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Dose and Time Relationships for Tumor Induction in the Liver and Esophagus of 4080 Inbred Rats by Chronic Ingestion of *N*-Nitrosodiethylamine or *N*-Nitrosodimethylamine[1]

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Abstract

A Weibull analysis is presented of the dose and time relationships for the effects on 4080 inbred rats of chronic ingestion in the drinking water of 16 different doses of *N*-nitrosodimethylamine (NDMA) or *N*-nitrosodiethylamine (NDEA). The sites chiefly affected were the liver (by both agents) and the esophagus (by NDEA only). Since the experiment continued on into extreme old age, effects became measurable at doses of only 0.01 to 0.02 mg/kg/day, which is an order of magnitude lower than previously achieved. (After only 2 years of treatment, however, the TD_{50} doses needed to halve the proportion of tumorless survivors would have been about 0.06 mg/kg/day of NDEA, or about 0.12 mg/kg/day of NDMA.) The general pattern of response was that the natural logarithm of the probability of remaining tumorless was given by the product of two terms, the first (the "Weibull *b* value") depending on the dose rate but not on the duration of exposure and the second depending not on dose at all but only on duration.

For all types of tumor the dependence on duration was fairly similar (and for each the second term was taken to be $-t^7$, where t = years of treatment), but for different types of tumor the dependence on dose rate was quite different. For esophageal tumors, the "Weibull *b* value" was approximately proportional to the cube of the dose rate of NDEA (males 21 d^3, females 11 d^3, where d = dose rate in mg/kg adult body weight/day), and the background incidence was unmeasurably low. For liver tumors induced by NDEA, the *b* value was approximately proportional to the fourth power of dose rate + 0.04 mg/kg/day [males, 19 $(d + 0.04)^4$; females, 32 $(d + 0.04)^4$], although the relationships were somewhat different for the different cell types of liver tumor. This one formula implies both approximate linearity at low doses and an approximately cubic relationship within the higher range of doses that was studied. For liver tumors induced by NDMA, the Weibull *b* value was approximately proportional to the sixth power of dose rate + 0.1 mg/kg/day [males, 37 $(d + 0.1)^6$; females, 51 $(d + 0.1)^6$], again with some variation between liver cell types, and again implying approximate linearity at low doses.

These algebraic formulae should, of course, be trusted only in the range of doses where they were derived, and particularly not above it. If that for NDMA is extrapolated to lower doses, however, it suggests that the tumor risks from 2 years of chronic exposure of such rats to very low dose rates of this agent would, in the absence of other causes of death, be on the order of 0.03% (males) or 0.04% (females) per μg per kg per day. Similar extrapolation using the formula for NDEA suggests that at very low dose levels the esophageal cancer risk would become much less important than the liver tumor risk and that the latter might be about 0.06% (males) or 0.1% (females)/μg/kg/day. (This is compatible with the observation that, at those moderately low dose levels where its effects are still directly measurable, NDEA appears to be about 2 or 3 times as potent as NDMA.)

Note that, among animals allowed to live out their natural life span (some of whom would die before completing 2 years of treatment but some of whom would survive substantially longer, and therefore suffer much higher tumor onset risks), the absolute risks produced by continuous treatment from 6 weeks of age onwards might be about 7 times as large as the 2-year risks, *i.e.*, averaging the two sexes, about 0.24% for each μg/kg NDMA and about 0.58% for each μg/kg NDEA. No direct estimate is obtainable from such data, of course, of the net effects of these agents on humans.

Introduction

In an unusually large dose-response experiment on the carcinogenic effects of NDEA[3] and of NDMA, 16 concentrations of these substances were administered chronically in the drinking water of 4080 inbred Colworth rats (Table 1). As expected, both agents produced large numbers of tumors of the liver; in addition NDEA produced large numbers of esophageal tumors. Experimental details have been reported separately, along with a crude description of the dose-response relationships for tumors of the liver and esophagus and tests of the statistical significance of any apparent effects on sites other than the liver and esophagus. The chief aim of the present paper is to provide a more precise characterization of the main (*i.e.*, liver and esophageal) dose-response relationships. This is of interest for two reasons.

First, there continues to be a considerable degree of interest in the extent to which the dose-response relationship at high dose levels can be used to predict the effects of much lower doses [for review, see Armitage (2)], and the present study provides an example of some real data spanning both an unusually wide range of high dose levels (from which various theoretical predictions can be constructed) and an unusually wide range of low dose levels (from which the accuracy of these predictions can be checked).

Second, there continues to be a considerable degree of interest in the mechanisms of carcinogenesis by chronic ingestion of low levels of nitrosamines, and if the relationship of nitrosamine carcinogenesis to dose and time in one inbred species could be characterized then this might constrain current speculations in various ways or draw attention to unexplained anomalies.

Structure of the Present Report

The experimental methods and basic results have been presented in a parallel report (1); therefore only a few details of the experimental methods will be recapitulated here. However, the statistical methods that are to be used (involving double Weibull distributions) may be unfamiliar to many readers, and so the ideas underlying these will be introduced and discussed at considerable length, in an effort to make most nonstatistical (and even some antistatistical) readers familiar enough with them to be comfortable with their meaning and to appreciate their advantages.

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[1] This experiment was commissioned by the Ministry of Agriculture, Fisheries and Food in consultation with the Department of Health and was executed at BIBRA and analyzed at Oxford.

[2] Retired.

[3] The abbreviations used are: NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; CI, cumulative incidence; ABWD, adult body weight per day; TD_{50}, that daily dose rate required to halve the proportion of tumorless survivors after 2 years of treatment; CL, confidence limits.

Table 1 *Initial distribution of 4080 weanling rats among groups (for further details, see Ref. 1, Table 1)*

Group (1–16)	Nitrosamine concentration (ppm v/v in water)	Av. group size			
		Male		Female	
		NDEA	NDMA	NDEA	NDMA
1	Control	240		240	
2–7	Low doses (0.033–1.056) in 2-fold steps	60	60	60	60
8–16	High doses (1.6–16.9) in smaller steps	60	60	60	60
Total		2040		2040	

A detailed understanding of exactly how the computer fits such models to experimental data is not needed (and thus is not given). However, a proper understanding of what such models offer can greatly clarify the interpretation of dose-response data that span both a high-dose range (where most animals develop tumors, and treatment chiefly affects the median time to tumor rather than the proportion of affected animals) and a low-dose range (where treatment chiefly affects the proportion of animals that develop tumors but has little effect on the distribution of ages at which first tumors arise). Indeed, without appropriate statistical methods an overall understanding of such dose-responses relationships is notoriously difficult to achieve. After the extended statistical introduction to double Weibull distributions, the main results will be presented and discussed in the usual way.

Selected Details of the Experimental Methods

Interim Sacrifices. One-tenth of the original animals were scheduled for sacrifice after 12 months and one-tenth after 18 months of treatment, but the remaining eight-tenths were scheduled to live their normal life span, with no terminal sacrifice. As a result, the experiment involved just over 3 years of treatment for some animals.

Context of Observation (Fatal/Incidental). At routine postmortem, all animals were examined for the presence of gross tumors, with special attention to the liver and esophagus. Where possible, liver tumors were then subdivided histologically as to cell type of origin (see below). For animals with tumors of any particular site (or cell type), an attempt was made to determine whether such tumors had been observed in a "fatal" context, *i.e.*, had contributed directly or indirectly to the death of the host, or in an "incidental" context.[4] Doubtful cases were classified as "probably fatal" or "probably incidental." The total proportion that fell into these two indeterminate classes was, however, only about 6% (3). Thus, separate analysis of them was not necessary, and the "fatal" category was extended to include "probably fatal," while the "incidental" category was extended to include "probably incidental." In the few livers or esophaguses that were autolyzed, cannibalized, or otherwise lost to histology, there is no record of the presence or absence of incidental tumors, but in some such cases observations by the animal house technicians could be used to indicate whether or not the animal was likely to have died *of* a neoplasm at one of those sites. Where such judgments were available they were accepted (and the lesions assumed to have been malignant, which would probably have been correct in most such instances).

Histology. All tumors from which sections were examined were classified simply as "benign" or "malignant," and if an animal had more than one tumor of some site (or, for the liver, cell type; see below), only the most malignant one was utilized. Subcategorization of the grade of malignancy was attempted, but in the view of the histologist (P. G.) this subcategorization was not consistent or reliable; therefore, no use has been made of it. The cell of origin of the liver tumors has been further subdivided into "liver cell," "bile duct," "mesenchymal" (*i.e.*, blood vessels), "Kupffer," and "not known" (due to autolysis, cannibalism, or loss of tissues). Analyses have been undertaken for the above four specific cell types and for "any liver."

Survival. Age-specific death rates from the aggregate of all causes other than tumors of the liver and esophagus were not significantly related to treatment. Consequently, in the first 8 dose levels most animals survived well into old age (median, 31 months of treatment for males and 28 months for females, *i.e.*, approximately 33 and 30 months of age), while in the top 8 dose levels most animals died of tumors.

Units of Time. These are measured (generally in years) from the start of chronic treatment at age 6 weeks, not from birth.

Units of Dosage. These are constant in the unusual units of ppm (v/v) in the water, but for uniformity with other reports they will be described in (approximate) mg/kg/day *adult* body weight, calculated on the approximation that adult males and females consumed about 41 and 72 ml/kg/day adult body weight, respectively. Although water and food were available *ad libitum*, water intake and body weight were not materially affected by treatment.

Descriptive Methods for Incidental and Fatal Tumors: Double Weibull Distributions

The response of a group of animals to a carcinogen is complex: some die of the tumor type of interest at one age, some die of it at another age, some die of unrelated causes at one age with the tumor type of interest as an "incidental" finding, some do so at another age, some die tumorless at one age, some do so at another age, and so on. For several years (4, 5), there have existed reasonably satisfactory ways of summarizing the age-specific death rates *from* the tumor type of interest by utilizing "Weibull" distributions (see below) with "shape" independent of dose but with "constant of proportionality" dependent on dose. These relate A, the probability that an animal would still be alive if tumors of the type of interest were the only cause of death, to t, the time in years since treatment began, by a formula such as

$$\log A \text{ decreases in proportion to } t^7 \qquad \text{(A)}$$

(N.B.: Since A must start off with the value 1, $\log A$ must start off with the value zero, and then as A becomes smaller $\log A$ must become negative.) The exponent 7 in the above equation is called the "shape" parameter, and as a rule it does not differ materially from one group to another (a value of 7 being one that fits the present data reasonably well; see "Appendix"). The constant of proportionality, by contrast, depends strongly on

[4] As discussed in the IARC report (3) and in our earlier report of these data (1), such contexts of observation are required not to determine the biological nature of the lesions, *i.e.*, chiefly sought histologically, of course, but merely to determine which denominator to relate them to statistically. For any reasonably short age range, the appropriate denominator for the *fatal* tumors found in it is the number of animals *still alive* and thus at risk of death from tumors. Conversely, the appropriate denominator for the *incidental* tumors found in it is the number of *deaths* from unrelated causes that bring animals to postmortem and thereby enable incidental tumors to be discovered.

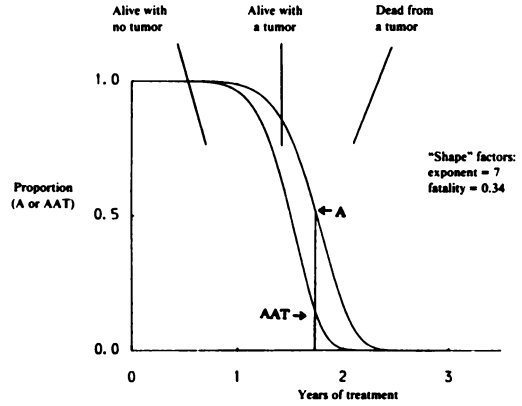


Fig. 1. Example of a "double Weibull" distribution, with $\log AAT = -0.3\ (t/1.5)^7$ and $\log A = -0.34 \times 0.3\ (t/1.5)^7$. The proportion of tumorless animals among the survivors at 21 months (see *vertical line*) is AAT/A and in general the log of the proportion of the survivors that are tumorless is $-0.66 \times 0.3\ (t/1.5)^7$, which is itself a Weibull distribution.

the dose rate, and it would be small for a low-dose group and large for a high-dose group. This type of Weibull formula can easily be adapted to describe purely incidental tumors, simply by defining AAT to be the probability that an animal would still be alive and tumorless (AAT) if tumors of the type of interest were the only cause of death, and then letting $\log AAT$ decrease in proportion to t^7, as in the formula[5]

$$\log AAT = -0.3\ (t/\text{med})^7 \qquad \text{(B)}$$

where "med" denotes the median time to the development of a tumor that would be detectable postmortem. One way of combining Equations (A) and (B) to provide a description of the pattern of both incidental and fatal tumors is to note that as AAT, the proportion *alive and tumorless*, decreases from its initial value of 1, then A, the proportion *alive*, must lag behind it. Consequently, we may write

$$\log A = f \cdot \log AAT$$

where the constant of proportionality, f, will be referred to as a "fatality factor," since it would be zero for a type of tumor that was never fatal, unity for a type that was instantly fatal, small (*e.g.*, 0.1) for a type that is unlikely and/or slow to cause death, and large (*e.g.*, 0.8) for a type that is likely to prove rapidly fatal. It is shown in a statistical appendix that a reasonably adequate fit to the present data on liver and esophageal tumors may be achieved by assuming that f is another "shape" parameter that does not depend on the dose level. (As noted above, however, f does depend on the tumor type being analyzed.)

Combination of Equations (A) and (B) leads to the form of the Weibull distribution that will be used to help summarize the present data:

$$\log AAT = -0.3\ (t/\text{med})^7 \text{ and } \log A = -f \times 0.3(t/\text{med})^7 \qquad \text{(C)}$$

where "med," the Weibull median, depends on the nitrosamine dose rate, but the "shape" (*i.e.*, the exponent 7 and the fatality factor f) does not. An example (with fatality factor f equal to 0.34) of such a "double Weibull" distribution is illustrated in Fig. 1.

[5] Note that the factor 0.3 is appropriate when "common" (*i.e.*, base 10) logarithms are used, for $\log (0.5) = -0.3$. If instead "natural" (*i.e.*, base e) logarithms were used, Equation B would be $\log_e AAT = -0.7\ (t/\text{med})^7$.

