

Letter to the Editor

Toxicity and Carcinogenicity

Chris Whipple¹

The article by Lauren Zeise, Richard Wilson, and Edmund Crouch on the "Use of Acute Toxicity to Estimate Carcinogenic Risk," in Volume 4, Number 3 of *Risk Analysis*, reported a good correlation between acute toxicity (i.e., LD50) and carcinogenic potency for chemicals tested (under "high, not lethal but usually toxic doses") in mice and rats by the National Cancer Institute. The authors note that, without long-term bioassay data, an estimate of carcinogenic potency could be based on acute toxicity and the observed correlation.

This result raises scientific issues quite distinct from the potential regulatory application described by the authors. It is my understanding that mechanisms underlying an acute toxic effect and a long-term carcinogenic effect are generally thought to be unrelated for most chemicals. In simple terms, carcinogens at low doses classified as genotoxic are thought to change genetic materials through a probabilistic process (e. g., the one hit and multistage models of carcinogenic potency refer to the number of stages of genetic transformation associated with carcinogenesis). Epigenetic carcinogens are not thought to act directly on DNA, but are thought to be associated with genetic changes. Acute toxics are thought to operate differently (e. g., by producing disequilibrium

or loss of function of some organ sufficient to produce a serious short-term response).

If these simplified views are roughly correct, one would not *a priori* expect a correlation between carcinogenic and acute toxicity, unless perhaps both effects correlate with the chemical activity of the substance, which I presume toxicologists have considered. My questions then are these:

- What are the implications of this correlation with regard to the current assumptions about mechanisms of action for carcinogenic and acute effects?
- Is this correlation observed for substances in which the evidence of carcinogenicity comes from exposures well below the levels which produce an acute effect? Is it observed with known human carcinogens?
- Does this correlation suggest that some observed carcinogenicity may be an artifact of an acute effect caused by a high test dose?
- If high dose carcinogenicity and acute toxicity involve similar mechanism, are these mechanisms likely to be active at low doses?

Although I am not a health scientist, it seems to me that this result is puzzling in view of current assumptions about toxic mechanisms. I would be interested to read the reactions of toxicologists and other health scientists to this finding.

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Letter to the Editor

Reply to Comments: On the Relationship of Toxicity and Carcinogenicity

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Dr. Whipple questions what the observed relationship between carcinogenicity and toxicity⁽¹⁾ means. We do not know. Death from toxic effects and cancer are such different end points that a relationship is unexpected, but there does seem to be a strong relationship. The Editor has suggested that we supply a few details while replying to Dr. Whipple's comments.

After analyzing approximately 200 results of animal cancer bioassays, we were struck by the infrequency with which relatively nontoxic chemicals exhibit potent carcinogenic effects. There seem to be no chemicals with the low toxicity of saccharin but the high carcinogenic potency of TCDD. This suggested some correlation between toxicity and carcinogenicity, and so we began a systematic study of the chemicals tested by the National Cancer Institute NCI,⁽²⁾ and National Toxicology Program (NTP).⁽³⁾ We detail the analysis and results elsewhere.^(5,11) Meanwhile, Parodi *et al.*,⁽⁴⁾ interested in finding good predictors of cancer potency, compared the carcinogenicity of 21 chemicals with acute lethal toxicity (LD50 in moles/kg-bodyweight) and measures of mutagenicity from three different tests—DNA fragmentation, DNA covalent binding, and the Ames test. They found LD50 the best predictor. All 21 of these chemicals were mutagens and capable of covalent binding with macromolecules. Parodi *et al.* suggested that their observation might be explicable because the toxic effects of these chemicals are due to this binding and not to other types of biological action. For the chemicals tested by the NCI and NTP the relationship is just as strong,⁽⁵⁾ but it

is difficult to suggest the reason for this strength because there is a much greater range of probable biological actions in the NCI/NTP chemicals.

