Implications of the Carcinogenic Hazard of Low Doses of Three Hepatocarcinogenic N-Nitrosamines¹

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The data of a large-scale experiment on single and combination effects of very low doses of the hepatocarcinogenic N-nitrosamines N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), and N-nitrosodiethanolamine (NDEIA) were modeled by new statistical methods to derive implications of the carcinogenic hazard of dose ranges so low as to result in long-term toxic effects only slightly different from the background. According to this model a linear relationship was found to exist between daily exposure to low N-nitrosamine levels and time to death with liver tumor. Extrapolation of these data to zero exposure suggested the occurrence of one to ten percent of spontaneous liver tumors in animals surviving more than 1000 days. At this advanced age a further reduction of carcinogen-induced liver tumor incidence does not contribute to a longer overall survival due to competitive, probably independent, causes of death. A quasi-threshold in terms of a “no-observed-effect level” can thus be derived from the data. The observed combination effect indicates a mere additivity in liver tumor occurrence, even at very low doses which alone cause no significant carcinogenic effect during the animal's lifetime. Within the combination of NDEA, NPYR and NDEIA, N-nitrosodiethylamine was found to be the most carcinogenic agent: it contributes by at least 17 orders of magnitude more to the relative risk of dying with liver tumor than the other two compounds, if the daily dose is increased by one unit (0.1 mg/kg). Likewise, NDEA shows the steepest slope when assessing the relationship between daily carcinogen doses and time to liver tumor occurrence; relative to NDEIA — the least potent carcinogen on a weight basis — and NPYR a 40-fold and 9-fold quicker appearance of liver tumors has to be expected, if the daily doses are increased by an equivalent amount.

Key words: Syncarcinogenesis — N-Nitrosamine — Low-dose hazard — Extrapolation — New statistical method

Recent studies on combination effects of carcinogens have mostly been performed with two agents (for review see 1), but complex mixtures of up to 40 carcinogens have also been used.²⁾ In view of the multitude of possible combination partners targeting one or more organs by various mechanisms of action, some very early studies focussed on syncarcinogenesis in the same target organ, such as skin³⁾ to facilitate observation of basic principles. Within this framework our approach has recently concentrated on using very low doses. In an experiment on the combination effect of very low doses of the three N-nitrosamines N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), and N-nitrosodiethanolamine (NDEIA), which cause similar organotropic carcinogenicity in rats,⁴⁾ the following observations were made.

Dose dependency of tumor formation was observed within the liver — the common target organ — even at very low levels of exposure to the single N-nitrosamines of their combination; the carcinogenic effects of the

N-nitroso compounds were simply summed in combination. The observed additivity was apparently linear. Doses which alone would presumably not have been carcinogenic, presented a significant cancer risk when administered in combination. The observed tumor incidences within the liver were near to a “no-observed-effect level” which can be defined in experimental systems when dosages are selected which are too low to allow the manifestation of tumors within the lifetime of the animal strain used. Since the experiment comprised 1800 rats, it is large-scale enough to ensure a reasonable data base for statistical evaluation of the underlying generally applicable relationships between low doses and carcinogenic hazard, to estimate the long-term toxic effects of dosages different from those of the experiment, and in addition, to delineate some general aspects of carcinogenesis near to the “no-observed-effect level.” The analysis was restricted to liver tumors. This approach seems to be justified since liver tumors were observed most frequently in the experiment and only they showed a clear dose-dependency in all carcinogen-treated groups. The fact that very few studies have focussed on combination effects of low doses of genotoxic carcinogens belonging to

¹ Dedicated to Prof. Dr. Dr. h.c. Wilhelm Doerr, on the occasion of his 75th birthday.

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classes that are environmentally important underlines the importance of this investigation. Implications of such research are to be expected for the extrapolation to human carcinogenesis.

MATERIALS AND METHODS

Chemicals NDEA, NPYR and NDEIA were synthesized and supplied by Dr. R. Preussmann (Institute of Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, FRG). The identity and purity (>99%) of the three test compounds was verified by chromatographic methods using detection both by thermal energy analysis and mass spectrometry.