Alternative Ways of Presenting Weibull Distributions. By choosing many quite different values for "med," Weibull distributions can predict either a high, early tumor yield, a high, late tumor yield, a moderate, late tumor yield, or a low, late tumor yield. This is illustrated in Fig. 2 for the hypothetical case of a tumor that is so rapidly fatal that it is virtually never seen as an incidental finding (*i.e.*, a tumor for which the fatality factor f equals 1). It shows what ordinary Kaplan-Meier survival curves (3) for death from such tumors would yield in a large 10-group experiment on animals with a life span of about 3 years where the Weibull medians were 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 years in groups 1 (high) to 10 (low), respectively.

Note that where the Weibull median is *less* than the normal life span (as in groups 1 to 5) most animals will develop the tumor type of interest, and the changes in the Weibull median will lead chiefly to changes in the times of tumor onset rather than to changes in the proportions of affected animals. Conversely, where the Weibull medians exceed the normal life span there will be large differences between the proportions of affected animals in different groups but there will be no substantial difference in the distribution of death times for those animals that actually do develop tumors. (Note also that the Weibull median for a particular group is in general *not* equal to the median of the *actual* tumor onset times in that group, unless nearly all the animals die of the tumor type of interest.)

Traditionally, the results of experiments in the high-dose range have often been described in terms of some measure of median-time-to-tumor, while those of experiments in the low-dose range have often been described in terms of some measure of percentage-of-affected-animals. The advantage of using Weibull distributions is that one single quantity (the Weibull median) can be used to describe the results of experiments in either dose range, or (as in the present study) in a wide dose range embracing both high and low doses. There are, however, two equivalent ways of writing the same Weibull distribution, the format of one of which emphasizes the dependence of the Weibull median on dose at high doses and the format of the other of which emphasizes the dependence of the proportion of affected animals on dose at low doses.

The first format is, as already discussed,

$$\log AAT = -0.3(t/\text{med})^7$$

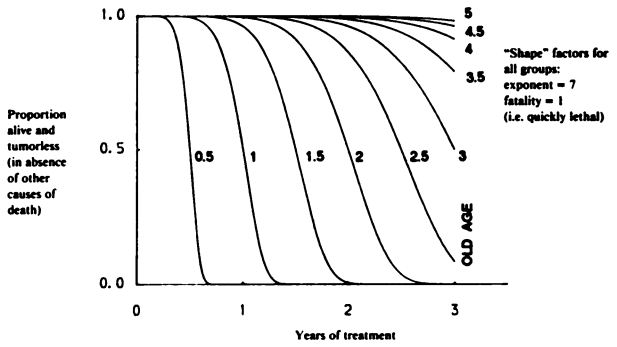


Fig. 2. Example of the dose-response relationship predicted by Weibull distributions for a hypothetical 10-group experiment in animals with a 3-year life span, where groups 1 to 5 (the high-dose groups) have Weibull medians of med = 0.5, 1, 1.5, 2, and 2.5 years, while groups 6 to 10 (the low-dose groups) have Weibull medians of med = 3, 3.5, 4, 4.5, and 5 years. In the high-dose groups, treatment affects the mean time to disease onset much more than it affects the proportion of affected animals. In the low-dose groups, however, the opposite is true, and treatment has a strong effect on the number of affected animals but no appreciable effect whatever on the mean (or median) time to tumor onset of those animals that actually develop tumors.

The second, entirely equivalent, format first introduces the concept of the cumulative incidence and then characterizes its relationship to dose. The *cumulative incidence* up to a certain time is obtained by dividing the prior life span of the animals into several short periods, noting for each period the probability that an animal that has no tumor at the start of it will develop a tumor during it, and then adding up these separate probabilities. Note that at low dose rates the cumulative incidence approximately equals the probability of prior tumor onset in the absence of other causes of death; *i.e.*, CI is approximately equal to $1 - AAT$. (The exact relationship can be shown to be $CI = -2.3 \log AAT$, but when the cumulative incidence is small this approximately equals $1 - AAT$.)

The second format for the Weibull distributions is thus

$$CI = -2.3 \log AAT = 0.69 \cdot t^7/\text{med}^7 = b \cdot t^7$$

where b is a dose-dependent constant of proportionality related to the Weibull median by the equation

$$b = 0.69/\text{med}^7 \quad \text{(D)}$$

At low doses, the formulation

$$CI = b \cdot t^7 \quad \text{(E)}$$

may be more attractive, while at high doses the formulation

$$\log AAT = -0.3\ (t/\text{med})^7 \quad \text{(F)}$$

may be preferred. Either, however, can be used throughout either dose range, or in a wide range of high and low doses, for they are statistically equivalent, and Equation D shows that if "med" is known then b can be calculated, and *vice versa*.

In general it may be easier to have some immediate feel for what a Weibull median means than for what a Weibull b value means (*e.g.*, medians of 1, 2, and 3 years correspond to b values of 0.69, 0.0054, and 0.00032!), and for this reason most of the descriptions of dose-response relationships that follow will involve medians. Moreover, no simple statistical distribution will exactly fit any data, and the medians are meaningful, and fairly robustly estimated, even if Weibull distributions are slightly inappropriate, or if the "shape" parameters are slightly wrong.

However, in discussing the nature of the dose-response relationship at low doses, the b values in the low-dosed groups are roughly proportional to the number of tumors that arise and thus provide a straightforward basis for extrapolation. (For example, at *low* doses the percentage of animals that would be expected, in the absence of other causes of death, to be affected after 705 days of treatment can be shown to be approximately 10,000 b.) Moreover, the b values may be somewhat more directly related to the underlying cellular processes of carcinogenesis than are the Weibull medians.[6] Consequently, some of the key relationships will for convenience be described both ways round.

Advantages of Using "Double Weibull" Distributions. The fact that only the Weibull median depends on treatment simplifies the complex problem of describing the ways in which differences in dose between one group and another affect the pattern of times at which animals die from or with the tumor type of interest, a problem that may be made still more complex by the prior deaths of some animals from other dose-related conditions. This can be reduced to the far simpler problem of describing how the dose in each group relates to the Weibull median in each group, perhaps by a simple plot of dose *versus* "med," for once "med" is known the Weibull formula completely specifies the distribution of tumor times.

Although these Weibull formulae may at first sight appear somewhat removed from reality, they can thus provide a remarkably economical summary of a large mass of experimental data. Moreover, the summary that they provide is likely (6) to be more directly related to the rates at which the actual cellular processes of carcinogenesis operate than are conventional summary statistics such as "percentage of tumor-bearing animals," "mean (or median) latency of the observed tumors," etc.

Computational Methods

The preceding introduction to Weibull distributions (and double Weibull distributions) was somewhat lengthy because readers without much feel for these distributions may gain little or nothing from the analyses that will follow. By contrast, many readers may pass over the present section on how such distributions were fitted to the actual data and move on directly to "Results."

Briefly, the method of maximum likelihood has been used throughout, both to select the dose-independent "shape" parameters and to fit the dose-dependent parameters (*i.e.*, the Weibull medians). In analyzing the effects of one agent (*e.g.*, NDEA) on one particular type of tumor (*e.g.*, malignant esophageal tumors) the following steps were taken. First, the 2 sexes and 16 dose levels of the test agent were treated as 32 different groups, to which a common exponent, a common fatality factor, and 32 different Weibull medians were to be fitted. Next, a likelihood[7] was written down for each animal in terms of the exponent k, the fatality factor f, and the median of that animal's group. Logarithms of these separate likelihoods were taken and all added together to give an overall log-likelihood. The values of k, f, and the 32 medians that maximized this joint log-likelihood were sought. For most types of tumor these "maximum likelihood" values for k were in the range of about 5 to 9, being generally about 6 for most types of NDMA-induced liver tumors and for NDEA-induced esophageal tumors, but about 8 for most types of NDEA-induced liver tumors. By fixing the

[6] If the time taken by a neoplasm to develop from its unicellular origin to a detectable size is negligible, then under simple multistage assumptions (6) the Weibull b values will be proportional to the product of all the rate constants for all the stages, and of some term(s) describing the extent to which partially altered cells have a selective advantage over their unaltered neighbors. This is a complex product, but at least it is a product of real things.

If, however, as is probably the case, the development of a neoplasm typically takes more than a few per cent of the life span, then perhaps the data will be better described by some more general Weibull distribution in which the cumulative incidence is proportional to some other power (less than 7) of t minus some fixed delay (*e.g.*, 0.25 year). Unfortunately, the appropriate delay cannot be predicted reliably and is surprisingly difficult to estimate reliably from the data since powers of (t − delay) may be uncomfortably close to being proportional to powers of t (5). (For example, human lung cancer death rates are approximately proportional both to the fourth power of age − 20 and to the seventh power of age.) Thus, the reason why rodent cumulative incidence rates appear to be proportional to (time)^7 may be because they are really proportional, with biologically meaningful constants of proportionality, to some lower power of, for example, (time − 0.25 year). If so, the b values relating cumulative incidence to (time)^7 may be less directly related to the underlying processes of carcinogenesis than simple multistage models would predict.

[7] For animals dying of such tumors at age t (*i.e.*, with such tumors observed in a "fatal" or "probably fatal" context) the contribution to the likelihood was equal to the downward slope of the graph of A against t at that time. For other animals (including those undergoing scheduled sacrifice), the likelihood was either A (if no postmortem was done), or AAT (if it was done and no such lesion emerged), or A-AAT otherwise (*i.e.*, if it was done and such a lesion was found in an "incidental" or "probably incidental" context).

Table 2 *Weibull medians for a combined analysis of benign and malignant esophageal neoplasms (using k = 7 and f = 0.065 for benign neoplasms and k = 7 and f = 0.46 for malignancies; for details, see Fig. 3)*

Treatment group	Male			Female		
	Estimated dose (mg/day/adult kg)	No. of rats with tumors	Weibull median (yr)	Estimated dose (mg/day/adult kg)	No. of rats with tumors	Weibull median (yr)
1	0	0		0	0	
2	0.001	0		0.002	0	
3	0.003	0		0.004	0	
4	0.005	0		0.009	0	
5	0.010	0		0.018	0	
6	0.020	3	3.77	0.036	3	3.25
7	0.041	16	2.69	0.072	19	2.24
8	0.061	32	2.08	0.107	21	1.98
9	0.082	37	1.68	0.143	32	1.58
10	0.102	45	1.62	0.179	29	1.46
11	0.122	48	1.48	0.215	42	1.17
12	0.163	41	1.45	0.287	37	1.10
13	0.204	49	1.14	0.358	44	1.01
14	0.245	46	1.06	0.430	41	0.89
15	0.326	47	0.96	0.573	36	0.81
16	0.653	46	0.71	1.146	26	0.66
Totals		410			330	

value of k arbitrarily first at 6 and then at 8 and in each case finding the best-fitting values for the common fatality factor and the 32 Weibull medians, it was discovered that the value of k had little influence on the values of f or of the medians. For simplicity, we therefore arbitrarily chose to impose a common exponent of $k = 7$ on all analyses and to select the value of f and the values of the medians that maximized the joint log-likelihood when the exponent was exactly 7.

Tumors of the bile ducts were almost all benign and were therefore analyzed as a homogeneous whole, irrespective of their degree of malignancy. For other sites, however, where there were substantial numbers of each type of neoplasm, two separate analyses (with two separate fatality factors) were undertaken, one for the benign and one for the malignant tumors, and for each different treatment group the results were then combined by direct addition of the two corresponding b values, as illustrated in Table 2 and Fig. 3. Consequently, for the analysis of "all esophageal tumors, malignant or benign" two separate fatality factors were required, one for malignant and one for benign neoplasms.

The resulting Weibull b values, or, equivalently, Weibull medians, are then tabulated and plotted in various ways (see "Results") against the dose rate. In interpreting such plots and tabulations it may be helpful to remember that, in the lower-dosed groups, the Weibull parameter b in some particular group is approximately proportional to N, the number of animals with such tumors in that group (and that in all groups the standard error of b is approximately equal to b divided by the square root of N).

Results

Esophageal Tumors

Results will be presented first for the esophagus and then for the liver. The esophageal analyses are easier to present because: (*a*) the spontaneous background rate of esophageal tumors is so low (no controls developed esophageal tumors, while 7 developed liver tumors) that the question of how to allow for the background does not arise; (*b*) whereas the liver tumors represent four anatomically distinct categories of neoplasm (liver cell, bile duct, mesenchyme, and Kupffer) the dose-response relationships of which must be examined separately, no such subcategorization of the esophageal neoplasms exists; and finally (*c*) whereas NDMA and NDEA both affect the liver, only NDEA affects the esophagus, so only one dose-response relationship, that for NDEA, need be examined for esophageal tumors.

Recapitulation of Weibull Definitions. For one particular type of tumor, in the absence of other causes of death and other tumors, the probability (AAT) of being alive and tumorless after t years of treatment would be given by

$$\log AAT = -0.3\ t^7/\text{med}^7 \qquad \text{(G)}$$

where "log" denotes the common, *i.e.*, base 10, logarithm and "med" is the group-specific Weibull median. Alternatively, if b, the Weibull constant of proportionality, is defined by the relationship

$$b = 0.69/\text{med}^7 \qquad \text{(H)}$$

then an equivalent way of writing Equation G can be shown to be

$$\text{CI} = b \cdot t^7 \qquad \text{(I)}$$

where "CI" denotes the cumulative incidence after t years of treatment. The need is therefore to describe how "med," or, equivalently, b, depends on treatment, *i.e.*, on the daily dose rate of nitrosamine (which will be written "mg/kg ABWD" to denote "estimated mg/kg adult body weight/day").

Effects of NDEA on Esophageal Tumors. The Weibull medians for esophageal tumors are plotted and tabulated against the NDEA dose rates (mg/kg ABWD) in Fig. 3*C* and in Table 2. The plotted medians in Fig. 3*C* define a reasonably straight line of slope approximately $-1/2.3$. It is not clear whether the line is exactly straight, and it is not clear exactly what its slope is; indeed, any slope from $-1/2.0$ to $-1/2.3$ would fit reasonably well, and the only reason for selecting $-1/2.33$ in preference to some adjacent value is for uniformity with the work of Druckrey (7).