The NCI/NTP tests are designed to find "carcinogens," so the doses used are the highest which can be tolerated without causing early death or certain other (noncarcinogenic) adverse effects. Two results are clear in the NCI/NTP series:

1. *No chemical in this series induced tumors in all dosed animals.* This result would certainly be expected if some low toxicity chemical had the high potency of TCDD. There are only a few chemicals for which almost 100% tumor incidence occurred in one or more of the species/sex combination tested, and where the lack of 100% incidence may be due to high early mortality. Examples are carbon tetrachloride, dibromochloropropane, and 4,4'-thiodianiline.
2. *A chemical was more likely to exhibit carcinogenicity if a clear toxic effect was elicited.* The NCI/NTP experiments were run as close to a maximum tolerated dose (MTD) as could be achieved, but the actual toxicity of the applied doses varied from experiment to experiment. We found that chemicals tested at a maximum dose which did not elicit a toxic effect (early deaths or a weight depression) rarely induced a significant increase in tumor rate. This is shown in Table I for male rats, and similar results were found in preliminary analysis of results in female rats.

These results, taken together, show that *chronic* toxicity and carcinogenicity are related. A more detailed study showed that acute LD50's are also

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Table I. NCI/NTP Experiments on the Male Rat: Chronic Toxicity vs. Determination of Carcinogenicity

<i>Toxicity indicated by weight depression^a</i>			
NCI/NTP Carcinogenicity Finding	Not toxic ^b	Toxic ^c	Total
Positive	11	58	69
Negative	51	68	119
Total	62	126	188

<i>Toxicity indicated by weight depression^a or reduced survival^d</i>			
NCI/NTP Carcinogenicity Finding	not Toxic ^b	Toxic ^c	Total
Positive	8	61	69
Negative	49	70	119
Total	57	131	188

^aWeight depression = $(W_d - W_c)/W_c$, where W_c and W_d are average weights in the control and high dose groups, respectively.

^bIndicates less than 5% weight depression at the 10th, 20th, and 30th week of the bioassay.

^cIndicates the converse of "Not toxic" explained above.

^dSignificant numbers of dosed animals (compared to controls) dying well before first observed tumor of significance.

quantitatively related to the carcinogenic potencies of these chemicals, with a very strong correlation for some classes of chemicals. We chose to use LD50 because this appears to be the only readily available and widely reported quantitative measure of toxicity.

The relationship between toxicity and carcinogenicity is not perfect: not all chemicals induced cancer at toxic doses. More than half of those chemicals tested at toxic doses in male rats were not considered carcinogenic (70 out of 131) under the conditions of the bioassays. This suggests that cancers are not simply due to those toxic effects which may be measured by weight depression—a conclusion which is reinforced by the few large scale experiments in which the dose-relationship for carcinogenicity has been extended down to doses well below those which are overtly toxic (e. g., the 2-AAF megamouse experiment, and the DMN and DEN experiments analyzed by Peto *et al.*).⁽⁶⁾

We can highlight the relationship between toxicity and carcinogenicity by plotting one against the other (Fig. 1). Every chemical would fall somewhere on this plot if it is assumed (as we do) that LD50 and carcinogenic potency can be defined for each chemical (we do not exclude $\beta = 0$).

On such a plot we can distinguish three regions:

- A: Chemicals in this region show up with significantly increased tumor rates at some site in standard bioassays performed at MTD. We can therefore measure the carcinogenic potency in such bioassays with reasonable accuracy. If the bioassays were performed at higher doses, high early mortality from toxic effects would limit their usefulness. Bioassays at lower doses would require much larger numbers of animals to see any effect—but the large experiments on DEN, DMN and AAF indicate that an effect would be seen, and the carcinogenic potency estimated from such low dose experiments is effectively equal to that seen in experiments performed near the MTD.
- B: Chemicals in this region will have no significant increase in tumor rates in standard bioassays performed at the MTD, and so the best estimate of carcinogenic potency will be very uncertain and may be zero. Detection of carcinogenicity in this region requires experiments with much larger numbers of animals. Such chemicals would currently be classified as "noncarcinogens under the conditions of the bioassay."
- C: Chemicals in this region would produce 100% incidence of tumors of a given type in standard bioassays with doses of MTD and MTD/2. The "maximum likelihood" estimate of potency with such results would be infinity. In order to get a good estimate of potency, a bioassay would have to be performed at a dose well below the MTD—and conversely, if a bioassay in which the dose used is well below MTD comes up with significantly increased tumor rates (but below 100%) then the chemical lies in this region of the plot.

