LETTERS

Chemical Carcinogens

Thomas H. Maugh II's recent articles on chemical carcinogens (Research News, 29 Sept. 1978, p. 1200; 6 Oct. 1978, p. 37) were both provocative and timely, although we were disturbed by several errors of fact and interpretation. Our concern stems in part from preliminary results of our 2-year study (*1*) investigating quantitatively the carcinogenic potencies of more than 500 chemicals adequately tested and reported in the literature. We have also quantified the sensitivity of negative cancer tests, expressing how potent a carcinogen would have to be to be detected by these tests.

A dispute of long standing in regulatory circles is whether chemical carcinogens exhibit "thresholds," or doses below which there is either no effect or an effect considerably less than that predicted by linear extrapolation from responses at higher doses. Two investigators are cited by Maugh in support of the "threshold theory," Gehring on vinyl chloride and Stokinger on chloroform. Their arguments are either misleading or inaccurate and do not support the contention that the risk to society from these chemicals is negligible because of thresholds.

The first argument is that mechanisms detoxifying carcinogens are overwhelmed at the high dose levels often used in rodent bioassays, and a disproportionately large carcinogenic effect occurs at these doses. As an example, Gehring implies that at dose levels of vinyl chloride above 50 to 150 parts per million, where detoxification mechanisms may be saturated, a disproportionate increase in carcinogenic effect in rodents would occur. Yet the animal bioassay results of Maltoni (*2*), Lee *et al.* (*3*), and Viola *et al.* (*4*) on vinyl chloride suggest, if anything, just the opposite: as inhalation exposures increase beyond 150 ppm, the increase in carcinogenic response is *less* than that predicted from a linear extrapolation of the responses in the dose range 0 to 150 ppm (Fig. 1). Thus, the risk from exposure to low levels of vinyl chloride might be more than a millionfold higher than Gehring's estimate (*5*). Based on recent pharmacokinetic studies on vinyl chloride (*6*), the explanation for this proportionately greater activity at low doses may be that the mechanisms that *activate* vinyl chloride to the proximate carcinogen are saturated at high doses.

A second argument cited in support of the threshold theory is that high doses of chemicals cause pathological damage to tissues and increase their susceptibility to cancer. At low doses few tumors should appear. However, the evaluation of chloroform bioassay data attributed to Stokinger in support of this argument is incorrect. Stokinger is cited as saying that rodents in the National Cancer Institute (NCI) test had "severely distended abdomens, markedly shortened life-spans, and extensive liver damage. . . . Tumors were observed in only the most debilitated animals." In fact, however, in the bioassay on mice, (i) abdominal distension was caused by the liver tumors induced by chloroform treatment; (ii) the life-span of three of the four dose/sex groups did *not* differ from the controls, although each group *did* develop a high incidence of liver tumors; and (iii) neither extensive liver damage (for example, necrosis), reduced weight gain, nor any other pathological sign of nonneoplastic debilitation was associated with chloroform treatment (*7*). Furthermore, in the NCI rat bioassay, chloroform treatment increased kidney and thyroid tumors significantly without any increase in nonneoplastic damage to these tissues.

The assertion that Roe "fed chloroform to mice, rats, and dogs at doses that did not produce pathological damage, and these animals show no evidence of malignant tumors," is incorrect in two respects: Roe's doses in rodents (i) *did* produce pathological damage and (ii) *did* produce malignant tumors, although Roe found no relationship between pathological damage and tumor incidence in a tissue (*8*). Roe's test on dogs was not as sensitive as his test on rodents: chloroform would have to be more than 5 times as potent (on a milligram per kilogram per day basis) in dogs as in rodents to be positive in his tests. Thus, Roe's experiments on the carcinogenicity of chloroform are consistent with those from NCI and support the conclusion that chloroform is a renal and hepatic carcinogen in rodents.