The relationship suggested by the straight lines with slope $-1/2.33$ in Fig. 3 is that

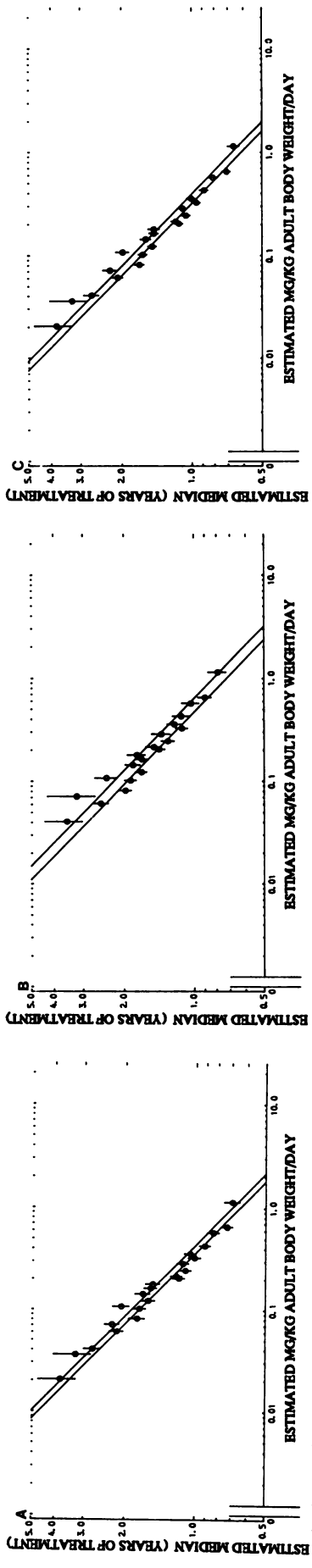


Fig. 3. Combined analyses. Combination of the analysis of benign esophageal tumors (*A*) with that of malignancies (*B*) yields an overall analysis of all esophageal neoplasms in NDEA-treated rats (*C*). ●, males; ○, females; *vertical lines*, approximate 95% confidence limits. Combination is by direct addition of the corresponding *b* values, a procedure that can be shown always to produce a combined median that is less than 10% more extreme than the more extreme of the two medians that are being combined. Thus, for example, in the group of females that received over 1 mg/kg/day, the Weibull median for benign tumors was about 0.7 years (*A*: this corresponds to a *b* value of about $0.69/0.7^7$, or 8.4), the Weibull median for malignant tumors was about 0.8 years, (*B*: this corresponds to a Weibull median of about $0.69/0.8^7$, or 3.3), and addition of the two corresponding *b* values yielded a total of about 11.7 (corresponding to a Weibull median of about $(0.69/11.7)^{1/7}$ years, *i.e.*, about 0.66 year, as in *C*). *A*, benign esophageal tumors (analyzed with $f = 0.065$). The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males (M) and females (F) of 0.072 and 0.086 mg/kg ABWD. NDEA: site, esophagus; $k = 7.00$, $f = 0.065$. Both sexes, benign only. TD_{50}: M, 0.07203, $b = 14.488$ (dose + 0.00)$^{3.0}$; F, 0.08608, $b = 8.491$ (dose + 0.00)$^{3.0}$. *B*, malignant esophageal tumors (analyzed with $f = 0.46$). The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.093 and 0.127 mg/kg ABWD. NDEA: site, esophagus; $k = 7.00$, $f = 0.460$. Both sexes, malignant only. TD_{50}: M, 0.09324, $b = 6.681$ (dose + 0.00)$^{3.0}$; F = 0.12665, $b = 2.666$ (dose + 0.00)$^{3.0}$. *C*, combined analysis of benign and malignant esophageal tumors, derived via addition of the *b* values corresponding to the Weibull medians in *A* and *B*. The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.063 and 0.079 mg/kg ABWD. NDEA: site, esophagus; $k = 7.00$, $f = 0.460$, 0.065. Both sexes, malignant and benign. TD_{50}: M, 0.06348, $b = 21.169$ (dose + 0.00)$^{3.0}$; F, 0.07859, $b = 11.157$ (dose + 0.00)$^{3.0}$.

$$(\text{mg/kg ABWD}) \cdot (\text{median})^{2.33} = \text{constant} \qquad \text{(J)}$$

and the fact that no clear departures from linearity are seen in Fig. 3 suggests that this relationship holds reasonably well at least throughout the range 0.02 to 1.0 mg/kg/day. Use of Equations H, I, and J yields an alternative way of presenting the same relationship in terms of the CI, namely,

$$\text{CI is approximately proportional to } (\text{mg/kg ABWD})^{7/2.33} \cdot t^7$$
$$\textit{i.e.}, \text{ to } (\text{dose-rate})^3 \cdot t^7 \qquad \text{(K)}$$

throughout the dose range 0.02 to 1 mg/kg ABWD. Thus, in the dose range where measurable effects are seen, every 2-fold reduction in the dose rate is likely to produce about an 8-fold reduction in the cumulative incidence.

Extrapolation to Low Doses. If such a dose-response relationship, or something similar to it, could be extrapolated downwards to dose levels so low that most animals do not develop tumors from the treatment, then in this low dose range the proportion expected to do so will likewise be reduced about 8-fold by every halving of the dose rate. This might lead to a natural explanation for the apparent "threshold" seen in these data at about 0.01 mg/kg ABWD [35 esophageal tumor-bearing animals in group 7, with 6 in group 6, and 0 in group 5 and in each lower-dosed group (Table 2)], because the dose rates are halved 5 successive times between groups 7 and 2. So, if 30–40 tumors would be expected in group 7, then 4 or 5 might be in group 6, 0.5 might be in group 5, and under 0.1 might be in groups 4, 3, and 2.

However, the fact that a particular mathematical relationship (Equation K) approximately matches the observed tumor yield in the dose range where measurably large effects are seen is *not* strong evidence that it must do so in any substantially lower dose range. For example, if the cumulative incidence were actually proportional to, say, $(\text{mg/kg} + 0.01)^4$ then this could provide virtually as good a fit in the dose range 0.02 to 1.0 as $(\text{mg/kg})^3$ does, yet would predict quite different behavior at low doses, with an excess risk that was approximately proportional to the dose rate at low doses (so that in the very low dose range a halving of the dose rate would only halve this risk). Conversely (but less plausibly), if the cumulative incidence were actually zero up to 0.005 mg/kg/day and proportional to some power of (mg/kg − 0.005) thereafter, then low doses (below 0.005 mg/kg ABWD) would be wholly without risk.

The problem of extrapolating dose-response relationships to very low doses has been discussed by many authors [*e.g.*, Crump *et al.* (8); Guess *et al.* (9); Peto (10); for review, see Armitage (2)]. The general conclusion is that the degree of curvature is unlikely to be greater in the very low dose range than it is at higher doses and that it may well be less, so that no matter what shape the dose response may appear to be at high doses, the excess risk may be approximately proportional to the effective dose at very low doses.

Applied to the present data, where among females the cumulative incidence at 3 years produced by a dose rate of 0.01 mg/kg is about 1%, these considerations suggest that the cumulative incidence at 3 years produced among females by even lower doses might be about as high as 1 in 1000 for every μg/kg ABWD, while conversely it might be about as low as is suggested by simple extrapolation of the lines in Fig. 3*C*, *i.e.*, approximately 10^{-5} times the cube of the dose rate in μg/kg ABWD. The implications of these opposite extremes are set out in Table 3.

Table 3 *Comparison of two alternative formulae for extrapolation to very low doses (below 0.01 mg/kg ABWD) of the dose-response relationship for the effects of NDEA on esophageal tumors among males*

N.B.: The effects among females would be about half as large.

Dose rate (μg/kg adult body wt/day)	Predicted cumulative incidence at 2 yr (%)	
	A. From linear extrapolation: 0.01% for every μg/kg ABWD	B. From cubic extrapolation: 10^{-6} cube of μg/kg ABWD
10	0.1	0.1
1	0.01	0.0001
0.1	0.001	0.0000001

Summary of Effects on Esophageal Tumors. Although there is, of course, some uncertainty as to the effects of dose rates less than 0.01 mg/kg ABWD, since they are so small that they cannot be measured reliably, a reasonably simple characterization of the effects of treatments in the dose range 0.02 to 1 mg/kg ABWD has been achieved, namely,

$$\text{Females: } \log_{10} AAT = -4.8\, d^3\, t^7$$

$$\text{Males: } \log_{10} AAT = -9.2\, d^3\, t^7$$

where d denotes the dose rate, in units of mg/kg adult body weight/day, t denotes the duration, in years, of treatment, $\log_{10}$ denotes common logarithms, and AAT denotes the proportion that would be alive and tumorless in the absence of other tumors or other causes of death.

Liver Tumors

These are somewhat less simple to describe than were the results from esophageal tumors, because there is an appreciable spontaneous liver tumor onset rate. This means that the data cannot be adequately described by any unmodified formula of the type

Weibull constant of proportionality, b, is proportional to some power of dose.

It may, however, be possible to describe the b values adequately by a modified formula, such as "value among controls *plus* a term proportional to some power of dose" or "some power of the sum of the effective background dose and the applied dose." The data for the two agents (first NDEA, then NDMA) will be presented separately.

Effects of NDEA on Various Types of Liver Tumor. Fig. 4 and Table 4 present the effects of NDEA treatment on the Weibull medians separately for each separate subtype of tumor. In theory, there are obvious advantages in looking separately at separate types of liver tumor, for in principle at least their shapes might be quite different. Indeed, there is direct evidence that their shapes are at least slightly different, for in dose levels 1 to 6, 66% (57 of 87) of liver tumors arose from parenchymal liver cells, while at dose levels 7 to 11 and 12 to 16, 88% (286 of 325) and 94% (405 of 430) did so. In practice, however, there are two disadvantages in separate examination of Figs. 4*A*, 4*B*, 4*C*, and 4*D*. First, whereas Fig. 4*A* is based on some 748 affected animals, Figs. 4*B*, 4*C*, and 4*D* are respectively based on 40, 42, and 13 affected animals. This is hardly an adequate basis for a 32-group dose-response relationship, and as a result the shapes of Figs. 4*B*, 4*C*, and 4*D* (but not Fig. 4*A*) are somewhat biased by the inevitable exclusion from particular graphs of those treatment groups that have no animals with the tumor type of interest. Second, whereas it is usually practicable to decide whether or not death was caused by liver tumors, it may be more difficult to decide which particular type of liver tumor caused death, especially at dose levels so high that the liver is likely to contain several tumors, yet the separate shapes of Figs. 4*A*, 4*B*, 4*C*, and 4*D* depend on this distinction. Consequently, despite the fact that the proportions of different types of tumor change as we go from low to high dose, there is also some interest in a graph relating NDEA dose rate to total liver tumor onset rates, irrespective of subsite of origin within the liver. This is given in Table 5 and Fig. 5 (and has the additional advantage of including the few animals who were believed to have died of a liver tumor but for whom no subsite of origin within the liver was recorded, due to loss of tissues).

Inevitably, since the control incidence is not zero the graph of log median against log mg/kg ABWD must (no matter what shape the dose-response relationship may be at low dose levels) flatten out at low doses, and this can be seen in Fig. 5. This non-zero background makes it a little difficult to provide a simple characterization of the entire dose-response relationship. Two alternative approaches are possible, and these are illustrated in Fig. 6. On the one hand one may relate the effect to some power of dose-plus-effective-background, as in Fig. 6*A*, while on the other one may relate the *excess*[8] effect to dose, as in Fig. 6*B*. These both provide a reasonable fit to the actual data, although they suggest very different effects at very low dose levels (Table 6). This again illustrates that reliable prediction of carcinogenic effects at very low doses is rarely possible, especially when, as here, the reliably measured effects at higher dose levels are not simply proportional to the dose rate.

Effects of NDMA on Various Types of Liver Tumor. Fig. 7 and Table 7 present the effects of NDMA treatment on the Weibull medians separately for each separate subtype of liver tumor. Unlike NDEA, where there were appreciable numbers for only one subtype (liver cell tumors), here there are appreciable numbers both for liver cell tumors and for bile duct tumors; indeed, for females the bile duct tumors considerably outnumber the liver cell tumors (Table 7). Moreover, the shapes of the dose-response curves for liver cell tumors and bile duct tumors appear rather different, especially for females (among whom bile duct tumors outnumber liver cell tumors in the range 0.05 to 0.5 mg/kg ABWD but not outside it). Despite this, for the reasons discussed above in relation to NDEA, the dose-response relationship for "all liver" is of some interest and is given in Table 8 and Fig. 8. The relationship is clearly nonlinear, but if it is replotted against dose rate +0.1 mg/kg ABWD, then a reasonably straight line does emerge (Fig. 9). This line is, however, probably more of a descriptive convenience than a source of any biological insight, inasmuch as even if any irregularities introduced by the mesenchymal and Kupffer tumors are ignored, the dose-response relationship for liver tumors is still the sum of two quite different dose-response relationships (those for liver cell and for bile duct tumors), both of which require separate consideration.

Comparison of Hepatocellular Dose-Response Curves for NDEA and NDMA (Figs. 4*A* and 7*A*). Except for the existence of a measurable background risk at zero dose, for NDEA the

[8] The excess in Fig. 6*B* is estimated by subtracting the Weibull b value for the controls of appropriate sex from that for each treated group. This yields, however, values that are statistically unstable for the lower-dosed groups (where the excess is not statistically significant). Thus, for example, the points for the pool of the 4 lowest treatment levels do not fall significantly below the line in Fig. 6*B*.

