | |
| **Esophagus** | | | | | | | | | | | |
| Basal cell carcinoma | | 14 | 15 | 10 | 13 | 18 | 3 | 17 | | 2 | 8 |
| Basal cell papilloma | | 11 | 4 | 5 | 5 | | | 6 | 1 | 2 | 4 |
| Metastatic pulmonary adenocarcinoma | | | | | | 1 | | | | | |
| Papilloma | | | 1 | 1 | 1 | | 1 | | | | |
| Squamous cell carcinoma | | | | 1 | 2 | | | | | | 1 |
| Undifferentiated carcinoma | | | | | 1 | | | | | | |
| **Heart** | | | | | | | | | | | |
| Endocardial sarcoma | | | | | | | 1 | | | | |
| **Intestine** | | | | | | | | | | | |
| Adenocarcinoma | | | | | | | 1 | | | | |
| **Kidney** | | | | | | | | | | | |
| Pelvic papilloma | | | | | | | | 1 | | | |
| Sarcoma | | | | | 1 | | | | | | |
| **Liver** | | | | | | | | | | | |
| Cholangiocarcinoma | | 1 | 1 | | | | | | | | |
| Neurosarcoma | | | | | | | | | 1 | | |
| Hemangioma | | | | | 1 | | | | | | |
| Hemangiosarcoma | | 18 | 2 | | | | | | | | |
| Hepatocellular carcinoma | | 17 | 9 | 1 | 1 | 5 | 5 | 3 | 1 | 6 | 4 |
| Hyperplastic nodule | 1 | 17 | 2 | | | 4 | 4 | 2 | 5 | 8 | 3 |
| Leukemia, early | 3 | 7 | | | | | 1 | 1 | | 3 | 1 |
| Lymphangioma | | | | | | | | | | 1 | |
| Undifferentiated carcinoma | | | 1 | | | | | | | | |
| **Lung** | | | | | | | | | | | |
| Adenocarcinoma | | | | | | 1 | | | | | 1 |
| Hemangiosarcoma (primary) | | | | | | | | 1 | | | |
| Metastatic adrenal carcinoma | | | | | | | | 1 | | | |
| Metastatic esophageal basal cell carcinoma | | | 1 | | | | | | | | |
| Metastatic hepatic hemangiosarcoma | | 12 | | | | | | | | | |
| Metastatic hepatocellular carcinoma | | 2 | 1 | | | | | | | | |
| Metastatic skin neurosarcoma | | | | | | | 1 | | | | |
| Metastatic splenic hemangiosarcoma | | | | | | 1 | | | | | |
| Metastatic uterine stromal cell sarcoma | | | | | | | | | 1 | | |
| **Lymph node** | | | | | | | | | | | |
| Hemangioma | | 1 | 1 | | | | 1 | 1 | | | |
| Leukemia, early | 1 | | | | | | | | | | 1 |
| Metastatic pulmonary adenocarcinoma | | | | | | 1 | | | | | |
| **Mammary gland** | | | | | | | | | | | |
| Adenoma | | | | | | 1 | 1 | | | | |
| Carcinoma | 5 | | | | | 1 | 5 | | 2 | 3 | 1 |
| Fibroadenoma | 7 | | | | | 2 | 5 | 5 | 4 | 4 | 2 |