Bioassay The methods of bioassay have been described in detail elsewhere.⁴⁾ In brief, 1800 male SD rats were treated as shown in Table I. The dosages had been set lower than those of previous experiments⁵⁻⁹⁾ to induce neoplasms towards the end of the animal's life span only. The aim of the study was to establish dose-response curves near to the no-observed-effect level for the individual N-nitrosamines as well as for their combination, and in addition, to determine possible combination effects.

Since all three carcinogens display similar organo-tropic carcinogenicity in rats, their common target organ — the liver — was used for detailed statistical analysis of tumor occurrence and combination effects following very low exposure levels. Our evaluation is implicitly based on the assumption that the incidence of other types of tumors and subsequent death is stochastically independent of the animal's fate caused by the liver tumor. Independence of those tumor risks will be difficult to confirm. From the fact that liver tumors were most frequently observed in this experiment and showed clear dose-dependency we conclude that any bias from this source is an order of magnitude smaller than the effects obtained in the present analysis. Furthermore, stochastic independence of death with liver tumor from death without fatal tumors could be assumed from the underlying experiment.

Statistical methods

Age-specific incidence: Time from the start of the experiment until death (age at death) and the absence or presence of a liver tumor were recorded for each animal of the 12 treatment groups and the control group. Age at death was analyzed by methods of censored survival times¹⁰⁾ taking death with liver tumor as the event of incidence and taking death without a liver tumor as the censoring event. Dose-dependent age-specific incidence was modeled by the proportional hazards model of Cox.¹¹⁾

For the investigation of a combination effect of the three compounds the following two multivariate proportional hazards models were fitted to the data:

$$\lambda(t|d_1, d_2, d_3) = \lambda_0(t) \exp(\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3) \quad (A)$$

and

$$\lambda(t|d_1, d_2, d_3) = \lambda_0(t) \exp(\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3 + \beta_4 d_1 d_2 d_3) \quad (B)$$

where the doses d_1 , d_2 and d_3 denote the three compounds NDEA, NPYR and NDEIA. The regression coefficients β_i describe the influence of the doses d_i and β_4 that of the combination, respectively, whereas $\lambda_0(t)$ denotes the baseline incidence rate if $d=0$. The mathematical symbol "exp" stands for the exponential function and means the power of the number e , e.g. $\exp(\beta_1 d_1) = e^{\beta_1 d_1}$.

The presence of a third-order combination effect — the only type of combination envisaged by the design — was tested by the null hypothesis $H_0: \beta_4 = 0$ versus $H_1: \beta_4 \neq 0$ by Wald's test as well as by the likelihood ratio test using the deviance between the two models. Relative risks of the single compounds or the combination were estimated as $RR_i = \exp(\beta_i)$. RR_i is equal to the factor by which the instantaneous risk of dying with the tumor of interest has to be multiplied if the dosage is increased by one unit. The validity of the proportional hazards model was checked by log(-log)-plots. In a preliminary analysis (results not shown here) the effects of each single compound as well as that of the combination were analyzed separately by using the univariate model.

Tumor-free survival: Curves of survival free of liver tumor were estimated for different combinations of dosages of the three compounds according to

$$S(t|d_1, d_2, d_3) = S_0(t)^{\exp(\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3)} \quad (C)$$

where $\beta_1, \beta_2, \beta_3$ were obtained from the fit to model (A) and where $S_0(t)$ was the estimate of the baseline survivor function according to Breslow.¹²⁾ Note that the survivor function S is a twofold power of S_0 (S_0 to the power of a number which itself is e to the power of the sum $\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3$). Plots of $S(t|d_1, d_2, d_3)$ were generated for ages larger than 500 days and surviving fractions from 1 to 0.75.