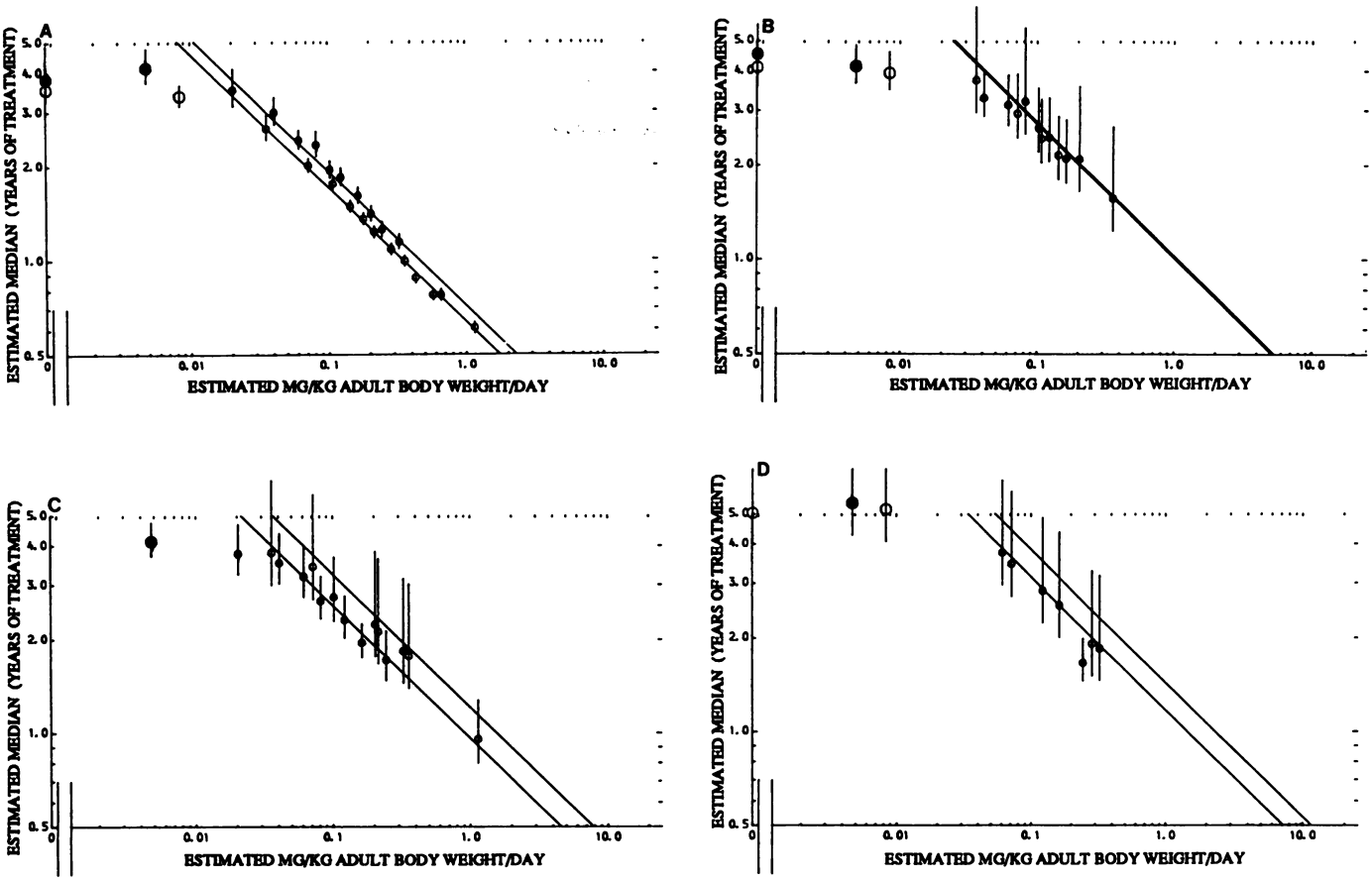


Fig. 4. Effects of NDEA on tumor induction in various different parts of the liver. *A*, liver cell; *B*, bile duct; *C*, mesenchyme; and *D*, Kupffer cell, with data for groups 2 to 5 pooled for statistical stability. ●, males (M); ○, females (F). *Small circles*, groups of about 60 animals each; *large circles*, groups of 240 animals. Lines of slope −1/2.3 are plotted for comparison. For the three less common types of tumor (*B*, *C*, and *D*), several groups have no such tumors and thus are not plotted. Hence, the plotted points are a biased sample, and a line through them will tend to overestimate the effects of treatment. Despite this, it is noteworthy that in the range 0.02 to 1.0 mg/kg ABWD the four graphs have strikingly similar slopes. *A*, NDEA, liver cell tumors, calculated with $f = 0.63$ (malignant) and 0.09 (benign). The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.091 and 0.069 mg/kg ABWD. NDEA: site, liver cell; $k = 7.00$, $f = 0.626$, 0.088. Both sexes, malignant and benign. TD_{50}: M, 0.09064; $b = 7.273$ (dose + 0.00)$^{3.0}$; F, 0.06886, $b = 16.584$ (dose + 0.00)$^{3.0}$. *B*, NDEA, bile duct tumors, calculated with $f = 0.11$ irrespective of malignancy. The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.20 and 0.21 mg/kg ABWD. NDEA: site, bile duct; $k = 7.00$; $f = 0.112$. Both sexes, any neoplasm. TD_{50}: M, 0.20495; $b = 0.629$ (dose + 0.00)$^{3.0}$; F, 0.21239; $b = 0.565$ (dose + 0.00)$^{3.0}$. *C*, NDEA, mesenchymal tumors, calculated with $f = 0.60$, irrespective of malignancy. The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.181 and 0.310 mg/kg ABWD. NDEA: site, mesenchyme; $k = 7.00$; $f = 0.595$. Both sexes, any neoplasm. TD_{50}: M, 0.18183, $b = 0.901$ (dose + 0.00)$^{3.0}$; F, 0.31048, $b = 0.181$ (dose + 0.00)$^{3.0}$. *D*, NDEA, Kupffer cell tumors, calculated with $f = 0.65$, irrespective of malignancy. The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males and females of 0.29 and 0.46 mg/kg ABWD. NDEA: site, Kupffer cells; $k = 7.00$, $f = 0.653$. Both sexes, any neoplasm. TD_{50}: M, 0.28599; $b = 0.232$ (dose + 0.00)$^{3.0}$; F, 0.45787; $b = 0.056$ (dose + 0.00)$^{3.0}$. *Bars*, 95% CL.

shape of the dose-response relationship for hepatocellular tumors (Fig. 4*A*) appears fairly similar to that for esophageal tumors (Fig. 3*C*), albeit perhaps with some slight downward curvature. Still, throughout the dose range above 0.02 mg/kg ABWD, both these graphs do have a fairly simple structure defining an approximately straight line of slope approximately −1/2.33. When we examine the dose-response relationship for the effects of NDMA on hepatocellular tumorigenesis (Fig. 7*A*), however, this simplicity of shape is no longer seen. Instead of the straight line seen for NDEA in the dose range above 0.02 mg/kg ABWD, a "shoulder" is seen for NDMA, involving a slope which, below 0.1 mg/kg ABWD, is shallower than above it.

The Weibull medians that are plotted in Figs. 4*A* and 7*A* provide a reasonably satisfactory visual format for describing the effects of high dose rates (above 0.1 mg/kg ABWD), but they provide a much less satisfactory visual format for describing the effects of the lower doses. As already noted, however, there exists an alternative format that may be preferred for describing the dose-response relationship at low doses, namely, use of Weibull "*b* values" rather than Weibull medians. Logically the two are equivalent[9] but at low dose levels the "*b* value" is approximately proportional to the number of affected animals, while the "median" is not. Table 9 therefore gives the *b* values for hepatocellular tumors, and Fig. 10 plots the first few of these *b* values against dose using an ordinary, nonlogarithmic scale to see whether the risks appear to be proportional to dose at low dose rates.

Examination of Fig. 10, coupled with comparison of Fig. 4*A* with Fig. 7*A*, indicates that although at dose rates of around 1 mg/kg ABWD NDEA and NDMA have similar effects, at dose rates of around 0.1 mg/kg ABWD the effects of NDMA are substantially less than those of NDEA. Indeed, the effects of 0.1 mg/kg ABWD of NDMA appear to be similar to those of barely half as much NDEA. Thus, on a *molar* basis, in this central dose range NDMA (with a molecular weight of 74.1) appears to be only about one-third as potent as NDEA (with a

[9] Given one, the other can readily be calculated from the relationship $b = 0.69/\text{median}$[7].

Table 4 *Relationship between NDEA dose rate and Weibull medians for the various subtypes of liver tumor (for details, see Fig. 4)*

Treatment group	Males								Females							
	NDEA dose rate[a]	A. Liver cell		B. Bile duct		C. Mesenchyme		D. Kupffer cell		NDEA dose rate[a]	A. Liver cell		B. Bile duct		C. Mesenchyme		D. Kupffer cell	
		N[b]	M	N	M	N	M	N	M		N	M	N	M	N	M	N	M
1	Zero	10[c]	3.82	3[c]	4.54	0[c]		0[c]		Zero	11[c]	3.53	4[c]	4.12	0[c]		1[c]	5.04
2	0.001	1	4.28	2	3.88	1	4.29	0		0.002	4	3.34	2	3.71	0		0	
3	0.003	2	4.02	0		0		0		0.004	4	3.30	0		0		0	
4	0.005	3	3.76	1	4.42	4	3.65	1	4.45	0.009	3	3.35	1	3.92	0		0	
5	0.010	0		2	3.84	1	4.27	0		0.018	4	3.47	2	3.78	0		1	4.25
6	0.020	5	3.51	0		3	3.78	0		0.036	10	2.64	1	3.75	1	3.81	0	
7	0.041	9	2.98	4	3.29	3	3.53	0		0.072	31	2.01	2	2.94	1	3.43	1	3.45
8	0.061	18	2.42	3	3.13	3	3.20	1	3.75	0.107	42	1.75	2	2.45	0		0	
9	0.082	10	2.34	1	3.22	4	2.67	0		0.143	45	1.48	2	2.16	0		0	
10	0.102	21	1.94	2	2.65	2	2.75	0		0.179	45	1.35	0		0		0	
11	0.122	20	1.84	2	2.47	4	2.32	1	2.84	0.215	45	1.23	0		1	2.12	0	
12	0.163	23	1.60	2	2.10	6	1.96	1	2.55	0.287	48	1.08	0		0		1	1.91
13	0.204	27	1.40	1	2.10	1	2.24	0		0.358	50	0.99	1	1.57	1	1.77	0	
14	0.245	28	1.25	0		3	1.72	4	1.66	0.430	52	0.88	0		0		0	
15	0.326	28	1.14	0		1	1.84	1	1.85	0.573	57	0.77	0		0		0	
16	0.653	47	0.77	0		0		0		1.146	45	0.61	0		2	0.96	0	
Totals		252		23		36		9			496		17		6		4	

[a] Estimated mg/kg adult body weight/day.
[b] N, number of tumor-bearing animals; M, median time to tumor (in absence of other tumors and other causes of death).
[c] Denominator = 240, four times that for treated group.

Table 5 *Relationship between NDEA dose rate and the Weibull medians for the aggregate of all liver tumors*

The tabulated medians derive from Weibull *b* values for the aggregated analysis, which were in turn derived by addition of a Weibull *b* value from an analysis of the malignant liver tumors with k = 7, f = 0.630, and a Weibull *b* value from an analysis of the benign liver tumors with k = 7, f = 0.081.

Treatment group (1 = control, 2–16 = NDEA-treated)	Males			Females		
	NDEA dose rate (mg/kg ABWD)	No. of liver tumor-bearing animals	Weibull median (yr)	NDEA dose rate (mg/kg ABWD)	No. of liver tumor-bearing animals	Weibull median (yr)
1	Zero	13[a]	3.67	Zero	16[a]	3.34
(2–5)	(0.005)	(19)[a]	(3.48)	(0.009)	(21)[a]	(3.19)
2	0.001	4	3.51	0.002	6	3.13
3	0.003	2	4.02	0.004	4	3.30
4	0.005	9	3.23	0.009	4	3.20
5	0.010	4	3.49	0.018	7	3.16
6	0.020	7	3.35	0.036	12	2.56
7	0.041	15	2.74	0.072	35	1.96
8	0.061	25	2.30	0.107	44	1.71
9	0.082	15	2.18	0.143	47	1.46
10	0.102	25	1.89	0.179	47	1.35
11	0.122	26	1.77	0.215	46	1.22
12	0.163	31	1.53	0.287	49	1.08
13	0.204	29	1.39	0.358	52	0.99
14	0.245	35	1.21	0.430	52	0.88
15	0.326	30	1.13	0.573	57	0.77
16	0.653	48	0.77	1.146	50	0.60
Totals		318			528	

[a] Denominator = 240, 4 times that for treated groups.

molecular weight of 102.1). In contrast, at higher doses (of about 1 mg/kg ABWD) the two substances appear approximately equipotent on a molar basis. Unfortunately, it is not possible to determine reliably how the ratio of their relative potencies varies below 0.01 mg/kg ABWD, because of the statistical problems that are introduced by random errors when small numbers of extra tumors are to be counted in the presence of an appreciable background. Thus, the ratio of their molar potencies might increase, decrease, or remain at about 1:3 at dose rates progressively lower than 0.01 mg/kg ABWD.

In summary, the molar ratio of the potencies of NDMA and NDEA for hepatocellular tumor induction is about unity at around 1 mg/kg ABWD, but decreases to about 1:3 at around 0.1 mg/kg ABWD.

Comparison of the Effects of NDEA and NDMA on the Remaining Types of Liver Tumor (Mesenchyme, Kupffer Cells, and Bile Ducts): *Mesenchyme*. Among the 1800 NDMA-treated animals, 94 developed a liver tumor that was classified histologically as "mesenchymal," while among the 1800 NDEA-treated animals only 42 did (Tables 7 and 4). Superficially, this comparison suggests that NDMA has a greater carcinogenic effect on the blood vessels than NDEA does. However, crude comparisons such as this cannot be trusted, for they do not take into account the fact that, except at the topmost dose level, the various NDMA-treated groups tended to live somewhat longer than did the corresponding NDEA-treated groups. (This was partly because the NDMA-treated animals had no esophageal tumors and partly because, except for the topmost dose level, a given concentration of NDMA tended to have less effect than

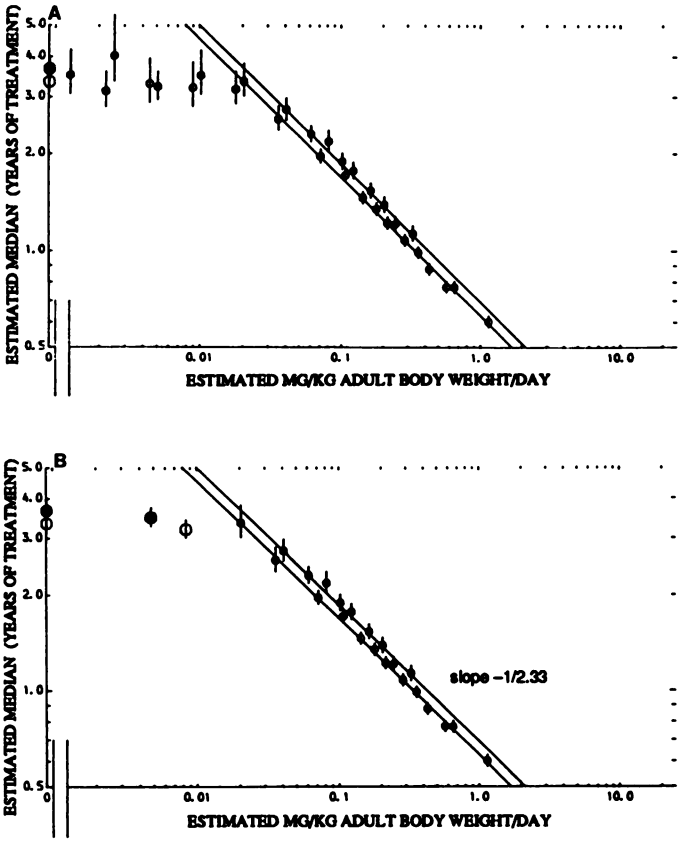


Fig. 5. Effects of NDEA on liver tumor induction (all subsites). *A*, direct plot; *B*, as *A* but with bottom four dose groups merged for statistical stability. *A*, NDEA, all liver tumors, calculated with $f = 0.08$ (benign) and 0.63 (malignant). The "maximum likelihood" lines of preselected slope −3/7 (*i.e.*, −1/2.33) cross the 2-year mark at dosage levels that suggest TD_{50} values for males (M) and females (F) of 0.084 and 0.067 mg/kg ABWD. (See, however, Fig. 6*A* for somewhat improved estimates of these TD_{50} values.) NDEA: site, any liver; $k = 7.00$, $f = 0.630, 0.081$. Both sexes, malignant and benign. TD_{50}: M, 0.084215, $b = 9.089\ (\text{dose} + 0.00)^{3.0}$; F, 0.06715; $b = 17.886\ (\text{dose} + 0.00)^{3.0}$. *B*, as in *A* but with the bottom four dose groups pooled for statistical stability (lines of slope −3/7, as in Fig. 6*A*). *Bars*, 95% CL.

did a similar concentration of NDEA on liver tumors.)