Table 1—*Continued*

| | Group 0 | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fibroadenoma, focal carcinoma | | | | | | | | 1 | | | |
| Fibroma | 4 | | | | | 1 | 2 | | 4 | 1 | 1 |
| Leiomyoma | | | | | | 1 | | | | | |
| Leiomyosarcoma | | | | | | 1 | | | | | |
| **Multiple organs** | | | | | | | | | | | |
| Leukemia, erythrocytic | 1 | | | | | | | | | | |
| Lymphosarcoma | | | | | | | 1 | | | | |
| Monocytic leukemia | 6 | 1 | | | 1 | 13 | 9 | 4 | 13 | 11 | 11 |
| **Nasal cavity** | | | | | | | | | | | |
| Adenoma | | | | | | 1 | | | | | |
| Basal cell carcinoma | | 1 | | | | 1 | 1 | | | | |
| Papilloma | | | | | | 1 | | | | | |
| **Oropharynx** | | | | | | | | | | | |
| Basal cell carcinoma | | | | | | | | | | 1 | |
| Basal cell papilloma | | | | | | | | | | 1 | |
| **Ovary** | | | | | | | | | | | |
| Carcinoma | | 1 | | | | | | | | | |
| Granulosa cell carcinoma | | | | | | | | | | 1 | |
| Neurofibroma | | | | | | | | | | 1 | |
| Neurosarcoma | | | | | | | | | 1 | | |
| **Pancreas** | | | | | | | | | | | |
| Hyperplastic nodule, acinar | | | 1 | | | | | | 1 | | 1 |
| Islet cell adenoma | 1 | | | | | | 1 | | | 1 | 1 |
| Islet cell carcinoma | | | | | | | 1 | 1 | | | |
| Islet cell neoplasm (autolytic) | | | | | | | | | | 1 | |
| **Pituitary** | | | | | | | | | | | |
| Adenoma | 4 | 1 | 1 | | | | 2 | 3 | 2 | | |
| Carcinoma | 4 | 1 | | | | 2 | 8 | 5 | 10 | 7 | 8 |
| **Rib** | | | | | | | | | | | |
| Osteogenic sarcoma | | | | | 1 | | | | | | |
| **Skin** | | | | | | | | | | | |
| Basal cell carcinoma | 1 | | | | | | 1 | | | | |
| Lipoma | | | | | | | 1 | | | | |
| Squamous cell carcinoma | 1 | | | | | | | | 4 | 2 | 1 |
| Neurosarcoma | | | | | | | 1 | | | | |
| **Spleen** | | | | | | | | | | | |
| Hemangioma | | | | | | | 1 | | | 2 | |
| Hemangiosarcoma | | | | | | 1 | | | 1 | | |
| Leukemia, early | 3 | 7 | | | | | 3 | 1 | | 3 | 1 |
| Monocytic leukemia | | | | | | | | | 1 | | |
| **Stomach, nonglandular** | | | | | | | | | | | |
| Basal cell carcinoma | | 1 | | | | 3 | | | | | |
| Basal cell papilloma | | 2 | 2 | | 1 | 3 | 4 | 2 | 1 | 2 | 5 |
| **Thyroid** | | | | | | | | | | | |
| C-cell adenoma | | | | | | 1 | 2 | 1 | | | 1 |
| C-cell carcinoma | 1 | | | | | 2 | 1 | | | | 1 |
| F-cell adenoma | 1 | | | | | | | | | | |
| F-cell carcinoma | | | | | | | | | 1 | | |
| **Tongue** | | | | | | | | | | | |
| Basal cell carcinoma | | | | | | 6 | 1 | 4 | | 1 | 2 |
| Basal cell papilloma | | | | | | 2 | 2 | 4 | | 1 | |
| Squamous cell carcinoma | | | | | | | | 2 | | | |
| **Uncertain histiogenesis** | | | | | | | | | | | |
| Adenocarcinoma | | | 1 | | | | | | | | |
| **Urinary bladder** | | | | | | | | | | | |
| Transitional cell carcinoma | 1 | | | | | | | | | | 1 |
| **Uterus** | | | | | | | | | | | |
| Adenocarcinoma | | | | | | | 1 | | | | |
| Endometrial polyp | 1 | | | | | 1 | 4 | | 5 | 4 | |
| Endometrial polyp, focal adenocarcinoma | | | | | | | | | | | 1 |
| Stromal cell sarcoma | | | | | | | 1 | | 2 | | |

The survival analyses are based on deaths from all causes as well as deaths from tumors of the upper gastrointestinal tract. (The cause of death was assigned by the pathologist from a review of the gross necropsy and a histological examination of the slides.)

In analyzing deaths from all causes, the only censored observations resulted from those relatively few animals, in several of the lower doses, which survived to the termination of the study at 130 weeks. In analyzing the survival data for deaths from tumors of the upper gastrointestinal tract, censoring also included those animals which died of a competing cause.