Potency of the individual nitrosamines and their combination: Within model (A) relative potencies of the compounds can be calculated directly as ratios of the regression coefficients β_i . Taking NDEA as reference $B_2 = \beta_1/\beta_2$ and $B_3 = \beta_1/\beta_3$ give the relative potency of NDEA with respect to NPYR and NDEIA: $B_2 d_1$ as a dose of NPYR and $B_3 d_1$ as a dose of NDEIA are equivalent to the dose d_1 of NDEA. Another potency measure applicable to each nitrosamine separately as well as to their additive combination was empirically obtained as follows: The univariate proportional hazards model was fitted to the data of the respective three experimental groups and the control group. Then the function of liver tumor-free survival was estimated on the basis of the fitted model as

$$S(t|d) = S_0(t)^{\exp(\beta d)} \quad (D)$$

From this we estimated the three quantiles $t_{0.90}(d)$, $t_{0.95}(d)$ and $t_{0.99}(d)$ of the liver tumor-free survival time and plotted them against dose.¹³⁾ The p -quantile $t_p(d)$ defined mathematically by

$$S(t_p(d)|d) = p \quad (E)$$

is the time up to which $(1-p)$ 100% of animals have died with a liver tumor found at necropsy if dose d had been given. The time $t_p(d)$ is long for low doses and short for high doses and its dose-dependent decrease can provide information on the potency of a carcinogen. We examined the functional dependency of $t_p(d)$ on dose d for the three selected percentages 90%, 95%, and 99% and observed a good agreement with linearity. Therefore, time to death of $(1-p)$ 100% of animals dying with liver tumor was fitted as a function of dose to the straight line

$$t_p(d) = a - bd \quad (F)$$

for each $p=0.90, 0.95$, and 0.99 and the negative slope b was taken as an empirical potency measure. Tentatively, confidence intervals for the slopes were calculated straightforwardly by linear regression methods applied to the pairs $(d_n, t_p(d_n))$. (Note that the dose levels used are discrete as a consequence of the piecewise constancy of the estimated survivor function S .)

Impact on the proportional hazards model: Modeling the quantile $t_p(d)$ as linear function of dose has consequences for the underlying semiparametric Cox model: $t_p(d) = a - bd$ forces the baseline survivor function (D) into a special form for large times t , namely $\exp[-\exp(m + nt)]$, which is mathematically equivalent to the survivor distribution of a special extreme value distribution,¹⁴⁾ where m and n are determined by the model parameters delineated above. It follows from the underlying Cox model that the intercept a may depend on p , but not the slope parameter b .

Relation to other potency measures: The potency measure b used here can be related to the index TD_{50} , the daily dose required to halve the probability of remaining tumorless at the end of the standard life span T_0 proposed recently by Sawyer *et al.*¹⁵⁾ TD_{50} can be generalized to TD_p , the daily dose required to get at time T $p\%$ tumors in those animals, which would remain tumorless if they were untreated, obtained formally as the solution of $S(T|TD_p) = (1-p) S_0(T)$. There is a simple relationship between the regression coefficient β in Cox's model $\lambda(t|d) = \lambda_0(t) \exp(\beta d)$ and TD_p , namely $\beta TD_p = \ln(1 + [\ln(1-p)/\ln S_0(T)])$. On the other hand, $t_p(d)$ as the solution of $S(t_p(d), d) = p$ gives the relationship $\beta d = \ln(\ln p / \ln S_0(t_p(d)))$. Therefore, both TD_p as a function of T and t_p as a function of d are defined through a simple equation involving the baseline survivor function S_0 . For TD_p time is fixed at first with reference to a standard situation, a dose is calculated depending on the percent-

age of observed tumors, and a fixed percentage, e.g., 50% is chosen for the assessment of potency. In our approach, a percentage of observed tumors is fixed at first, a time is calculated depending on the dose, and this dose-response relationship is quantitated in terms of its negative slope b .

Statistical software: Statistical calculations were performed by the program package SURVIVAL developed by the Department of Biostatistics at the DKFZ¹⁶⁾ and were run on an IBM 3090 computer.