This difference in survival has to be allowed for when comparing the effects on mesenchymal tumorigenesis of the two agents. Because of it, even if similar concentrations of NDMA and NDEA had similar effects on mesenchymal cells, one would expect about twice as many mesenchymal tumors among NDMA-treated animals as among NDEA-treated animals (Table 10), which is just about as observed. Closer examination shows that this overall similarity conceals a real excess of mesenchymal tumors when the top NDMA-treated group is compared with the top NDEA-treated group, counter-balanced by, in aggregate, a slight shortfall of mesenchymal tumors when the 14 other NDMA-treated groups are compared with the corresponding NDEA-treated groups. It is, however, not clear how much emphasis to give to such an irregularity, and certainly at dose levels below 10 ppm v/v no evidence whatever remains of any greater effect of NDMA—rather the reverse, in fact. Since, moreover, the molecular weight of NDMA is less than that of NDEA, a given *molar* concentration of NDMA would actually have significantly *less* carcinogenic effect on the mesenchymal cells than the corresponding molar concentration of NDEA would have.

Kupffer Cells. Kupffer cell tumors are so rare (11 among NDMA-treated animals *versus* 12 receiving NDEA) that the number of affected animals in most groups is zero, so for those groups the Weibull b values are zero and the Weibull medians cannot be estimated or plotted (Figs. 4*D* and 7*D*). A dose-response relationship from which have been removed only those groups that had no affected animals is automatically biased; thus examination of Figs. 4*D* and 7*D* could be misleading. Just as for mesenchymal tumors, however, adjustment for longevity

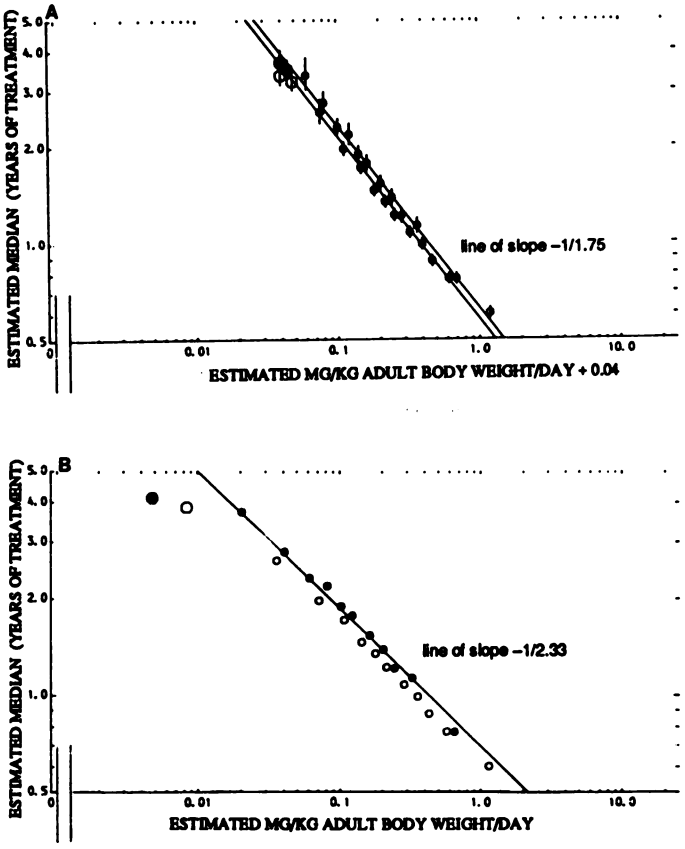


Fig. 6. Effects of NDEA on liver tumor incidence (all subsites from Fig. 5*B*), replotting (*A*) risks *versus* dose rate plus 0.04 mg/kg ABWD, and (*B*) excess risks *versus* dose rate. The excess risks in *B* were estimated by subtraction of the control b values from the b values for the treated groups. *A*, NDEA, all liver tumors. Risk *versus* dose rate plus 0.04 mg/kg. The "maximum likelihood" lines of preselected slope −4/7 (*i.e.*, −1/1.75) cross the 2-year mark at dosage levels that suggest TD_{50} values for males (M) and females (F) of 0.090 and 0.074 mg/kg ABWD. Because the fit of the straight lines in this graph is better than that in Fig. 5, these TD_{50} values are likely to be more reliable than those suggested by the lines in Fig. 5. NDEA: site, any liver; $k = 7.00$, $f = 0.630, 0.081$. Both sexes, malignant and benign. TD_{50}: M, $0.09044 + 0.04$, $b = 18.703\ (\text{dose} + 0.04)^{4.0}$; F, $0.07397 + 0.04$, $b = 32.093\ (\text{dose} + 0.04)^{4.0}$. *B*, NDEA, all liver tumors. Excess risk *versus* dose rate. The line that is plotted is an arbitrary one of slope −3/7 (*i.e.*, −1/2.33), plotted near the points merely for visual comparison with the slope suggested by them. It is not a line that has been formally fitted to the points.

Table 6 *Comparison of two alternative formulae for extrapolation to very low dose rates (below 0.01 mg/kg ABWD) of the dose-response relationship for the effects of NDEA on liver tumors*

Note: Even the middle column in this table may slightly (*e.g.*, by a factor of about 2; see "Discussion") underestimate the true effects of NDEA at low dose-levels.

Dose rate, d (mg/kg ABWD)	Predicted cumulative incidence at 2 years (%)	
	A. Assuming CI = $32\ (d + 0.04)^4 t^7$, as indicated by the line in Fig. 6*A*	B. Assuming extra CI = $9\ d^3 t^7$, as indicated by the line in Fig. 6*B*
0.06	41	25
0.03	8.8	3.1
0.01	1.5	0.1
0.003	0.35	0.003
0.001	0.1	0.0001
0.0001	0.01	0.0000001

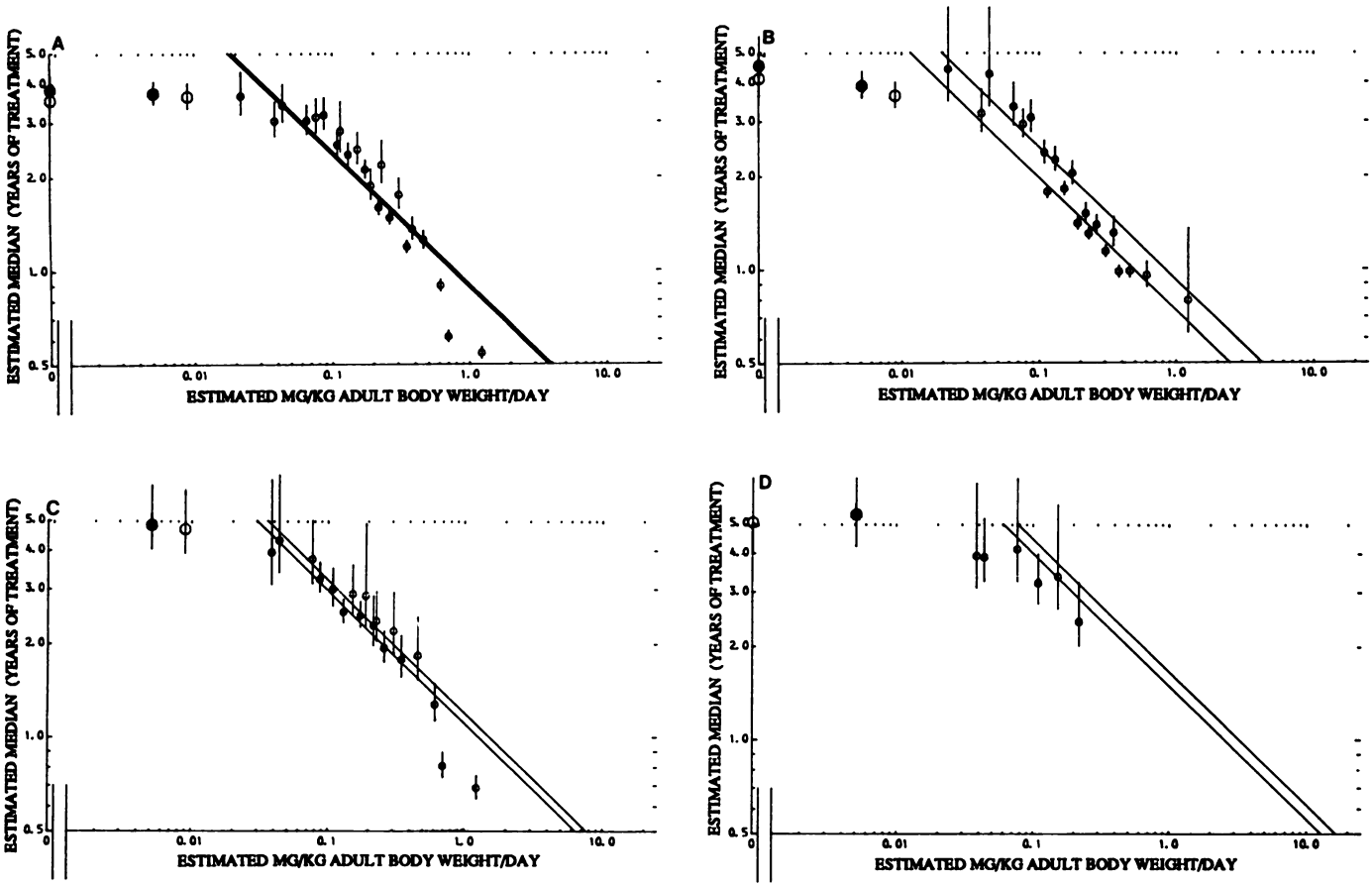


Fig. 7. Effects of NDMA on tumor induction in various parts of the liver. *A*, liver cell; *B* bile duct; *C*, mesenchyme; *D*, Kupffer cell. Data for groups 2 to 5 pooled for statistical stability. ●, males (M); ○, females (F). *Small circles*, groups of 60 animals each; *large circles*, groups of 240 animals. Lines of slope −1/2.3 are plotted for comparison, but in no case (except perhaps Fig. 4*D*, where there are insufficient data to tell) do they appear to provide a good fit to the data at high doses. *A*, NDMA, liver cell tumors, calculated with $f = 0.61$ (malignant) and $f = 0.07$ (benign). Although "maximum likelihood" lines of slope −3/7 (*i.e.*, −1/2.33) are plotted, they fit the data so poorly that the TD_{50} values they suggest cannot be accepted: see Fig. 9*A* for a more satisfactory set of TD_{50} estimates. NDMA: site, liver cell; $k = 7.00$, $f = 0.613, 0.071$. Both sexes, malignant and benign. TD_{50}: M, 0.14775, $b = 1.679$ (dose + 0.00)$^{3.0}$; F, 0.15736, $b = 1.390$ (dose + 0.00)$^{3.0}$. *B*, NDMA, bile duct tumors (nearly all of which were benign), calculated with $f = 0.21$ irrespective of malignancy. As with *A*, the fit of the maximum likelihood lines of slope −3/7 to the data is not satisfactory. NDMA: site, bile duct; $k = 7.00$, $f = 0.206$. Both sexes, any neoplasm. TD_{50}: M, 0.16553; $b = 1.194$ (dose + 0.00)$^{3.0}$; F, 0.09731; $b = 5.877$ (dose + 0.00)$^{3.0}$. *C*, NDMA, mesenchymal tumors (nearly all of which were malignant), calculated with $f = 0.92$ irrespective of malignancy. As with Fig. *A*, the fit of the maximum likelihood lines of slope −3/7 to the data is not satisfactory. NDMA: site, mesenchyme; $k = 7.00$, $f = 0.917$. Both sexes, any neoplasm. TD_{50}: M, 0.25253; $b = 0.336$ (dose + 0.00)$^{3.0}$; F, 0.30245; $b = 0.196$ (dose + 0.00)$^{3.0}$. *D*, NDMA, Kupffer cell tumors (all of which were malignant), calculated with $f = 0.87$. The data are too sparse to check on the quality of fit of the maximum likelihood lines of slope −3/7. (The apparently poor fit is not informative and arises merely because groups in which no such tumors arose pull the lines upwards.) NDMA: site, Kupffer cells; $k = 7.00$, $f = 0.869$. Both sexes, any neoplasm. TD_{50}: M, 0.50779; $b = 0.041$ (dose + 0.00)$^{3.0}$; F, 0.65037; $b = 0.020$ (dose + 0.00)$^{3.0}$. *Bars*, 95% CL.

suggests (Table 10) that if equal concentrations of NDMA and NDEA had similar effects on the Kupffer cells then one would expect about two-thirds of the total of 23 Kupffer cell tumor-bearing animals among the NDMA-treated groups and one-third among the NDEA-treated animals. In fact, however, they are observed to be distributed about equally (11 and 12, respectively) between the two agents. This shortfall is only marginally significant statistically, but again when it is remembered that the molecular weight of NDMA is less than that of NDEA it appears again that if equimolar concentrations of NDMA and NDEA were compared, the NDEA would have a slightly greater carcinogenic effect on the Kupffer cells.