Final tumor incidences were analyzed for those dosed groups where survival patterns did not preclude meaningful comparisons, *i.e.*, either no statistical difference between survival curves was detected, or, if a difference was detected, the group with the shortened life span also displayed a higher tumor incidence. In Tables 2 and 3, the numbers of animals with malignant and benign tumors are added to the numbers with benign tumors only. The comparisons of the control group *versus* a single dosed group were made with the one-sided Fisher exact test (5). The tests for positive trend (also one-sided) were based on the logistic model of Cox (1). Departure from linear trend was tested by the $\chi^2$ test; this 2-sided test provides an indication of the adequacy (or inadequacy) of the

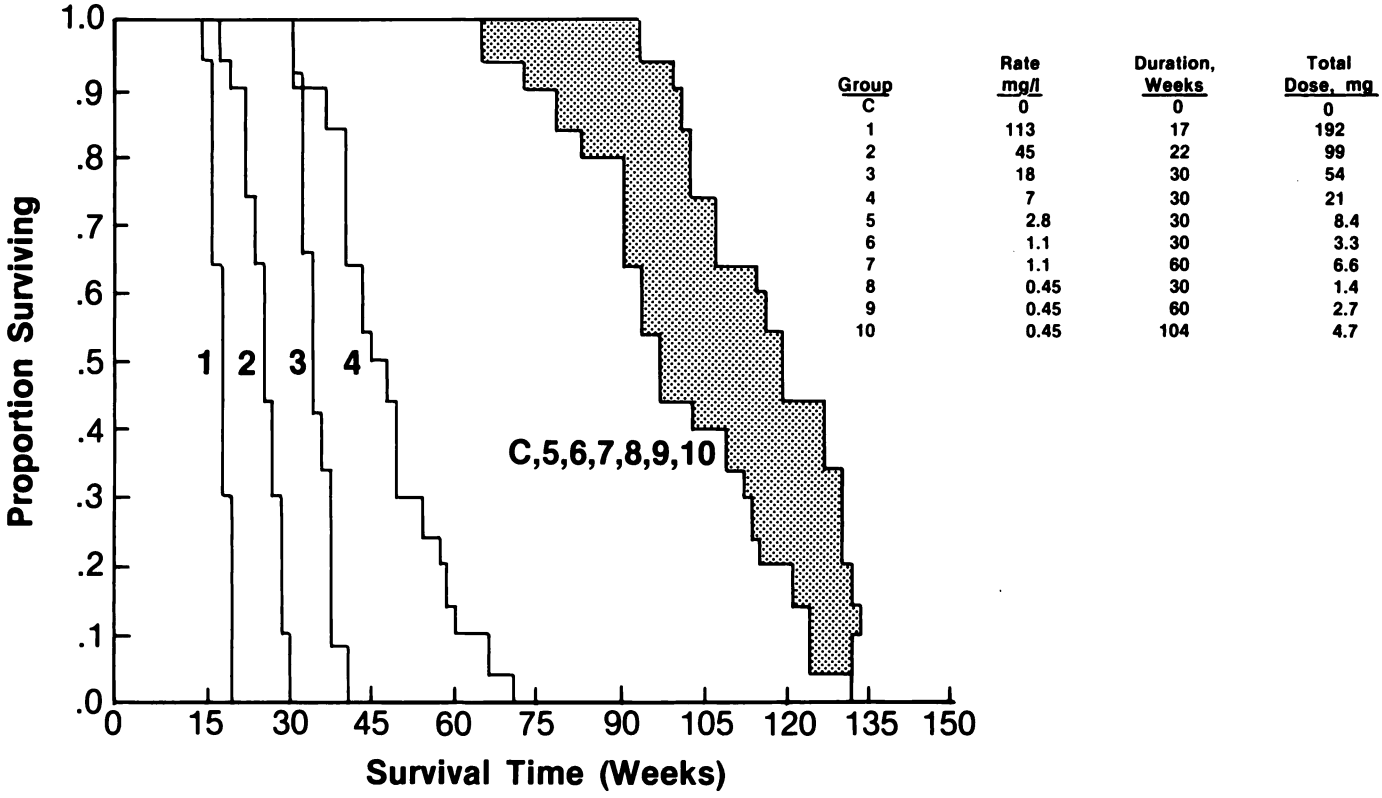


Chart 1. Kaplan-Meier survival curves with respect to death from all causes.