The width of the strip defining Region A depends principally on the number of animals in the experimental groups, the background tumor rate, and the spacing of the dose groups in the experiment—the first two mainly affect the boundary between A and B, the last the boundary between A and C.

The two results of the NCI/NTP series outlined above can be visualized easily on this plot. The first simply suggests that there are no chemicals (or very

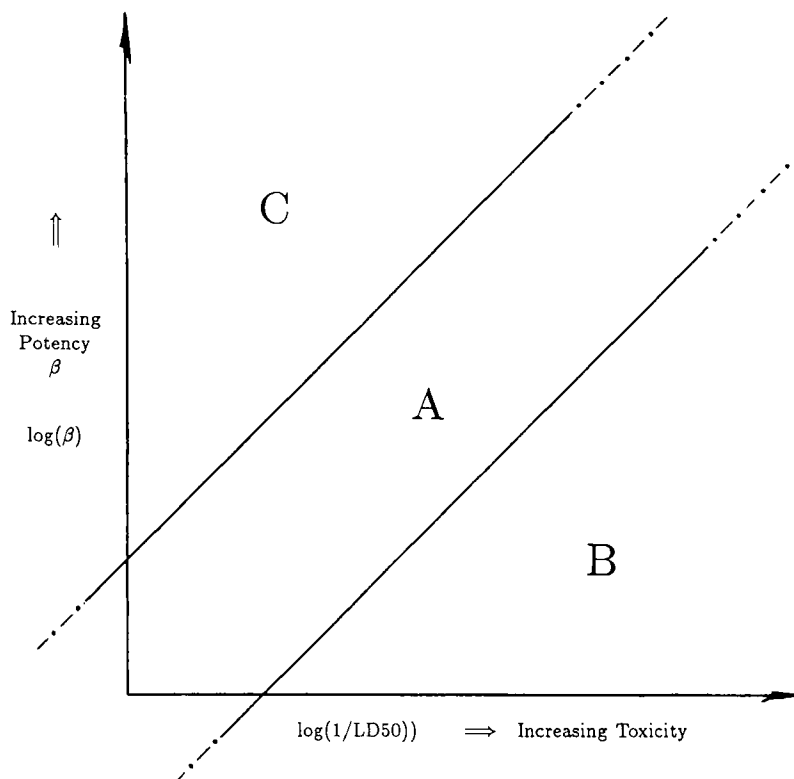


Fig. 1. Plots of LD50 vs. cancer potency (β) for standard bioassays performed using maximum tolerated doses (MTD). Region A: Rates for a given type of tumor are significant, but not all dosed animals have the tumor. Region B: Insignificant tumor response for all sites. Region C: 100% tumor incidence for a single site.

few) which lie in Region C. The second simply indicates approximately where the band A lies (quantitatively).

Bernstein *et al.*⁽⁷⁾ have demonstrated that the observed interspecies relationships between measured values of potencies can arise artifactually, if chemicals which lie in Regions C and B are censored from the observations. We want to stress that it is nature which appears to be doing the censoring from Region C, not us. 100% tumor incidence is simply not observed. Further, in looking at the relationship between carcinogenic potency and acute toxicity, we deliberately ignored insignificant results (Region B) since we were interested in getting a worst case estimate—how potent a chemical might be.

In Figs. 2 and 3 we show examples of experimental plots of the form of Fig. 1. We have censored Region B from these plots by excluding all chemicals in which the carcinogenic potency (β) was not significant at the 0.05 level. However, it is nature which

has censored Region C. All the points corresponding to chemicals in this series, which are not plotted in Fig. 2, must lie in the right lower portion of the plot.