Bile Duct. For bile duct tumors the situation is quite different, for the excess among the NDMA-treated animals is so large (427 affected animals, compared with only 33 NDEA-treated animals affected) that it cannot be entirely ascribed to differences in longevity (Table 10). Indeed, even after allowing for differences in longevity, the analysis in Table 10 suggest that, at a given concentration (in ppm v/v) of nitrosamine, the age-specific onset rate of bile duct tumors is about 3.8 times as great for NDMA as for NDEA (with 95% confidence range of 3 to 5). However, at a given concentration in ppm v/v the ratio of the *molar* concentrations of NDMA and NDEA is 1.5:1. Moreover, the slopes of the lines in Fig. 6 suggest that the Weibull *b* values are approximately proportional either to the third power of the dose rate or to the fourth power of (the dose rate plus a small background term). Therefore, a 1.5-fold difference in dose rate would produce at least a 3-fold difference in the Weibull *b* value and hence in the age-specific tumor onset rate. This analysis suggests that, despite appearances, equimolar concentrations of the two agents actually produce rather similar age-specific onset rates of bile duct tumors. This somewhat surprising conclusion may be made more plausible by visual comparison of the dose-response relationships for bile duct tumors that are illustrated in Figs. 4*B* and 7*B*; if these are superposed and then moved sideways slightly to allow for the differences in molecular weight, the plotted points for NDMA and for NDEA are intermingled.

Summary. For any particular NDMA (MW 74.1) dose rate of around 0.1–0.5 mg/kg ABWD, the dose rate of NDEA (MW 102.1) required to produce the same age-specific onset rate of bile duct tumors would be approximately equimolar, that re-

Table 7 *Relationship between NDMA dose-rate and Weibull medians for the various subtypes of liver tumor (for details, see Fig. 7)*

Treatment group	Males								Females							
	NDMA dose rate[a]	A. Liver Cell		B. Bile duct		C. Mesenchyme		D. Kupffer cell		NDMA dose rate[a]	A. Liver cell		B. Bile duct		C. Mesenchyme		D. Kupffer cell	
		N[b]	M	N	M	N	M	N	M		N	M	N	M	N	M	N	M
1	Zero	10[c]	3.82	3[c]	4.54	0[c]		0[c]		Zero	11[c]	3.53	4[c]	4.12	0[c]		1[c]	5.05
2	0.001	4	3.66	2	4.02	0		0		0.002	2	3.86	1	4.27	1	4.31	0	
3	0.003	3	3.74	3	3.73	0		1	4.39	0.005	2	3.80	4	3.43	0		0	
4	0.005	2	3.97	2	3.96	1	4.39	0		0.010	4	3.31	1	4.04	0		0	
5	0.011	4	3.55	2	3.95	1	4.37	0		0.019	2	3.75	4	3.34	1	4.18	0	
6	0.022	4	3.63	1	4.42	0		0		0.038	6	3.02	4	3.18	1	3.94	1	3.94
7	0.044	5	3.40	1	4.25	1	4.31	2	3.90	0.076	6	3.10	9	2.94	2	3.75	1	4.13
8	0.065	8	3.03	4	3.34	0		0		0.115	3	2.81	39	1.78	0		0	
9	0.087	7	3.16	7	3.09	7	3.23	0		0.153	7	2.46	33	1.82	3	2.90	1	3.37
10	0.109	13	2.54	13	2.39	5	3.00	3	3.21	0.191	7	1.88	44	1.41	1	2.86	0	
11	0.131	14	2.37	12	2.26	12	2.54	0		0.229	4	2.19	48	1.30	3	2.38	0	
12	0.174	19	2.12	12	2.04	10	2.47	0		0.306	7	1.75	46	1.14	2	2.21	0	
13	0.218	27	1.59	16	1.51	3	2.30	2	2.42	0.382	13	1.36	44	0.98	0		0	
14	0.261	32	1.48	18	1.39	7	1.94	0		0.459	20	1.26	38	0.99	2	1.84	0	
15	0.348	44	1.20	8	1.31	4	1.78	0		0.612	40	0.90	10	0.96	5	1.27	0	
16	0.697	46	0.61	0		10	0.81	0		1.224	41	0.54	1	0.79	12	0.69	0	
Totals		242		104		61		8			175		330		33		4	

[a] Estimated mg/kg adult body weight/day.
[b] N, no. of tumor-bearing animals; M, median time to tumor (in absence of other tumors and other causes of death).
[c] Denominator = 240, 4 times that for treated groups.

quired to produce the same age-specific onset rate of mesenchymal (and, perhaps, Kupffer cell) tumors would be about 0.7 of the NDMA molarity, and that required to produce the same age-specific onset rate of hepatocellular tumors would be about 0.3 of the NDMA molarity. Due to random fluctuations, the ratios for lower doses are not known reliably, and in view of the odd findings in the highest group (at about 1 mg/kg ABWD) neither are the ratios for higher doses.

Discussion

This experiment has included the range of moderately high doses studied by Druckrey (7), and in that range the dose-response relationship (dose rate × median$^{2.3}$ ≅ constant) that he reported has been nicely confirmed, at least for NDEA. However, among animals at any one particular dose level, the extraordinarily sharp dependence of tumor onset times on duration of exposure that Druckrey described in that dose range has been replaced in the present study by a more ordinary "Weibull" distribution, in which at a given dose level the disease onset rate is proportional to only about the seventh power of the duration of exposure.

The present experiment has also included a range of moderately low dose rates, below those studied by Druckrey. In this lower range some significant carcinogenic effects have been picked up, but for liver tumors these are not what would have been predicted by simple extrapolation downwards of the dose-response relationships that are found at higher doses both in Druckrey's and in the present experiment.

The overall relationships observed in the present experiment were approximately:

Site	Sex	Agent	Cumulative incidence[a]
Esophagus	F	NDEA	$11.16\ d^3\ t^7$
Esophagus	M	NDEA	$21.17\ d^3\ t^7$
Liver (all sites)	F	NDEA	$32.09\ (d + 0.04)^4\ t^7$
Liver (all sites)	M	NDEA	$18.70\ (d + 0.04)^4\ t^7$
Liver (all sites)	F	NDMA	$51.45\ (d + 0.1)^6\ t^7$
Liver (all sites)	M	NDMA	$37.43\ (d + 0.1)^6\ t^7$

[a] Approximate cumulative incidence observed after administration of d mg/kg ABWD for t years. N.B.: It can be shown that $-\log_e AAT$ is equal to the cumulative incidence.

The discussion of these observations will be divided into two parts: (*a*) a discussion, which is of necessity somewhat hypothetical, of low-dose extrapolation; and (*b*) some discussion of the effects actually observed in the experimental dose range, chiefly addressing the question of what types of further research are now needed.

1. Estimation of the Effects on Colworth Rats of Very Low Dose Levels of Nitrosamines

General Principles. It has already been emphasized, in Tables 3 and 6, that no purely mathematical arguments can yield from these data reliable estimates of the effects of very low doses of nitrosamines. Some extra biological assumptions must be made in order to produce such estimates, and the estimated risks may depend quite strongly on the detail of those assumptions. There are, however, some quite plausible general arguments that can in the present instance greatly reduce that uncertainty.

The fundamental assumption (which was mentioned with the results for esophageal tumors) is that if the disease onset rate for animals of a particular age is plotted against the chronic dose rate, then the resulting graph may be approximately

Table 8 *Relationship between NDMA dose rate and the Weibull medians for the aggregate of all liver tumors*

The tabulated medians derive from Weibull *b* values for the aggregated analysis, which were in turn derived by addition of a Weibull *b* value from an analysis of the malignant liver tumors with $k = 7, f = 0.708$ and a Weibull *b* value from an analysis of the benign liver tumors with $k = 7, f = 0.164$.

Treatment group (1 = control, 2–16 = NDMA-treated)	Males			Females		
	NDMA dose rate (mg/kg ABWD)	No. of liver tumor-bearing animals	Weibull median (yr)	NDMA dose rate (mg/kg ABWD)	No. of liver tumor-bearing animals	Weibull median (yr)
1	Zero	13	3.67	Zero	16	3.35
(2–5)	(0.005)	(23)	(3.41)	(0.009)	(22)	(3.23)
2	0.001	5	3.53	0.002	4	3.50
3	0.003	7	3.30	0.005	6	3.22
4	0.005	5	3.47	0.010	5	3.20
5	0.011	6	3.36	0.019	7	3.10
6	0.022	5	3.52	0.038	12	2.73
7	0.044	9	3.12	0.076	18	2.62
8	0.065	12	2.83	0.115	42	1.72
9	0.087	19	2.72	0.153	43	1.76
10	0.109	35	2.14	0.191	51	1.34
11	0.131	38	2.00	0.229	55	1.26
12	0.174	41	1.84	0.306	56	1.10
13	0.218	48	1.38	0.382	58	0.94
14	0.261	56	1.26	0.459	59	0.94
15	0.348	56	1.12	0.612	57	0.82
16	0.697	59	0.61	1.224	58	0.53
Totals		414			547	

straight, or it may exhibit upward curvature, but it is unlikely to exhibit much downward curvature. The theoretical arguments for this derive from fairly general considerations about multistage models for the processes of carcinogenesis (8). More importantly, the practical arguments for it derive from observations of a variety of dose-response relationships in both humans [*e.g.*, Doll & Peto (1)] and animals [*e.g.*, Lee and O'Neill (12)]. In particular, the relationships in the present experiment between the Weibull *b* values and the nitrosamine dose levels exhibit very strong upward curvature at high dose levels (where *b* is approximately proportional to the cube of the dose rate), and even at low dose levels, as, *e.g.*, in Figs. 10 and 11, there is no evidence of downward curvature.

The second assumption is that there *might* be some "background" exposure of control animals. This is defined to mean exposure to agents or processes that, although different from the carcinogen being studied, produce changes (*e.g.*, in DNA) that are functionally similar to (even if chemically different from) those it produces (13). This means that the effective total dose (TD) of the test agent is equivalent to a background dose (BD) plus an applied dose (AD) (so TD = AD + BD). Next, consider a graph of total risk *versus* total dose; a hypothetical example of such a graph is given in Fig. 12*A*. It is, of course, not possible to predict reliably the shape of such a graph (except to note that if it does have any curvature then this is likely to be upward rather than downward). In particular, it is not possible to predict reliably whether its slope at zero total dose would be zero, or whether it would be positive. However, consider instead the corresponding graph of risk *versus applied* dose (Fig. 12*B*). If there is any "background" risk and any (effective) "background" dose, then this graph of added risk against applied dose will probably start with a positive slope. In other words, *if* there is any appreciable background of tumors produced by mechanisms analogous to those involved with the test agent, *then* at low applied dose levels the added risk is likely to be approximately proportional to the applied dose. This conclusion is, moreover, likely to hold reasonably well (unless any rate-limiting enzyme systems become induced or saturated) in the range of doses where the added risk does not greatly exceed the background. Analogous arguments underlie the fact that no statistical analysis of any experimental or epidemiological dose-response relationship can ever validly[10] exclude the possibility of approximate "linearity" (*i.e.*, proportionality between risk and dose) emerging not far below the range of doses the effects of which have been measured reasonably accurately.

Thus far, it has been noted that (*a*) low-dose linearity could well obtain (especially where the background risks are appreciable) and that (*b*) low-dose linearity can never validly be excluded. This does not, however, add up to strong evidence for low-dose linearity in any particular case, *unless* an approximately linear dose-response relationship can be reliably observed at moderately low doses.

Applications of General Principles to Present Data. For esophageal tumors, the background is unmeasurably low (probably less than 1 in 1000, since no esophageal tumors developed among the 480 controls, the 480 animals in NDEA dose levels 2 to 5, and the 1800 NDMA-treated animals). Consequently, although low-dose linearity is certainly consistent with the esophageal dose-response relationship (Table 3), there is no strong evidence for it, no useful estimate of its likely magnitude is available, and the data are equally consistent with nonlinearity and even with the presence of a threshold.

For liver tumors, however, the background rate is appreciable, with 6% of the controls affected (or 8% if the animals undergoing scheduled sacrifice after only 12 or 18 months are excluded). The existence of this background makes low-dose linearity more probable, at least in the range of doses that produce extra effects that do not greatly exceed this background. For NDEA the data in Figs. 10 and 11 provide a nonsignificant suggestion of low-dose linearity, as does the graphical presentation in Fig. 6*A* and the corresponding analysis in Table 6*A*. However, the analyses in Fig. 6*B* and Table 6*B* indicate that the existence of such low-dose linearity cannot be regarded as proved by these analyses. For NDMA, there is some evidence for low-dose linearity due to the existence of an appreciable background and a range of

[10] This is because (*a*) no experimental data can distinguish reliably between a particular nonlinear relationship of risk to dose and the same type of relationship of risk to dose-plus-background, and (*b*) in choosing models to such data that are intended to form a basis for predictions about the effects of low doses, "background" should obviously be a parameter to be estimated, rather than a parameter that is arbitrarily forced to be zero.