Table 2

*Analyses of the incidence of tumors at specific sites in female rats treated with NDEA for 0, 30, 60, and 104 weeks at a common dose rate of 0.45 mg/liter*

| | | No. of animals with tumors | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Esophagus | | | | | Liver | |
| Duration (wk) | Total dose (mg) | Carcinoma[b] | Carcinoma and/or papilloma[c] | Forestomach papilloma | Tongue, oropharynx carcinoma | Upper gastrointestinal tract[a] (carcinoma or papilloma) | Carcinoma | Carcinoma or hyperplastic nodule |
| 0 | 0 | 0/20 (0)[d] | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 1/20 (5) |
| 30 | 1.35 | 0/20 (0) | 1/20 (5) | 1/20 (5) | 0/20 (0) | 2/20 (10) | 1/20 (5) | 6/20 (30) |
| | | NS[e] | NS | NS | NS | NS | NS | $p = 0.046$ |
| 60 | 2.70 | 2/20 (10) | 3/20 (15) | 2/20 (10) | 2/20 (10) | 6/20 (30) | 6/20 (30) | 11/20 (55) |
| | | NS | NS | NS | NS | $p = 0.010$ | $p = 0.010$ | $p = 0.00062$ |
| 104 | 4.68 | 9/20 (45) | 13/20 (65) | 5/20 (25) | 2/20 (10) | 14/20 (70) | 4/20 (20) | 7/20 (35) |
| | | $p = 0.00061$ | $p = 0.00001$ | $p = 0.024$ | NS | $p \leq 0.00001$ | NS | $p = 0.022$ |
| Test for positive trend | | $p = 0.38 \times 10^{-5}$ | $p = 0.26 \times 10^{-7}$ | $p = 0.0044$ | NS | $p = 0.27 \times 10^{-7}$ | $p = 0.010$ | $p = 0.012$ |

[a] Animals with at least one tumor (carcinoma or papilloma) of the esophagus, forestomach, tongue, or oropharynx.
[b] Animals with a carcinoma only or a carcinoma and papillomas.
[c] Animals included in preceding column plus those with only papillomas.
[d] Numbers in parentheses, percentage.
[e] The probability level for the Fisher exact test for the comparison of the control group with a treated group is given beneath the incidence of tumors in that treated group when $p < 0.05$; otherwise, not significant (NS) is indicated.

Table 3

*Analyses of the incidence of tumors at specific sites in female rats treated with NDEA at 4 dose rates for 30 weeks*

| Dose rate (mg/liter) | No. of animals with tumors | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Esophagus | | Forestomach | | Tongue | | | Liver | | |
| | Carcinoma[b] | Carcinoma and/or papilloma[c] | Carcinoma[b] | Carcinoma and/or papilloma[c] | Carcinoma[b] | Carcinoma and/or papilloma[c] | Upper gastrointestinal tract[a] (carcinoma or papilloma) | Carcinoma | Carcinoma plus hyperplastic nodule |
| 0 | 0/20 (0)[d] | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 0/20 (0) | 1/20 (5) |
| 0.45 | 0/20 (0) | 1/20 (5) | 0/20 (0) | 1/20 (5) | 0/20 (0) | 0/20 (0) | 2/20 (10) | 1/20 (5) | 6/20 (30) |
| | NS[e] | NS | NS | NS | NS | NS | NS | NS | $p = 0.046$ |
| 1.1 | 3/20 (15) | 4/20 (20) | 0/20 (0) | 4/20 (20) | 1/20 (5) | 3/20 (15) | 11/20 (55) | 5/20 (25) | 8/20 (40) |
| | NS | NS | NS | NS | NS | NS | $p = 0.00007$ | $p = 0.024$ | $p = 0.0098$ |
| 2.8 | 18/19 (95) | 18/19 (95) | 3/19 (16) | 6/19 (32) | 6/19 (32) | 7/19 (37) | 18/19 (95) | 5/19 (26) | 8/19 (42) |
| | $p < 0.00001$ | $p < 0.00001$ | NS | $p = 0.0083$ | $p = 0.0083$ | $p = 0.0033$ | $p < 0.00001$ | $p = 0.020$ | $p = 0.0076$ |
| Test for positive trend | $p = 0.31 \times 10^{-14}$ | $p = 0.48 \times 10^{-13}$ | $p = 0.012$ | $p = 0.0019$ | $p = 0.00020$ | $p = 0.00011$ | $p = 0.33 \times 10^{-12}$ | $p = 0.0074$ | $p = 0.016$ |

[a] Animals with at least one tumor (carcinoma or papilloma) of the esophagus, forestomach, or tongue.
[b] Animals with a carcinoma only or a carcinoma and papillomas.
[c] Animals included in preceding column plus those with only papillomas.
[d] Numbers in parentheses, percentage.
[e] The probability level for the Fisher exact test for the comparison of the control group with a treated group is given beneath the incidence of tumors in that treated group when $p \leq 0.05$; otherwise, not significant (NS) is indicated.

hypothesized linear dose-response model. The trend tests are described by Thomas *et al.* (15).