RESULTS

The raw data of liver tumor incidences as shown in Table I were analyzed for the presence of a combination effect of the three compounds by using multivariate proportional hazards models as shown in equations (A) and (B). The results of the fit are shown in Table II; they indicate no significant third-order combination effect, since the product term of the three dosages ($d_1 \times d_2 \times d_3$) does not improve the quality of the fit. This is confirmed by the deviance of the two models, which equals 1.34 with a corresponding non-significant P -value of 0.25. In an additional analysis no influence of quadratic or cubic terms of the dose was found. Furthermore, the test for an additive combination effect (based on the sum of the three dosages of the combination part of the experiment instead of their product as shown in equation (B)) also showed no significant effect of that term.

Based on these results, model (A) was used for estimating curves of survival free of liver tumor within as well as below the experimental dose range according to equation (C). The curves of the highest and lowest experimental dosages (scaled by a factor of 10) together with an extrapolated tenfold lower dose are depicted in Fig. 1 a-d. No systematic or serious deviations were found when those curves were calculated for the actually used doses and were compared with the respective Kaplan-Meier estimates. Clearly, the extrapolations to lower dosages than experimentally used show a minor reduction of age-specific liver tumor incidence as compared to that which was achieved by an equivalent reduction within the experimental dose range. This means that the additional gain in liver tumor-free survival — if the dosages were reduced to one-tenth of the lowest experimental dose — is estimated to be maximally one-fifth of that gain in liver tumor-free survival, which was observed in the experiment.

The relative importance of the three carcinogens within the combination can be ranked according to the increases in relative risk of dying with liver tumor if the daily N-nitrosamine doses are increased by one unit (0.1 mg/kg). According to Table II, an increase of the NDEA dose would influence the combination's carcinogenic effect most heavily, as can be derived from the

Table I. Experimental Design and Results of a Carcinogenesis Experiment with N-Nitrosodiethylamine (NDEA), N-Nitrosopyrrolidine (NPYR) and N-Nitrosodiethanolamine (NDEIA) in Male SD Rats

Group no.	No. of animals	Single dose administered/day ^{a, b)}			Median survival time (95% conf. limits)	Incidence of malignant tumors (%)	Incidence of liver tumors (%)
		NDEA	NPYR	NDEIA			
1	500	—	—	—	931 (910–945)	29	0.6
2a	80	0.1	—	—	854 (816–892) ^{c)}	65	45.0 ^{d)}
2b	80	0.032	—	—	879 (812–939) ^{c)}	34	3.8 ^{d)}
2c	80	0.01	—	—	914 (879–1017)	29	2.5 ^{d)}
3a	80	—	0.4	—	951 (854–1001)	44	21.3 ^{d)}
3b	80	—	0.133	—	897 (875–948)	35	5.0 ^{d)}
3c	80	—	0.04	—	926 (907–989)	29	1.3
4a	80	—	—	2.0	928 (897–985)	35	7.5 ^{d)}
4b	80	—	—	0.63	934 (908–990)	36	1.3
4c	80	—	—	0.20	916 (889–968)	36	2.5 ^{d)}
5a	100	0.032	0.13	0.63	914 (875–983)	48	16.0 ^{d)}
5b	240	0.01	0.04	0.2	944 (908–983)	33	4.2 ^{d)}
5c	240	0.0032	0.013	0.063	937 (903–965)	31	1.7

a) mg/kg body wt.

b) N-Nitrosamines were administered via the drinking water 5×/week.

c) $P < 0.005$ versus control according to the Wilcoxon rank sum test.¹⁰⁾d) $P < 0.05$ versus liver tumor incidence in controls according to Peto *et al.*²⁴⁾Table II. Results of the Fit of the Multivariate Models (A)^{a)} and (B)^{b)}