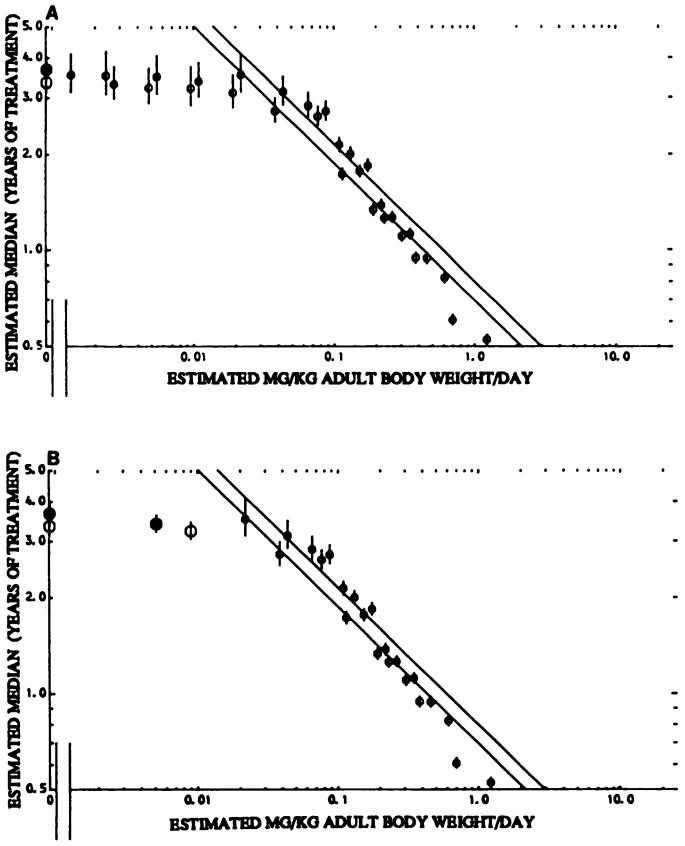
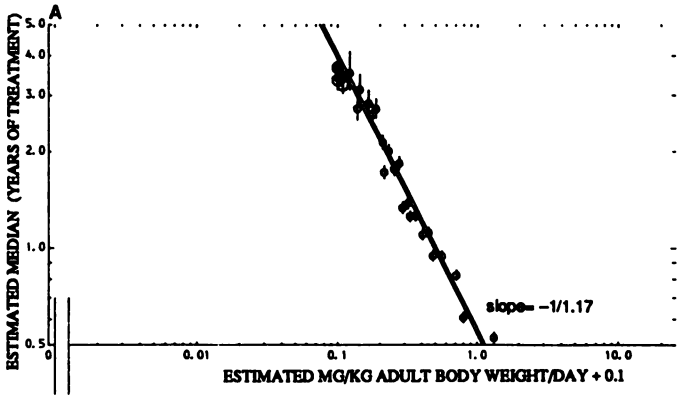


Fig. 8. Effects of NDMA on liver tumor induction (all subsites). *A*, direct plot; *B*, as *A* but with bottom four dose groups merged for statistical stability. *A*, NDMA, all liver tumors, calculated with $f = 0.16$ (benign) and 0.71 (malignant). Although "maximum likelihood" lines of slope −3/7 (*i.e.*, −1/2.33) are plotted, they fit the data so poorly that the TD_{50} values they suggest cannot be accepted: see Fig. 9*A* for a more satisfactory set of TD_{50} estimates. NDMA: site, any liver; $k = 7.00, f = 0.708, 0.164$. Both sexes, malignant and benign. TD_{50}: M, 0.11743, $b = 3.344$ (dose + 0.00)$^{3.0}$; F, 0.08547, $b = 8.673$ (dose + 0.00)$^{3.0}$. *B*, as in *A* but with the bottom four dose groups merged for statistical stability. (The plotted lines of slope −3/7 cannot be trusted; see above.) *Bars*, 95% CL.

dose rates [those below 0.1 mg/kg ABWD] in which the excess risk does appear to be approximately proportional to the dose rate [Fig. 11; see also Table 11 and Fig. 9*A*, the line in which suggests[11] that liver tumor risks are approximately proportional to (dose + 0.1 mg/kg ABWD)^6]. It should be appreciated, however, that this conclusion is based on very small numbers of tumors. Because of this linearity at moderately low doses, there is, at least in the one instance of NDMA-induced hepatomas, reason to suppose that the excess risks at very low dose rates are, although they cannot be measured directly, approximately proportional to the applied dose rate. Moreover, it is possible that the risks per unit dose at these *very* low dose rates will be approximately the same as the risks per unit dose observed directly at those *moderately* low dose rates of NDMA where the risk is approximately proportional to the dose rate. From Fig. 11, it appears that a dose rate of 0.05 mg/kg ABWD (which is within this moderate range) will increase the Weibull b value to about 5×10^{-4} (males, 3×10^{-4}; females, 7×10^{-4}). Alternatively, a similar estimate may be obtained from the line in Fig. 9*A*. These estimates suggest that, in the absence of other causes of death, the mortality-adjusted excess risks at very low dose levels in a standard 2-year experiment with NDMA starting at 6 weeks of age would be of the order of 0.03% for males and 0.04% for females per μg per kg ABWD. With the type of life span achieved in the present study, however, the excess risks from lifelong exposure starting at 6 weeks of age and not adjusted for mortality would probably be about 7 times as large as this, to judge from the ratios of the b value to the numbers of affected animals discussed in the notes to Table 11.

Although no equally reliable direct estimate of the likely effects of low doses of NDEA is available, the fact that at dose rates of about 0.1 mg/kg ABWD a given dose of NDEA appears to be equivalent to about 2 or 3 times that dose of NDMA suggests that for NDEA the risks from 2 years of exposure at very low doses might be approximately 0.08%/μg NDEA/kg ABWD.

Moreover, this and not the esophageal cancer risk is likely to be the dominant contributor to the effects of low dose rates of NDEA because the fact that the esophageal dose-response relationship has no measurable background level (and no good evidence of any divergence from a simple third-power dose-response relationship) indicates that at very low dose levels the effects of NDEA on liver tumorigenesis will exceed those on



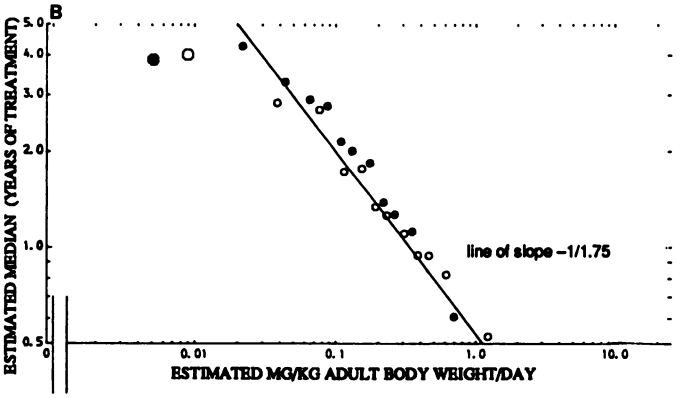


Fig. 9. Effects of NDMA on liver tumor incidence (all subsites, from Fig. 8*B*), replotting (*A*) risks *versus* dose rate + 0.1 mg/kg ABWD, and (*B*) excess risks *versus* dose rate (estimated by subtraction of the Weibull b value for the corresponding control group from those for the treated groups). *A*, NDMA, all liver tumors. Risk *versus* dose rate + 0.1 mg/kg. The "maximum likelihood" lines of preselected slope −6/7 (*i.e.*, −1/1.17) cross the 2-year mark at dosage levels that suggest TD_{50} values of 0.129 (male; M) and 0.117 (female; F) mg/kg ABWD. Because the fit of these lines to the data appears better than that of the lines in Figs. 7 and 8, the TD_{50} values that it suggests are likely to be more reliable than those suggested by the earlier figures. NDMA: site, any liver; $k = 7.00, f = 0.708, 0.164$. Both sexes, malignant and benign. TD_{50}: M, 0.12912 + 0.10, $b = 37.431$ (dose + 0.10)$^{6.0}$; F, 0.11729 + 0.10, $b = 51.449$ (dose + 0.10)$^{6.0}$. *B*, NDMA, all liver tumors. Excess risk *versus* dose rate. The *line* that is plotted is an arbitrary one of slope −4/7 (*i.e.*, −1/1.75), plotted near to the *points* merely for visual comparison with the slope suggested by them. It is not a line that has been formally fitted to the points. *Bars*, 95% CL.

[11] Formally, the line in Fig. 9*A* suggests that the Weibull b values for NDMA-treated animals are approximately equal to 50 (dose + 0.1 mg/kg ABWD)^6; this suggests that the Weibull b values at zero and at 0.05 mg/kg ABWD will be approximately 0.5×10^{-4} and 5.7×10^{-4}, respectively.

Table 9 *Hepatocellular tumorigenesis described by Weibull b values*

N.B.: These *b* values may be more attractive than medians are for characterizing and comparing the effects of various *low* dose rates. To give the *b* values some intuitive meaning, note that at low, although not at high, doses, the percentage of animals that would, in the absence of other causes of death, be affected by hepatocellular tumors after 705 days of treatment is approximately[a] $b.10^4$, and thus, for readability of the low-dose effects, values of $b.10^4$ have been tabulated.

NDEA, males			NDMA, males			NDEA, females			NDMA, females		
Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$
Zero	10	0.59	Zero	10	0.59	Zero	11	1.01	Zero	11	1.01
0.001	1	0.26	0.001	4	0.79						
0.003	2	0.41	0.003	3	0.67	0.002	4	1.50	0.002	2	0.54
0.005	3	0.65	0.005	2	0.45	0.004	4	1.63	0.005	2	0.61
0.010	0	0.00	0.011	4	0.97	0.009	3	1.47	0.010	4	1.59
0.020	5	1.05	0.022	4	0.83	0.018	4	1.15	0.019	2	0.66
0.041	9	3.29	0.044	5	1.33	0.036	10	7.71	0.038	6	3.00
0.061	18	14.07	0.065	8	2.96	0.072	31	53.18	0.076	6	2.50
0.082	10	18.02	0.087	7	2.20	0.107	42	138.4	0.115	3	5.00
0.102	21	65.94	0.109	13	10.15	0.143	45	442.7	0.153	7	12.89
0.122	20	97.22	0.131	14	16.60	0.179	45	840.4	0.191	7	84.30
0.163	23	256.5	0.174	19	36.25	0.215	45	1,644	0.229	4	28.44
0.204	27	644.5	0.218	27	265.1	0.287	48	4,002	0.306	7	136.8
0.245	28	1,444	0.261	32	449.8	0.358	50	7,220	0.382	13	808.2
0.326	28	2,722	0.348	44	1,988	0.430	52	17,366	0.459	20	1,407
0.653	47	42,225	0.697	46	210,184	0.573	57	42,206	0.612	40	14,783
						1.146	45	222,377	1.224	41	488,768
Lowest 4 non-zero doses combined:											
0.005	6	0.33	0.005	13	0.72	0.008	15	1.44	0.009	10	0.85

[a] Strictly, it is $100\,[1 - \exp(-b \times (705/365)^7)]$, *i.e.*, $100\,[1 - \exp(-100b)]$. The *b* values have been estimated by addition of the two separate *b* values generated by the fit of a double Weibull distribution with exponent 7 to the malignant tumors and, separately, to the benign tumors. The four (independently estimated) fatality factors used in fitting these distributions were: NDEA, malignant, $f = 0.63$; NDEA, benign, $f = 0.09$; NDMA, malignant, $f = 0.61$; NDMA, benign, $f = 0.07$.

esophageal tumorigenesis. Therefore, in estimating the net (all sites) tumorigenic effects of very low doses of NDEA, only its effects on the liver need be considered, so the wide uncertainty about its likely effects on the esophagus does not matter much.

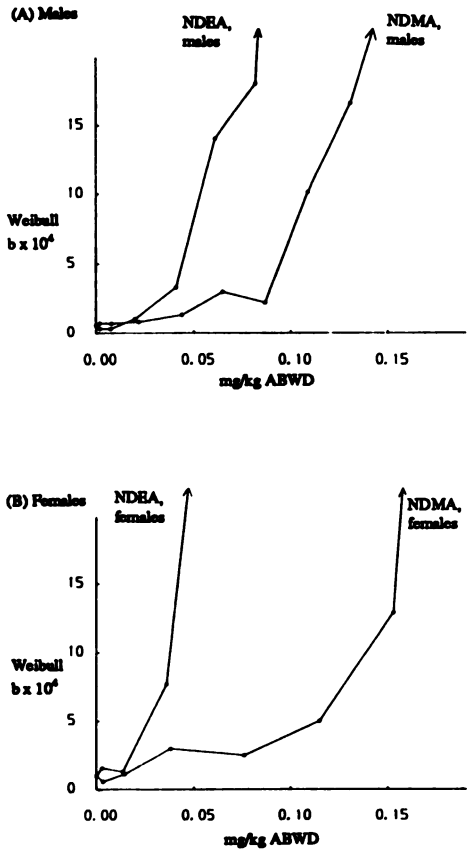


Fig. 10. Weibull *b* values comparing the effects of low doses of NDEA and NDMA on hepatocellular tumorigenesis (from Table 9). For each sex, the lowest four non-zero dose levels are pooled, for statistical stability.

Summary. The best estimate of the likely carcinogenic effects on Colworth rats of very low doses of NDMA and NDEA is that (*a*) their effects on the liver will exceed their effects on all other sites and (*b*) in the absence of other causes of death, in a 2-year experiment starting at 6 weeks of age their effects per μg per kg ABWD would probably be to produce, respectively, liver tumor risks of about 0.03 to 0.04% (NDMA) and about 0.06 to 0.1% (NDEA). In the presence of other causes of death, lifelong exposure from week 6 onwards (not truncated after 2 years of treatment) would probably yield risks about 7 times as large as these.

Public Health Implications of Low-Dose Extrapolations. What is really wanted, of course, is not the lifelong risk/μg/kg/day for Colworth rats but rather the corresponding lifelong risk for humans in a heterogeneous, wild population. This cannot, however, be inferred directly from the present experiment. For rats, lifelong risks of about 0.04%/μg/kg/day have been demonstrated, but although the lifelong human risks might happen to be similar to this, they might easily be a few orders of magnitude different from it in either direction.[12]

Testing of Theoretical Models for Low-Dose Extrapolation. Various methods have been proposed for extrapolating from dose levels high enough to have a clearly observable effect down to very low dose levels. Those that allow in their mathematical formulation for the *possibility* of some effective background dose all tend to produce reassuringly similar upper confidence limits for the estimated effects at low dose levels (2). By contrast, those [such as Mantel and Schneiderman's (5) formulation of the "probit" model, or Cornfield's (6) use of power-law relationships] that inadvertently conceal in their mathematical formulation the rigid assumption that no such background

[12] This is because for humans to avoid cancer throughout their large bodies throughout their long life span means that we require controls on the processes of carcinogenesis that are millions of times stricter than those required by small, short-lived rodents. This figure derives chiefly from consideration of our life span, which is about 30 time that of rats. Since cancer onset rates rise as at least the fourth or fifth power of duration of exposure, we need protection by a factor of 30^4 or 30^5 throughout our large bodies: for discussion, see Doll and Peto (14), Section 4.2.

Table 10 *Comparison of the effects of equivalent concentrations of NDMA and NDEA on various types of liver tumor*

Note: The number of tumor-bearing animals observed is contrasted with the number that would have been expected if, among animals of the same sex and age given the same concentrations ppm v/v of nitrosamine, both the death rate *from* the tumor type of interest and the prevalence per survivor *of* incidental such tumors were the same for NDMA and for NDEA. For details, see the IARC report (3).

	NDEA		NDMA		Variance, V, of $O - E$	P	Estimated onset rate ratio[a]
	Observed TBA[b]	Expected TBA	Observed TBA	Expected TBA			
Bile duct	33	113.2	427	346.8	59.7	<0.0001	3.8
Kupffer	12	7.4	11	15.6	3.9	<0.05	3.2
Mesenchyme	42	43.8	94	92.2	21.3	NS	1.0

[a] Ratio of age-specific onset rate among NDEA-treated animals to that among NDMA-treated animals, estimated as exp $[(O - E)/V]$. For estimation of the corresponding ratio of effective doses, see text.