The correlations are superior for those data in which the measured LD50 is believed to be most reliable [Fig. 2(B)], and for some particular classes of chemicals (e. g., Fig. 3). Because these plots give the upper envelop of possible values, risk estimates for NCI type chemicals from the LD50 using the best fit line are conservative.

The potency used in these figures is the same as we have used before,^(1,8,9) defined as the parameter β relating lifetime cancer incidence (R) in a population to an average daily dose (d) and background incidence (α):

$$R = 1 - (1 - \alpha) \cdot \exp\left\{\frac{-\beta \cdot d}{1 - \alpha}\right\}$$

where d is in mg/kg/day, and β is in mg/day/kg. For details on estimation, see Crouch.⁽⁹⁾

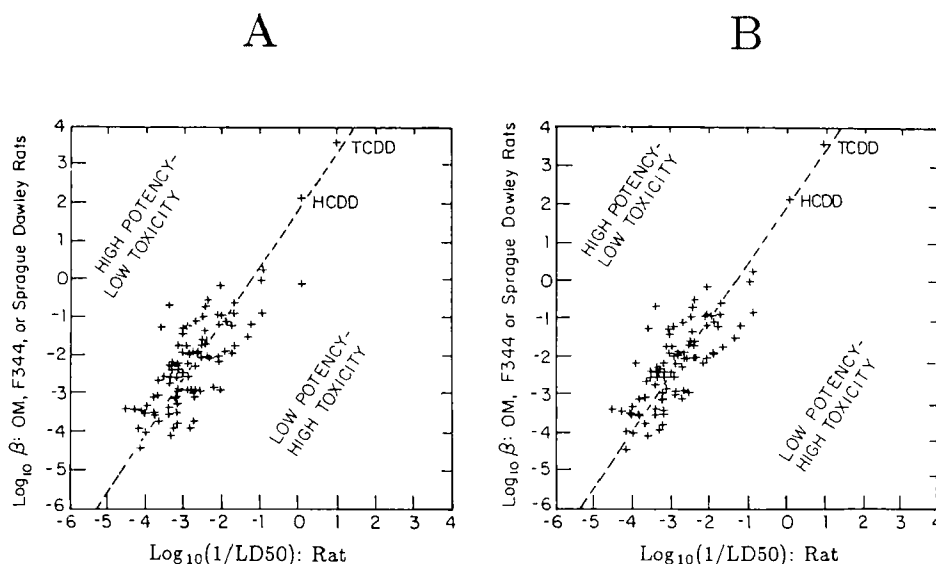


Fig. 2. LD50 vs. carcinogenic potency (β) measured in NCI/NTP rat experiments for (A) all cases where values for potency and LD50 were available; (B) cases with reliable LD50 values. Sometimes acute LD50 values were larger than a dose applied each day and tolerated in an NCI/NTP bioassay. This can happen when the LD50 is poorly measured or misreported; the NCI animals can be less sensitive than those used in acute toxicity studies; or the chemical may be rapidly metabolized and excreted, enabling the consumption of amounts greater than the LD50 in feeding experiments. If the published LD50 was smaller than a dose for which there was high survival, it was excluded from the analysis leading to Fig. 2(B).

We can illustrate the relationship between potency and toxicity in another way. The bioassays of the NCI/NTP series were carried out as close to the MTD as possible, and so we will not be far wrong if we assume the maximum dose used in the bioassay is equal to the MTD. Figure 4 shows histograms of the product $\beta \cdot D$, where β is the carcinogenic potency, and D is the maximum dose applied in the bioassay, for those results in which β is significant. One can alternatively think of this product as proportional to $D/CD50$, where CD50 is the median cancer dose (by analogy with LD50), since potency is approximately $\log_e(2)/CD50$.

For small values of $\beta \cdot D$, a large amount of chemical (compared to the toxic dose) is required to achieve carcinogenic effects, while for large values of $\beta \cdot D$ the cancer dose is small compared to the toxic dose. The figure shows that large values of $\beta \cdot D$ are strongly suppressed. On a plot like Fig. 1, constant values of $\beta \cdot D$ correspond to lines of unit-positive slope—like those sketched separating the regions. A value of $\beta \cdot D$ greater than 5, or perhaps 3.3, should probably be considered to be in Region C.