	Regression parameter	$\beta^c)$	SE ^{d)}	RR ^{e)}	P-value ^{f)}	Relative potency of NDEA ^{g)}
Equation (A) ^{a)}						
NDEA	β_1	48.1	3.0	7.4×10^{20}	10^{-5}	1
NPYR	β_2	6.9	0.8	914.7	10^{-5}	7
NDEIA	β_3	0.8	0.2	2.2	0.0008	60
NDEA + NPYR + NDEIA	β_4	—	—	—	—	—
Equation (B) ^{b)}						
NDEA	β_1	48.6	3.1	1.2×10^{21}	10^{-5}	
NPYR	β_2	7.1	0.8	1.2×10^3	10^{-5}	
NDEIA	β_3	0.9	0.2	2.5	0.0001	
NDEA + NPYR + NDEIA	β_4	-127.0	111.5	0.0	0.3	

a) (A) $\lambda(t|d_1, d_2, d_3) = \lambda_0(t) \exp(\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3)$.b) (B) $\lambda(t|d_1, d_2, d_3) = \lambda_0(t) \exp(\beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3 + \beta_4 d_1 d_2 d_3)$.

c) Statistically estimated value of the respective regression parameter.

d) Standard error.

e) Relative increase in the risk of dying with liver tumor if the dosage is increased by one unit.

f) According to Wald's test (Kalbfleisch & Prentice, 1980).

g) Relative potency of NDEA calculated as β_1/β_2 for NPYR and β_1/β_3 for NDEIA.

relative risk of dying with liver tumor, which rises by 17 or more orders of magnitude if the NDEA dose is increased as compared to an equivalent increase of the NPYR and NDEIA doses. This is corroborated by the

relative potencies of 7.0 and 60.1 of NDEA compared to NPYR and NDEIA; i.e., for an equivalent risk of dying with a liver tumor the NPYR dose has to be 7-fold that of NDEA and 60-fold increased in the case of NDEIA.

Fig. 1. Curves of survival free of liver tumor for ages greater than 500 days and survival probabilities from 100% to 75% are shown for N-nitrosodiethylamine (a), N-nitrosopyrrolidine (b), N-nitrosodiethanolamine (c) and their combination (d). The dose range comprises the highest and lowest experimental dosages (dashed and solid lines, respectively) and, additionally, a tenfold lower dose (chaindashed lines). All curves are calculated from the fitted model (C).

In order to further quantify the potency of the individual nitrosamines and their combination, the fit to the proportional hazards model was used to calculate the 1%, 5%, and 10% quantiles of time to death with liver tumors (Fig. 2). From this graph the time can be assessed for any dose after which 1%, 5% or 10% of the animals have died with liver tumors, if treated with the individual N-nitrosamines (Fig. 2a–c) or their specific combination used in the experiment (Fig. 2d). The decreasing dose-response curves could be well fitted by straight lines according to the equation $t_p(d) = a - bd$. In this context the intercept a denotes the time by which a certain percentage of liver tumors has occurred spontaneously (Table III). This time varies in a relatively narrow range when estimated by using the data obtained from the single N-nitrosamines or their combination: from 1042–1058 days of age (1% of rats die spontaneously with liver

tumor) to 1219–1295 days (10% of rats die spontaneously with liver tumor, Table III).

The negative slope b of the regression lines offers a potency measure defined as decrease in the manifestation time of liver tumor incidence if the dose is increased by one unit (0.1 mg/kg N-nitrosamine) (Table III). Relative to NDEIA — the least potent carcinogen — the time to death with liver tumor per dose unit (0.1 mg/kg) is decreased by factors of 40 and 4 for NDEA and NPYR, respectively, and by a factor of 3 for the combination.

DISCUSSION

The present study was designed to estimate carcinogenic effects in the rat liver caused by exposure to very low levels of the N-nitrosamines NDEA, NPYR, NDEIA or their combination. The statistical evaluation of this

Fig. 2. Estimation of the relationship between individual daily N-nitrosamine dose and time to death with liver tumors. The time until which 1% (square), 5% (bullet) or 10% (triangle) of the animals have died with liver tumor due to administration of N-nitrosodiethylamine (a), N-nitrosopyrrolidine (b), N-nitrosodiethanolamine (c) or their combination (d) is indicated by the straight chaindashed, solid and dashed lines, respectively. All points are calculated from the proportional hazards model (D) fitted to the single nitrosamines and their combination.

study gives evidence of the underlying combination effect, and of carcinogenesis near to the "no-observed-effect level," and the implications of the results, and possible extrapolation to the human situation, are discussed.