[b] TBA, tumor-bearing animals; $O - E$ = Observed − expected; NS, not significant.

effects can possibly exist can produce what appear to be statistical guarantees of extraordinarily low risks. Such guarantees have been criticized on theoretical grounds (8), and it may therefore be of interest to test in practice what would happen if some such model was fitted to the top 8 dose groups in the present experiment and then used to predict what to expect at lower doses.

For NDEA, no gross discrepancies would be apparent (although if our estimate that 1 μg/kg ABWD produces a 2-year risk of about 0.08% is accepted, then many of the theoretical models would greatly underestimate this risk). For NDMA, however, some quite marked discrepancies would arise if the general philosophy of, for example, Cornfield (6) were used to extrapolate downwards from doses of above 0.1 mg/kg ABWD, because it would then be inferred that the age-specific risks were proportional to about the fourth power of the dose rate (*i.e.*, that every 10-fold reduction in dose would produce a 10,000-fold reduction in effect). Although this is approximately

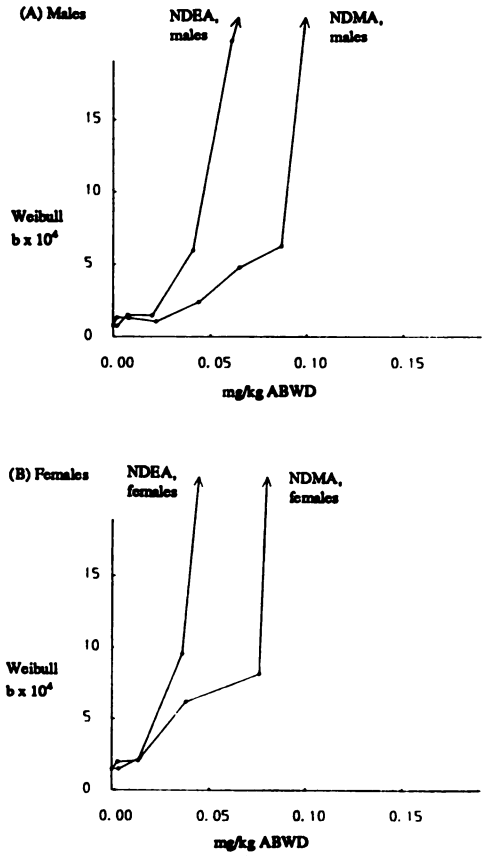


Fig. 11. Weibull b values comparing the effects of NDEA an NDMA on the aggregate of all liver tumors.

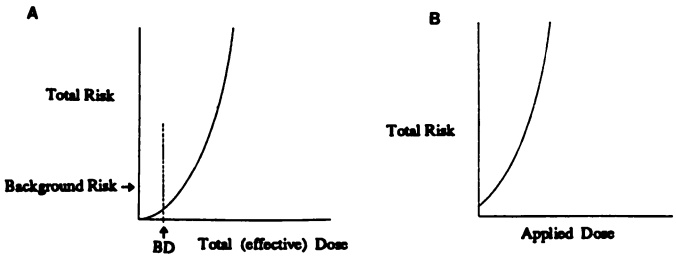


Fig. 12. Plausibility of linearity at low applied doses when appreciable "background" exposure exists. Total risk = added risk + background risk. Total (effective) dose = applied dose + background dose, where the background dose represents the net exposure to agents or processes that produce DNA changes functionally equivalent to those produced by the test agent. *A*, total risk *versus* total (effective) dose. *B*, added risk *versus* applied dose (by deletion of parts of *A* representing doses or risks below the background levels among untreated animals). Note that the positive slope at zero applied dose in *B* depends only on the assumption that functionally similar processes are already producing some risk but does not depend on the detailed shape of the dose-response relationship in the deleted parts of *A*.

true in the dose range above 0.1 mg/kg ABWD, it ceases to be true at about 0.1 mg/kg ABWD. As the relationship converts from a fourth-power relationship to a linear relationship, the 10-fold decrease in dose as we go from 0.1 to 0.01 mg NDMA/kg ABWD produces only a 100-fold (and not a 10,000-fold) decrease in the Weibull b value. Thus, the use on the upper Weibull b values of the type of extrapolation technique suggested by Cornfield (6) would result in about a 100-fold underestimation of the effects of 0.01 mg/kg ABWD that have been observed directly[13] in this experiment. This error (which results from having ignored the possibility of a background dose) is likely to become far worse at even moderately lower doses. Indeed, if, as is probably the case, the excess risks at low doses are approximately proportional to the applied dose rate, then the error involved in using such methods to extrapolate down by just a further 10-fold reduction in dose (*i.e.*, to 1 μg/kg ABWD) would probably be about 100,000-fold.

2. Observed Effects in the Experimental Range of Doses

Biological Interpretation of the Observed Dose-Response Relationships. There are several substantial sources of difficulty in interpreting the observed dose-response relationships biologically.

First, although the dose rate to the entire liver is known, the effective dose rates to the individual parenchymal cells are probably extremely heterogeneous. At any one particular dose

[13] A formal test for trend of the aggregated dose-response relationships for NDMA and NDEA in the control and lowest four dose groups only is reported in the parallel paper that describes more fully the methods of the present experiment; the 1-tailed p value is 0.02, and in view of the continuity with the highly significant effects at slightly higher doses this constitutes some evidence of a real effect of these dose levels.

Table 11 *All liver tumorigenesis, hepatocellular plus other, described by Weibull b values*[a]

NDEA, males			NDMA, males			NDEA, females			NDMA, females		
Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$	Dose rate	TBA	$b.10^4$
Zero	13	0.78	Zero	13	0.78	Zero	16	1.50	Zero	16	1.50
0.001	4	1.06	0.001	5	1.02						
0.003	2	0.42	0.003	7	1.63	0.002	6	2.37	0.002	4	1.07
0.005	9	1.90	0.005	5	1.14	0.004	4	1.63	0.005	6	1.94
0.010	4	1.09	0.011	6	1.43	0.009	4	2.01	0.010	5	2.02
0.020	7	1.47	0.022	5	1.04	0.018	7	2.19	0.19	7	2.53
0.041	15	5.98	0.044	9	2.41	0.036	12	9.57	0.038	12	6.18
0.061	25	20.38	0.065	12	4.77	0.072	35	62.1	0.096	18	8.15
0.082	15	29.96	0.087	19	6.27	0.107	44	161.8	0.115	42	152.5
0.102	25	80.97	0.109	35	34.18	0.143	47	497.1	0.153	43	130.5
0.122	26	129.3	0.131	38	54.10	0.179	47	869.3	0.191	51	915.8
0.163	31	352.7	0.174	41	97.51	0.215	46	1,721	0.229	55	1,397
0.204	29	708.8	0.218	48	792.3	0.287	49	4,078	0.306	56	3,514
0.245	35	1,813	0.261	56	1,345	0.358	52	7,478	0.382	58	10,394
0.326	30	2,911	0.348	56	3,169	0.430	52	17,392	0.459	59	10,544
0.653	48	42,931	0.697	59	230,990	0.573	57	42,390	0.612	57	27,425
						1.146	50	241,570	1.224	58	619,882
Lowest 4 non-zero doses combined:											
0.005	19	1.12	0.005	23	1.30	0.008	21	2.05	0.009	22	1.89

[a] Notes as for Table 9, except that the respective fatality factors utilized were 0.63, 0.08, 0.71, and 0.16. These same data are also available, in an alternative format, in Tables 5 and 8. Excluding the animals scheduled for early sacrifice, about 10% of the control and low-dosed (groups 2–5) animals in Table 11 developed liver tumors, and the pooled b value for these groups is about 1.44×10^{-4}, suggesting that by about 2 years 1.44% would, in the absence of other causes of death, have developed a spontaneous tumor. Thus, the ratio of the lifelong risk to the 2-year risk is about 10/1.44, or 7-fold.

rate, the total risk of hepatocellular cancer arising is the sum of the risks for the heavily exposed cells, the risks for the moderately exposed cells, and the risks for the slightly exposed cells. To judge by the overall shape of the dose-response relationship, this sum is likely to be dominated by the contributions of the few most heavily exposed cells. If, as recommended by Hoel *et al.* (17), attempts are made to determine levels of DNA alkylation as indices of cellular exposure to the ultimate carcinogen, it would be desirable to measure not only the mean values but also their statistical distribution in the cells of the zones of the hepatic lobule. This would, of course, be an unnecessary refinement if the effect on each cell were simply proportional to the chronic dose rate, but the fact that it may not be is one of the main reasons for wanting to study the dose response of the degree of DNA alkylation.

A second difficulty, which applies chiefly to the bile duct, mesenchymal, and Kupffer cell tumors, is that these cell types may not be able to activate the nitrosamine molecules to any significant extent. Consequently, they may suffer exposure chiefly or wholly as a result of moderately long-lived activated molecules diffusing out of the parenchymal cells in which they are formed and into other types of cell. It is not known what factors affect such diffusion, but the complexity of this process reemphasizes the complexity of interpretation of dose-response relationships in whole animals.

Next, many of the nitrosamine dose levels that have been studied constitute such a gross insult to the liver that its normal ordered structure is seriously disrupted. [For detailed tabulations of the prevalence of various indices of such disruption, see the accompanying report on these data (1)]. The target cells for carcinogenesis may therefore be undergoing unusually rapid division to repair gross organ damage, and the normal stem cell hierarchy may be disordered. To judge by the well-known enhancing effects of partial hepatectomy, the enhancing effects of such damage may be very important contributors to the carcinogenic effects of high dose levels.

Finally, despite the unusual size of this experiment (and the unusually long life span of the test animals, which allowed effects to be demonstrated down to doses as low as 0.01 to 0.02 mg/kg ABWD), there is still such substantial statistical uncertainty at the lower dose levels that the detail of the dose-response relationships there is obscured.

In summary, a detailed mechanistic understanding of the entire dose-response relationship is not available, but there are many ways in which these data should stimulate progress toward additional mechanistic insights.

Appendix: Interpretation of the Exponent of Time

Throughout the present report, there has been extensive analysis and discussion of the way in which the excess age-specific onset rates depend on the dose rate, but there has been little discussion of the remarkable way in which the excess dose-specific onset rates depend upon the duration of exposure. As has already been noted, however, the CI[14] is proportional to about the *seventh* power of the duration of exposure. Thus, a 2-fold decrease in the duration of exposure will produce about a 100-fold decrease in the cumulative incidence.

There was some slight variation between the best-fitting exponent for one type of tumor and that for another type of tumor[15] but all of the best-fitting values were in the range 5 to 9, so a 2-fold decrease in duration of exposure would always produce a vast decrease in the cumulative incidence.

It might be thought that the imposition of an exponent of 7 in the statistical analysis of a neoplasm for which the actual exponent was either 6 or 8 might seriously bias the apparent

[14] The CI, defined in the "Statistical Methods," is the integral of the incidence rates up to a given age. When the cumulative incidence is small then it approximately equals the probability (in the absence of other causes of death) of a tumor arising by a given age, but when it is large then this probability is given by $1 - \exp(-CI)$. In the double Weibull distributions used in the present paper, the CI is equal to $b \cdot t^7$, where b is the dose rate-dependent Weibull constant of proportionality and t is the duration, in years, or chronic treatment.

[15] An exponent of 8 provided a significantly better fit than one of 7 for the NDEA-induced liver tumors, and an exponent of 6 provided a significantly better fit than one of 7 for the esophageal tumors and for the NDMA-induced liver tuomrs. Separate estimation of k for the high-dose groups and for the low-dose groups produced no discrepancies, however. However, for a given tumor type, the best-fitting value of k often tended to be slightly lower for benign than for malignant tumors, as might be expected if there were a final rate-determining step from benign to malignant.

shape of the dose-response relationship, but in practice when duplicate analyses of particular dose-response relationships were undertaken with exponents 6 and 8, no material changes were produced either in the overall fatality factor or in the group-specific medians. In each case, in the range of doses where substantial numbers of neoplasms arose, the slope of the graph of log median against log NDEA dose was about $-1/2.33$, as in Fig. 3 (esophageal tumors) and Fig. 5 (liver tumors). This slope indicates that even though, as already discussed, an enormously strong *dose*-response relationship exists (whereby a doubling of the dose produces nearly a 10-fold increase in the cumulative incidence), an even stronger effect of *duration* of exposure exists (whereby a doubling of duration produces over a 100-fold increase in the cumulative incidence). For esophageal tumors, for example, the relationship suggested by Fig. 3 is that the cumulative incidence is approximately proportional to

$$(\text{dose rate})^3 \times (\text{duration})^7$$

Why might this be? Taken at face value, it suggests *either* that in the chain of cellular events which culminates in cancer there are some rate-determining processes that are not strongly affected by treatment *or* that old age *per se* (whatever that may mean) in some way predisposes to cancer. However, at least for agents that have important effects on the first stage of the multistage process of carcinogenesis, there is no evidence that "age" *per se* enhances their effects—rather the reverse, in some instances (18). Moreover, in a parallel experiment older Colworth rats were less susceptible to liver carcinogenesis by nitrosamines, and in some other systems as well [*e.g.*, Peto *et al.* (19)] where there is a strong dependence of cumulative incidence on duration of exposure it appears that age *per se* has no substantial relevance to the reasons for this.

Mathematically, the simplest explanation of the seventh-power dependence on duration of exposure might be that there are about seven rate-determining processes involved in the alteration of a normal to a neoplastic cell. However, although this may be simple mathematically, until recently it has not been simple for a cell biologist to produce plausible suggestions as to what these seven different stages might actually represent. There are alternative mathematical approaches that can, by invoking selective proliferation of partially altered cells and/or a period of growth between the genesis of a cancer and its detectability, yield a seventh-power dependence of risk on time with fewer than seven rate-determining processes (6). Moving up from the other end of the scale, the molecular biologists have evidence for the complementary involvement of multiple oncogenes in human cancer (20). Recent data from Vogelstein's laboratory (21) provide evidence of seven to eight genetic events in human colon cancer. As an understanding of these steps emerges, perhaps the mathematics and the biology will converge into a synthesis that represents a proper understanding of all the main rate-determining processes that are usually involved in carcinogenesis and that predicts both the dose and the time dependence of experimental carcinogenesis. Until that is achieved, however, the extraordinarily strong dependence of the cumulative incidence on duration of exposure stands as a warning to the cell biologists that much probably remains undiscovered even among the *rate-determining* processes of carcinogenesis, which, after all, are likely to represent the most important processes from the point of view of cancer prevention.

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Note: Public-use copies of the full data are available from R.G.

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