## RESULTS

The survival patterns of the treated rats are shown in Chart 1. The rate of mortality of the rats in the 4 highest dose groups was dose related, and virtually all of these animals died with tumors of the upper gastrointestinal tract and/or liver tumors, the latter being common in Groups 1 and 2. As shown in Chart 2, plotting the total dose against the median survival of the rats in Groups 1 to 4 gave a straight line semilogarithmically. Pairwise comparisons of the survival distributions indicated that Groups 1, 2, 3, and 4 were significantly ($p < 0.0001$) different from each other and from each of the remaining test groups. Among the remaining test groups (Chart 1, *shaded area*), the differences among mortality patterns were, for the most part, not statistically significant, with the exceptions noted as follows. Group 5 animals died sooner than did those in Group C (controls), 6, and 8, and Group 10 animals died sooner than those in Group 8; $0.02 < p < 0.05$ (2-tailed) for these 4 comparisons.

All neoplasms observed are listed by treatment group in Table 1. The esophagus and liver were the organs where most of the compound-related tumors were observed. For much of the analysis which follows, tumors of the forestomach, tongue, and oropharynx were combined with those of the esophagus and discussed as tumors of the upper gastrointestinal tract. Chart 3 shows the Kaplan-Meier survival curves with respect to deaths caused directly or indirectly by tumors of the upper gastrointestinal tract. The incomplete survival curves of treatment Groups 1 and 2 indicate that many of the deaths in these groups were attributed to causes other than tumors of the upper gastrointestinal tract, most notably tumors of the liver. Pairwise comparisons of the survival distributions for Groups 2, 3, and 4 indicated that they are significantly ($p < 0.0001$) different from each other and from each of the other groups. In Group 1, so few of the deaths were attributed to tumors of the




Chart 2. Dose response of NDEA in rats. Median time of death of rats with tumors following administration of NDEA at different dose rates leading to various cumulative doses.

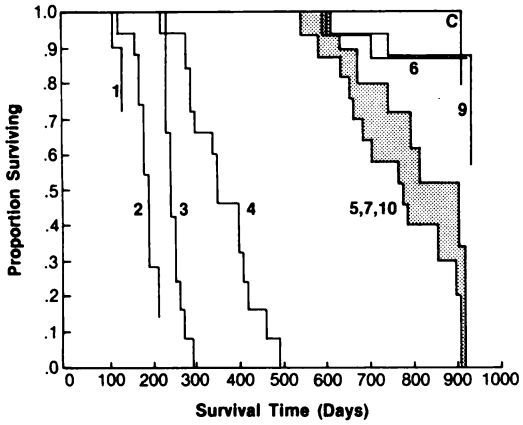


Chart 3. Kaplan-Meier survival curves with respect to death by tumor of the upper gastrointestinal tract.

upper gastrointestinal tract that all comparisons of this group with other groups fall short of statistical significance. The survival patterns for Groups 5, 7, and 10 lie within the *shaded area*; they were not statistically different from each other. None of the animals in Group 8 died from tumors of the upper gastrointestinal tract. Animals in Groups C, 6, and 9 generally died from causes other than tumors of the upper gastrointestinal tract, although some few tumors were observed in these organs.

Table 2 gives tumor incidences for organs of the upper gastrointestinal tract and the liver for those treatment groups given 0.45 mg/liter for 30, 60, and 104 weeks. For every organ-tumor combination except carcinomas of the tongue and oropharynx, the test for a positive dose-related trend was highly significant. Note that an incidence of 1 in 20 appears in the 30-week group for several organ-tumor combinations. Normally, an incidence this low would evoke little interest, but when reviewed as part of a highly significant trend, the possibility that it may be compound related cannot be overlooked. Table 3 contains the analysis of tumor incidences in rats treated for 30 weeks at rates of 0.45, 1.1, and 2.8 mg/liter. Here also, the tumor incidences in the various organ-tumor combinations are strongly dose related, with the tests for positive trend showing a high degree of statistical significance.

Among the liver tumors, hemangioendothelial sarcomas were common only among rats receiving the highest concentration of NDEA. At the lower concentrations, those liver tumors that appeared were all hepatocellular carcinomas.