If the MTD and carcinogenic potency were unrelated, we would expect to see many more chemicals

with high values of $\beta \cdot D$. This can be confirmed by randomizing the potency values amongst the doses. The resultant histograms are also shown in Fig. 4. The deficiency of high $\beta \cdot D$ values in the observations is obvious. (The presence of nonsignificant observations complicates this comparison a little, but when these are included in the randomization, half of them are placed in the 0–0.2 bin, and the other half are indeterminate—but no distribution of them to the histogram can recover the experimentally observed histogram).

Getting around to the specific questions asked by Dr. Whipple, we do not know what, if any, implications there are for current assumptions about mechanisms of action for carcinogens. Correlations such as these have been ignored because there is no obvious biological relationship between the end points, although the observed relationships are not inconsistent with the current understandings of carcinogenicity and toxicity. There are some features in common to both acute lethal toxicity and chemical carcinogenesis. The chemical has to be absorbed, transported around the body, and possibly metabolized; active metabolites have to reach the site of action. As Parodi *et al.* point out, covalent binding

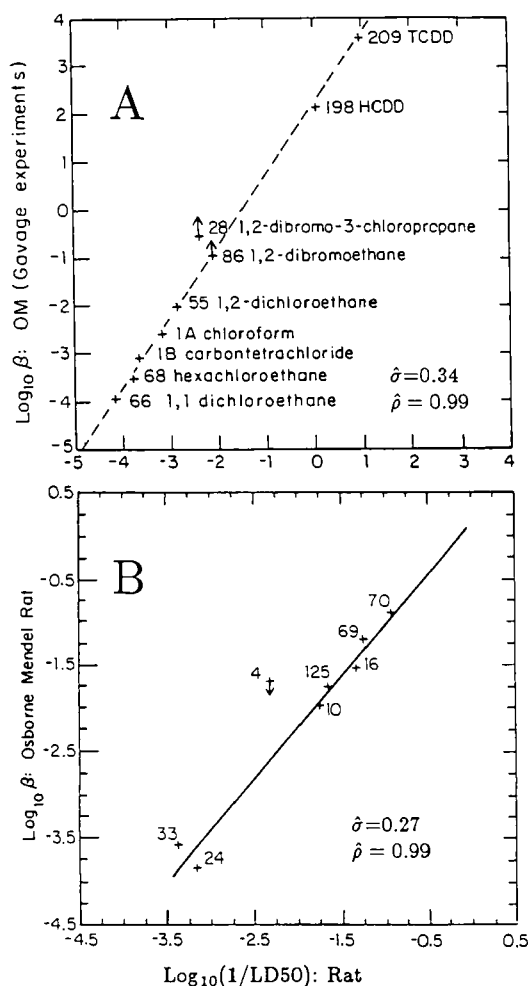


Fig. 3. Rat LD50 vs. rat potency measured in Osborne Mendel (OM) rats. Chemical numbers match the NCI/NTP *Technical Report* numbers. (A) Chemicals given orally by gavage. Potency may be underestimated (\uparrow) for the only 2 brominated alkanes tested because tumor incidence approached 100%. (B) Organophosphorous pesticides given in feed experiments. The potency was not statistically significant for dimethoate (chemical no. 4), and so the upper 95% confidence bound is plotted (\downarrow). Note: $\hat{\sigma}$ is the estimated error in the potency prediction at the geometric mean of the LD50 values used in the regression; $\hat{\rho}$ is an estimate of the correlation coefficient.

with macromolecules, an important determinant of mutagenic potency, could also be the main cause of toxicity in some cases.