Regarding the combination effect it has to be admitted that the large number of animals necessary to assess carcinogenic effects of low doses at a high level of accuracy caused restrictions in the design of the experiment with respect to the dose combinations to be tested. Only the combination of all three compounds was used, while

the three second-order combinations were omitted, so our ability to detect the presence of combination effects and to identify their type is limited. The data obtained, however, do not support the presence of any synergistic or antagonistic effect, but instead, the liver tumor risk can be described in this case solely by a multiplicative model of the age-dependent hazard of dying with a liver tumor. According to the statistical model which was used to fit the basic experimental data (A) the following conclusion can be drawn on carcinogenesis following low doses of genotoxic agents: within the original experimen-

Table III. Results of Linear Regression Applied to the Quantile Plots^{a)}

Carcinogen	Quantile (%)	a^b (days)	b^c (days)	95% confidence interval of b	Correlation
NDEA	90	1258	486	450-524	-0.98
	95	1188	466	439-594	-0.98
	99	1058	480	432-528	-0.96
NPYR	90	1237	55	44-67	-0.94
	95	1163	46	35-58	-0.91
	99	1047	60	39-80	-0.93
NDEIA	90	1295	14	8-20	-0.96
	95	1204	12	9-16	-0.96
	99	1042	11	6-15	-0.96
NDEA+	90	1219	31	29-33	-0.99
NPYR+	95	1158	31	28-33	-0.99
NDEIA	99	1048	36	32-41	-0.99

a) Linear regression $t_p = a_p - bd$, with t =time in days, d =individual dose per day.

b) Parameter a corresponds to the estimated time of liver tumor occurrence at zero exposure.

c) Parameter b denotes the decrease in manifestation time of liver tumors (in days) that corresponds to a 0.1 mg/kg increase of N-nitrosamine dose; the ratio of the combination is 0.05:0.2:1.

tal dose range a linear relationship exists in SD rats between daily N-nitrosamine exposure and the time to death with liver tumor. This is unusual, since many drugs display a linear increase in their effect on a logarithmic dose scale, only.¹⁷⁾ In previous experiments a linear relationship was found to exist between mean tumor induction times and corresponding daily doses of NDEA and NDEIA on a log-log scale.^{5,7)} The doses of the present experiment, however, were considerably lower than those investigated previously and no evidence against linearity can be derived from the data. The fit of the data to a straight line (Fig. 2a-d) as assessed by the product correlation coefficient appears to be reasonably good with $r > 0.96$ for all plots except those of NPYR, which showed a weaker correlation, but still larger than 0.91 (Table III). The potency measure obtained as the negative slope of these curves (b) is suited to quantify the differences in risk inherent to the various carcinogens and offers a frame to assess, e.g., the combination's potency. It was derived directly from the univariate regression model applied to the dose-response data of each compound separately, obtained from the fit to the proportional hazards model. It is tempting to extrapolate the quantile lines to doses lower than those experimentally used, and even to zero exposure, which is equal to the intercept of the regression lines. Thus, the opportunity is given to quantify the time to occurrence of up to 10% of spontaneous liver tumors at least approximately, if the variability of the slope and intercept estimates is considered and if a possible underestimation of the variability of the slope by use of the two-step procedure is taken into

account. The numerical value of the 10% quantile at zero exposure has to be interpreted with caution, however, since it is beyond the real life expectancy of the animals. The observed percentage of preneoplastic liver foci, which increased dose-dependently above the spontaneous background,⁴⁾ on the other hand, gives a hint that such a high incidence of liver tumors might have occurred spontaneously if the animals had lived long enough. This view is based on the observation, that preneoplastic liver foci — though spontaneously occurring at an advanced age — predict the appearance of subsequent liver cancer reasonably well.¹⁸⁻²¹⁾ Since both liver tumors and preneoplastic liver lesions appear in untreated animals at considerably high incidence, the question may be raised as to whether the carcinogen's activity merely enhances a physiological process, inherent to or concomitant with the process of aging.