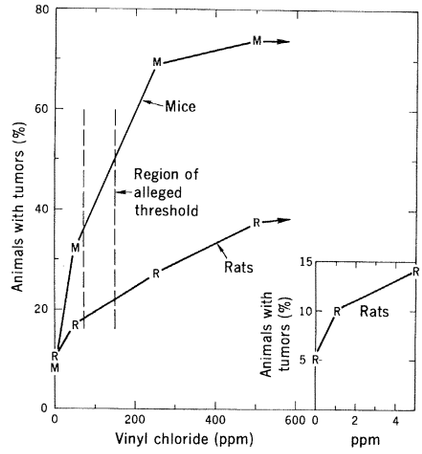

Fig. 1. Tumors from inhaled doses of vinyl chloride (*2*). The percent of Sprague-Dawley rats or Swiss mice with tumors is plotted against the vinyl chloride concentration in air (4 hours daily) over two dose ranges, 0 to 500 ppm (lifetime observation) and 0 to 5 ppm (87-week observation). There is no evidence for a threshold.

Thresholds, or a disproportionately small effect at low doses, may someday be demonstrated for certain carcinogens, but there is no evidence that they are the general case or that they have been demonstrated for vinyl chloride or chloroform.

In addition to the possibility of thresholds, Maugh also suggests that predicting human risk from animal cancer tests is extremely difficult because of large variations in carcinogenic response between sexes and species. Whether this is a difficulty, however, can only be decided by a systematic, quantitative analysis of the data, including an analysis of the power of negative cancer tests. Our preliminary results using this type of analysis suggest that there is generally good agreement between species, sexes, and strains. Considering the more than millionfold range in carcinogenic potency observed among chemical carcinogens, even an order-of-magnitude agreement between sexes and species would be quite useful, especially in supporting priorities for regulation.

With respect to the relationship between carcinogenic potency and mutagenic potency from short-term assays, Maugh refers to the reservations expressed by Ashby and Styles that such a relationship is tenuous, at best. Yet Maugh fails to mention, as we observed elsewhere (*9*), that their reservations were based on experiments with serious conceptual and technical errors. While we would not expect that the correlation would be precise, preliminary evidence (*10*) suggests that a battery of short-term tests may be quite useful in approximately estimating carcinogenic potency and thus in helping to set priorities among large numbers of new mutagens that have not been tested in animals.

Maugh also quotes Lijinsky as saying the *Salmonella* (Ames) test is not a useful qualitative predictor of carcinogenicity on the basis that it has only a 55 percent correlation for a group of polycyclic hydrocarbons that he tested for both mutagenicity and carcinogenicity (*11*). However, the Ames test has been extensively validated in three independent studies (*12, 13*), all of which agreed that approximately 90 percent of the carcino-

gens from a wide variety of classes were mutagens in the test. Furthermore, Lijinsky's study does show that the *Salmonella* mutagenicity assay is an excellent test for the detection of carcinogenic polycyclic hydrocarbons: 88 percent of the carcinogenic hydrocarbons analyzed (14 out of 16) were mutagenic in *Salmonella*. This agrees with a larger study on polycyclics by Coombs *et al.* (*14*), in which 38 out of 38 carcinogenic polycyclics were mutagenic in the test.

Lijinsky obtained the "55 percent correlation" estimate by combining his results on the 16 carcinogens with the 8 "noncarcinogens," of which 8 out of 8 were mutagens. Unfortunately, Lijinsky accepts the results of limited or inadequate cancer tests as definitive evidence that a chemical is a "noncarcinogen." But the word "noncarcinogen" has meaning only in the context of an analysis of the *power of the test*. We have discussed elsewhere (*15*) the problems with using Lijinsky's insensitive cancer tests for determining whether or not a chemical is a "noncarcinogen." His analysis of carcinogenicity was based entirely on skin painting studies, which are considerably less sensitive than the standard NCI feeding studies and do not meet NCI criteria for an adequate test. It remains problematical whether his skin painting studies would have detected very many of the known human carcinogens at the doses used, although the *Salmonella* test detects almost all of these (*13*).