Farber⁽¹⁰⁾ stresses that the interaction of a chemical with cellular DNA is necessary but not sufficient for initiation. A round of cell proliferation—to fix the modified DNA—is also required. Thus chemicals that can induce cell death, with consequent reparative proliferation, can play a major role in the induction

of cancers in tissues that are normally nondividing (e.g., liver and kidney). The commonality of these processes may exist even where lethal effects involve sites entirely different from the cancer site. For example, halogenated insecticides and industrial solvents cause liver cancer in mice, and liver, kidney, and endocrine cancers in rats; whereas, their acutely lethal effect is on the central nervous system. The commonality may occur because at lethal and sublethal doses all these compounds also produce secondary toxic effects, such as liver and kidney damage; some affect hormonal imbalances. Such secondary toxicities may be more directly related to the processes involved in the carcinogenesis. (For some classes of chemicals, lethal doses may be much smaller than secondary toxic doses; in which case such secondary effects could not be directly observed). For a class of chemicals with high LD50- β correlations (e.g., chlorinated alkanes), the secondary and primary toxicities could be nearly proportional, so that a chemical's LD50 would be an indicator of activity in a variety of biological processes.

It is not clear what Dr. Whipple means when he says, "exposures well below the levels which produce an acute effect." Many of the NCI/NTP carcinogens were tested at dose rates such that daily doses (or the bolus doses for gavage experiments) were substantially lower than doses producing acute effects. The cumulative doses could nevertheless be very high—many times the (acute) LD50. We used the LD50 as a toxicity measure simply because of its availability for chemicals that have not been tested in long-term bioassays—so that our results could be applied. The doses in the NCI/NTP bioassays were, however, close to a MTD for chronic effects.

If Dr. Whipple is asking about substances that elicit carcinogenic effects in standard bioassays at dose rates well below a chronic MTD, we would like to hear about such substances—for those are the ones whose absence is providing the correlation. The cases in which such effects have been observed are just those on which large-scale experiments have been performed, with large enough numbers of animals to detect small carcinogenic responses. The potencies derived from such experiments have generally agreed well with those estimated from high dose experiments. What is required to destroy the correlations is a set of chemicals for which in standard bioassays, carcinogenic effects are seen at dose rates of the order of 10^{-5} of the MTD.

For "human carcinogens," chemicals that can be directly shown to cause cancer in humans, we