The observed good agreement of the b estimates, which have to be identical for theoretical reasons, supports the appropriateness of this empirical measure in our analysis. Confidence intervals of the slope b have been calculated tentatively in order to quantify the variability of b . Because of serial correlations of the calculated dose-time pairs their actual sizes may be larger, and therefore, they should be used cautiously. The observed linear relationship must not hold for bioassays with a larger proportion of tumors ($1 - p > 0.10$) and therefore b can not be extrapolated with respect to $p < 0.90$; it even might not exist in this form, if, e.g., the relationship is no longer linear for $p < 0.90$. But we can still recommend the general approach in this case.

Cox's proportional hazards model¹¹⁾ was applied for the evaluation of the time to death with liver tumor, where absence of a liver tumor was taken as a censored observation.¹⁰⁾ Previous arguments favored this model for analysis of carcinogenicity data obtained from NDE1A²²⁾ compared to a parametric model such as the log normal- or the Weibull model. Its underlying assumption was assessed by the log(-log)-plot of the survival curves from this experiment and no serious deviations from parallelism were observed. Only the curve for the control group showed a somewhat different course; but this was based on too few observed events to consider it as a significant deviation.

The interpretation of time-to-death data in terms of tumor incidence used here assumes that for animals getting a liver tumor this neoplasm becomes the leading cause of death, and that this death comes rather quickly. The longer the animals could have lived with an unobservable liver tumor, and the more concurrent neoplasms present in the animal may have been related to its death, the more biased would be the statement on tumor incidence. In the worst case, however, the data can still be taken as they are, namely times-to-death with liver tumor. This points to the serious problem of occult tumor analysis in carcinogenesis^{23, 24)} for which at present no clear solution is available, if no assumptions about cause of death are made and if it is difficult to preplan interim sacrifice times.

Cox's model, being based on tumor-free survival data, also offers insight into complementary aspects of the influence of aging: A tentative extrapolation to carcinogenic effects of dosages as low as one-tenth of the lowest experimental N-nitrosamine doses (Fig. 1a-d) indicates a remarkably lower gain in liver tumor-free survival than that obtained by an equivalent reduction above this dose. This finding is dependent on the remaining low life expectancy of the animals when liver tumors were observed. All liver tumors of this experiment occurred in rats older than two years; the lowest experimental dosages and

assumed lower carcinogen levels are associated with corresponding times to liver tumor of 1100-1250 days, i.e., a time when less than 20% of the control rats were alive.⁴⁾ Therefore, if the incidence of liver tumors would be reduced by lowering the exposure to the N-nitroso compounds no measurable increase in liver tumor-free survival could be expected due to the limitation of the overall survival. At the dose range of N-nitrosamines allowing a median survival of more than 900 days a further reduction of carcinogen-induced liver tumor occurrence would not contribute to a longer overall survival of the animals due to competitive, probably independent, causes of death. In other words, a quasi-threshold of carcinogen exposure has been reached below which further reduction may alter the cause of death or the incidence of non fatal liver neoplasms but not the life expectancy. This quasi-threshold can be developed from the described model and might then have implications for the assessment of exposure limits of genotoxic carcinogens.

With regard to human exposure to low levels of different carcinogens, the following implications may be valid. a) The elimination of genotoxic carcinogens from the human environment will contribute to reduced tumor occurrence even if no firm relationship to a certain type of cancer has been established, since this will reduce the summation of small carcinogenic effects of genotoxins.²⁵⁾ b) Such a reduction will not necessarily translate into longer tumor-free survival if the neoplasms usually occur at the end of an individual's lifetime, which is in accord with the hypothesis that below a certain carcinogen exposure no observable carcinogen-related effects will occur within an individual's lifetime. c) A background of cancer incidence probably exists independently of exogenous induction and seems to be age-related only, so therefore will not be amenable to primary prevention. The latter view is in accord with recent epidemiologic estimates on the proportion of preventable cancers in certain organs.²⁶⁾

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