### *Pathological Findings*

**Neoplasms of Esophagus.** Carcinomas and papillomas of the esophagus were mainly basal cell with areas of squamous cells and with formation of keratin. The degree of differentiation was related to the dose of chemical. The carcinomas at the highest doses generally were poorly differentiated and invasive, and those at the lower doses were well differentiated. There also were more carcinomas per rat at the higher doses. Rats with carcinomas generally also had papillomas and hyperplasia. Rats without neoplasms had basal cell hyperplasia.

The histopathology of preneoplastic and neoplastic lesions of the esophagus in rats ingesting diethylnitrosamine has been described and illustrated previously (12).

Carcinomas of the esophagus were large, invasive, and multiple. Some rats with large invasive carcinomas of the esophagus developed obstruction of the esophagus, which decreased food consumption, which led to emaciation. Many rats with carcinomas or papillomas of the esophagus often died from abscesses in the lung, secondary to aspiration of keratin and/or squamous metaplasia of the bronchi and bronchioles. There was marked formation of keratin in the esophagus.

**Neoplasms of Tongue.** Carcinomas of the tongue were well- and poorly differentiated basal and squamous cell carcinomas, which were invasive with focal abscess formation. The histology of these neoplasms was similar to the neoplasms of the esophagus.

Large invasive carcinomas of the tongue contributed to or caused the death of rats.

**Neoplasms of Forestomach.** Neoplasms of the forestomach were mainly papillomas with some carcinomas. They did not contribute to the death of the rats.

**Neoplasms of Liver.** Rats given the highest doses of NDEA developed both hepatocellular carcinomas and hemangiosarcomas, and they were multiple. They also were larger in rats on the high doses than in those on the lower doses. The cause of death of the rats with hemangiosarcomas was rupture of the sarcoma with massive bleeding into the peritoneum. Twelve rats in Group 1 had metastatic hemangiosarcoma to the lungs; 2 other rats had metastatic hepatocellular carcinomas. One rat in Group 2 had metastatic hepatocellular carcinoma to the lung.

The hepatocellular carcinomas included both well- and poorly differentiated neoplasms in rats on the high doses and well-differentiated neoplasms in those on the lower doses. Rats with hepatocellular carcinomas also developed hyperplastic nodules and severe diffuse atypical hyperplasia. There also were hyperplastic nodules in some rats on the lower doses that did not have carcinomas.

The histopathology of carcinomas and sarcomas of the liver in rats has been described and illustrated (13). Hyperplastic nodules, considered as precursors of carcinomas, have been described in rats given *N*-2-fluorenyldiacetamide (11).

**Neoplasms in Other Organs.** Rats in Group 8 developed stromal cell sarcomas of the uterus, one with lung metastases. (These neoplasms generally are rare in untreated animals and do not metastasize.) One rat in Group 7 had a large adrenocortical carcinoma with lung metastases.

Rats given the lower doses also often developed monocytic leukemia or carcinomas of the pituitary. The monocytic leukemia involved multiple organs and usually caused death of the animals. The carcinomas of the pituitary were large enough to result in death of the animals.

## DISCUSSION

There was a good correlation between the biological behavior of the neoplasms of the liver and esophagus and the dose of NDEA. Carcinomas were larger, invasive, and poorly differentiated, as well as multiple, in rats given the higher doses. Carcinomas of the esophagus and sarcomas of the liver resulted in the death of the rats.

While it is possible to be reasonably certain that the tumors that were found in both treated and untreated animals [and the latter group compared well with a large survey of tumors appearing in untreated F-344 rats (6)] were not related to the

treatment, those tumors which were found only rarely in the treated groups might or might not be related to the nitrosamine treatment. Only much larger experiments can answer this question.

The trends in the statistical analysis of these results show that the treatment with NDEA led to either an elevated incidence of upper gastrointestinal tract neoplasms and liver tumors, or to a reduced survival, or both. Even 0.45 mg/liter given for only 30 weeks seems to be effective, as shown by the trends in Table 2. The total dose received by each animal in this group was only 1.35 mg, suggesting that in rats NDEA is a carcinogen of very great potency. These doses are lower than those used by Druckrey *et al.*, and the response of our Fischer rats seems to be somewhat greater than his BDIX rats.