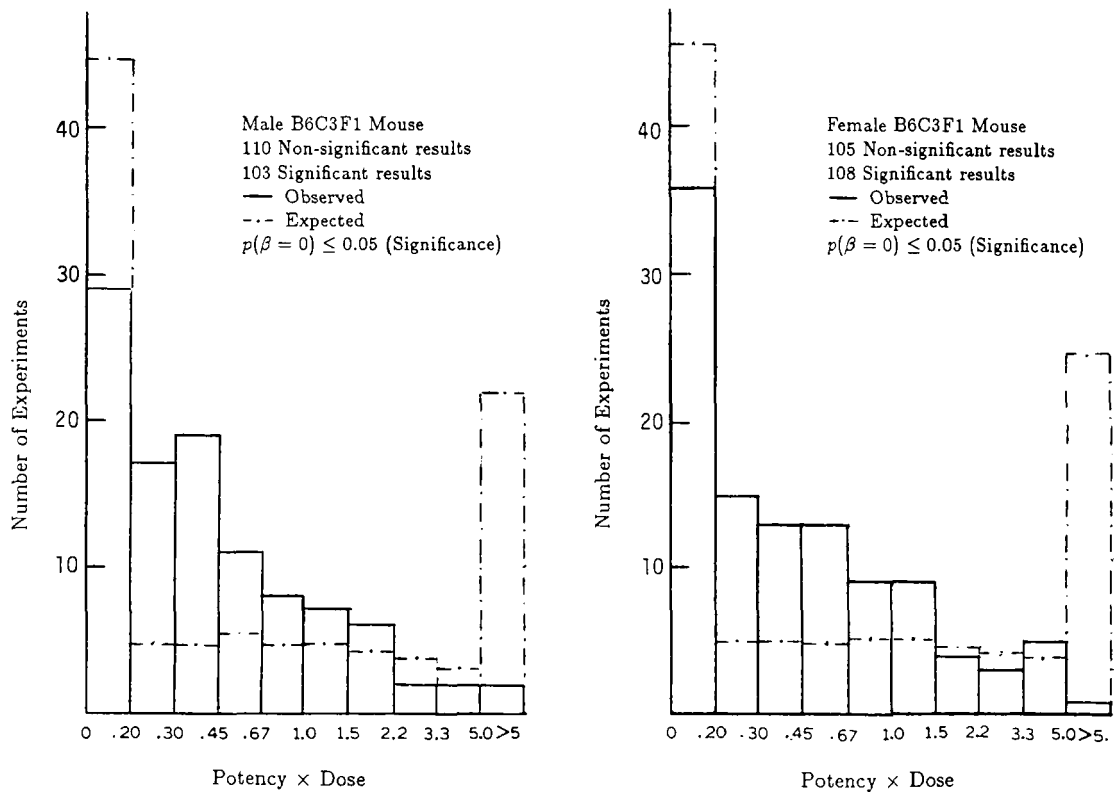


Fig. 4. Potency \times dose vs. the number of NCI/NTP experiments.

lack data. Much animal data on human carcinogens is difficult to analyze because of the nonstandard protocols used. However, several chemicals in the Parodi *et al.* comparisons discussed above were known human carcinogens.

The existence of a correlation between toxicity and carcinogenicity is necessary to support a hypothesis that some observed carcinogenicity is an artifact of a toxic effect (not necessarily acute) of high doses. As mentioned above, we do not think this is particularly likely—although there may be such cases—and we can make no comment on the question of whether a common toxic-carcinogenic mechanism acts both at high and low doses.

REFERENCES

1. L. Zeise, R. Wilson, E. A. C. Crouch, "Use of Acute Toxicity to Estimate Carcinogenic Risk," *Risk Anal.* **4**, 187-199 (1984).
2. National Cancer Institute (NCI), *Carcinogenesis Technical Report Series* (Public Health Service, National Institutes of Health, Bethesda, Maryland, 1976-1980).
3. National Toxicology Program (NTP), *Technical Report Series* (Public Health Service, National Institutes of Health, Research Triangle Park, 1982).
4. S. Parodi, M. Taningher, P. Boero, L. Santi, "Quantitative Correlations Amongst Alkaline DNA Fragmentation, DNA Covalent Binding, Mutagenicity in the Ames Test, and Carcinogenicity for 21 Compounds," *Mutation Res.* **93**, 1-24 (1982).
5. L. Zeise, *Surrogate Measures of Human Cancer Risk* (Ph.D. Dissertation, Harvard University, Cambridge, Massachusetts 1984).
6. R. Peto, R. Gray, R. Brantom, P. Grasso, *Effects on Two Tonnes of Inbred Rats of Chronic Ingestion of diethyl or Dimethyl-Nitrosamine: An Unusually Detailed Dose-Response Study* (Imperial Cancer Research Fund Cancer Studies Unit, Radcliffe Infirmary, Oxford, 1982).
7. L. Bernstein, L. S. Gold, B. N. Ames, M. C. Pike, D. G. Hoel, "Some Tautologous Aspects of the Comparison of Carcinogenic Potency in Rats and Mice," *Fund. Appl. Toxicol.* **5**, 79-86 (1985).
8. E. A. C. Crouch, R. Wilson, "Regulation of Carcinogens," *Risk Anal.* **1**, 47-57 (1981).
9. E. A. C. Crouch, "Uncertainties in Interspecies Extrapolation of Carcinogenicity," *Environ. Health Perspect.* **50**, 321-327 (1983).
10. E. Farber, "Cellular Biochemistry of the Stepwise Development of Cancer with Chemicals: G. H. A. Clowes Memorial Lecture," *Cancer Res.* **44**, 546-5474 (1984).
11. L. Zeise, E.A.C. Crouch, "A Possible Relationship Between Toxicity and Carcinogenicity," *J. Am. Coll. Toxicol.*, in press (1985).