Two features of the results deserve mention: (*a*) at the lowest doses of NDEA, tumors of the upper gastrointestinal tract were frequent, showing that these organs of the Fischer rat are extremely sensitive to the carcinogenic action of very small amounts of this carcinogen; (*b*) at the low doses at which survival of treated and control animals was similar, there is no indication of an increase in incidence of any of the apparently "spontaneous" tumors, suggesting that the process by which these tumors arise is unaffected by NDEA, even though it exerts a strong carcinogenic effect in other organs and tissues. Table 3 shows the increased incidence of upper gastrointestinal tract tumors when higher doses of NDEA were given for 30 weeks only. In the rats given 0.45 ppm NDEA for 104 weeks (Table 2), 14 of the 20 animals (70%) were found to have tumors of the upper gastrointestinal tract. It is reasonable to suggest that concentrations of nitrosamine considerably below this might pose a risk to humans if continued for a large part of a human lifetime. Concentrations of nitrosodimethylamine ranging up to 50 ppb have been found in beer (14), and similar concentrations of nitrosopyrrolidine have been found in fried bacon (4). There have been other reports of nitrosamines at the several ppb level in many sources of environmental contact with humans.

## REFERENCES


1. Cox, D. R. The regression analysis of binary sequences (with discussion). J. Roy. Stat. Soc., *20:* 215–242, 1958.
2. Druckrey, H., Schildbach, A., Schmahl, D., Preussmann, R., and Ivankovic, S. Quantitative Analyse der carcinogenen Wirkung von Diathylnitrosamine. Arzneimittel-Forsch., *13:* 841–846, 1963.
3. Eisenbrand, G., Habs, M., Schmähl, D., and Preussmann, R. Carcinogenicity of *N*-nitroso-3-hydroxypyrrolidine and dose-response study with *N*-nitrosopiperidine in rats. *In: N*-Nitroso Compounds: Analysis, Formation and Occurrence. IARC Scientific Publication No. 31, pp 657–663. Lyon, France: International Agency for Research on Cancer, 1980.
4. Fazio, T., White, R. H., Dusold, L. R., and Howard, J. W. Nitrosopyrrolidine in cooked bacon. J. Assoc. Off. Anal. Chem., *56:* 919–921, 1973.
5. Fisher, R. A. Statistical Methods for Research Workers, Ed. 10, pp. 96–97. New York; G. E. Stechert & Co., 1946.
6. Goodman, D. G., Ward, J. M., Squire, R. A., Chu, K. C., and Linhart, M. S. Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol., *48:* 237–248, 1979.
7. Kaplan, E. L., and Meier, P. Non-parametric estimation from incomplete observations. J. Am. Stat. Assoc., *53:* 457–481, 1958.
8. Lijinsky, W., and Taylor, H. W. Relative carcinogenic effectiveness of derivatives of nitrosodiethylamine in rats. Cancer Res., *38:* 2391–2394, 1978.
9. Peto, R., and Peto, J. Asymptotically efficient rank invariant test procedures (with discussion). J. Roy. Stat. Soc. Ser. A, *135:* 185–206, 1972.
10. Preussmann, R., Schmähl, D., and Eisenbrand, G. Carcinogenicity of *N*-Nitrosopyrrolidine: dose-response study in rats. Z. Krebsforsch., *90:* 161–166, 1977.
11. Reuber, M. D. Development of preneoplastic and neoplastic lesions of the liver in male rats ingesting .025 percent *N*,2-fluorenyldiacetamide. J. Natl. Cancer Inst., *34:* 697–724, 1965.
12. Reuber, M. D. Histopathology of preneoplastic and neoplastic lesions of the esophagus in rats ingesting diethylnitrosamine. J. Natl. Cancer Inst., *58:* 313–321, 1977.
13. Reuber, M. D., and Lee, C. W. Effect of age and sex on hepatic lesions in Buffalo strain rats ingesting diethylnitrosamine. J. Natl. Cancer Inst., *41:* 1133–1140, 1968.
14. Spiegelhalder, B., Eisenbrand, G., and Preussmann, R. Contamination of beer with trace quantities of *N*-nitrosodimethylamine. Food Cosmet. Toxicol., *17:* 29–31, 1979.
15. Thomas, D. G., Breslow, N., and Gart, J. J. Trend and homogeneity analyses of proportions and life table data. Computers Biomed. Res., *10:* 373–381, 1977.