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References and Notes

1. N. K. Hooper, C. Sawyer, R. Peto, R. Harris, A. Friedman, B. N. Ames, in preparation.
2. C. Maltoni, in *Conferences on Cell Proliferation*, vol. 4, *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winsten, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), book A, pp. 119-146.
3. C. C. Lee, J. C. Bhandari, M. Winston, W. B. House, R. L. Dixon, J. S. Woods, *J. Toxicol. Environ. Health* **4**, 15 (1978).
4. P. L. Viola, A. Bigotti, A. Caputo, *Cancer Res.* **31**, 516 (1971).
5. Gehring's estimate, based on an extrapolation from rodent data, is that the risk of liver angiosarcoma in *humans* is about 10^{-8} from exposure for over half a lifetime to 1 ppm of vinyl chloride. This contrasts with a risk of liver angiosarcomas in *rats* of greater than 10^{-3} (and mammary carcinomas of greater than 10^{-2}) from exposure for over half a lifetime to 1 ppm (*2*); in extrapolating to humans we estimate the risk of cancer might be in the range of 10^{-2} to 10^{-1}.
6. P. G. Watanabe, J. A. Zempel, D. G. Pegg, P. J. Gehring, *Toxicol. Appl. Pharmacol.* **44**, 571 (1978); P. J. Gehring, P. G. Watanabe, C. N. Park, *ibid.*, p. 581. It should also be noted that, if Maugh's figure 1, which suggests a threshold, is replotted with dose on a linear scale, the data fit equally well a straight line passing through the origin, suggesting no threshold.
7. "Report on the carcinogenesis bioassay of chloroform" (National Cancer Institute, Bethesda, Md., 1 March 1976); "Individual animal pathology table for chloroform" (National Cancer Institute, Bethesda, Md., 24 September 1976).
8. F. J. C. Roe, unpublished preliminary report of long-term tests of chloroform in rats, mice, and dogs. In four strains of mice, tumors developed in tissue which showed no other pathological damage, and tumors did not develop in tissue which showed pathological damage: ICI-Swiss mice had kidney tumors but no kidney damage; CBA and CF/1 mice had kidney damage but no kidney tumors; and ICI-Swiss mice had liver damage but no liver tumors.
9. B. N. Ames and N. K. Hooper, *Nature (London)* **274**, 19 (1978).
10. M. Meselson and K. Russell, in *Conferences on Cell Proliferation*, vol. 4, *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winsten, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), book C, pp. 1473-1481.
11. A. W. Andrews, L. H. Thibault, W. Lijinsky, *Mutat. Res.* **51**, 311 (1978).
12. I. F. H. Purchase, E. Longstaff, J. Ashby, J. A. Styles, D. Anderson, P. A. Lefevre, F. R. Westwood, *Nature (London)* **264**, 624 (1976); T. Sugimura *et al.*, in *Fundamentals in Cancer Prevention*, P. N. Magee *et al.*, Eds. (University of Tokyo Press-University Park Press, Baltimore, 1976), p. 191.
13. J. McCann, E. Choi, E. Yamasaki, B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5135 (1975); J. McCann and B. N. Ames, *ibid.* **73**, 950 (1976); in *Conferences on Cell Proliferation*, vol. 4. *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winsten, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), book C, pp. 1431-1450.
14. M. M. Coombs, C. Dixon, A.-M. Kissonerghis, *Cancer Res.* **36**, 4525 (1976).
15. B. N. Ames and L. Haroun, in preparation. For example, Lijinsky (*11*) lists benzo[*e*]pyrene as one of the eight "noncarcinogens." The *Salmonella* test shows that benzo[*e*]pyrene is a mutagen, but it is only 1/360 as potent as benzo[*a*]pyrene. Other short-term tests using animal cells also show that benzo[*e*]pyrene is a mutagen. Before Lijinsky can conclude that these tests are unreliable because benzo[*e*]pyrene is not a carcinogen, he should show that the power of the cancer test is such that it could detect a chemical 1/360 as potent as benzo[*a*]pyrene.

In his review (Research News, 6 Oct. 1978, p. 40) of some of the arguments concerning the existence of threshold doses in carcinogenesis, Thomas Maugh reproduces a graph [from David B. Clayson (*1*, p. 164)] as the basis for some people's argument that high dose-low dose extrapolation of animal data (and hence extrapolation from animal to man) is a nearly impossible task. The graph reproduced is incomplete in that the dose scale—log dose—that was in the original article is left out.

That graph implies a biological model—the logarithm of the percent of animals developing a tumor is linearly related to the logarithm of the dose. Not one of the extrapolation techniques that I know (Mantel-Bryan, Crump *et al.*, Albert-Altschuler, one-hit, multi-hit, log-logistic, and others) uses this model. I know no theory of carcinogenesis that is consistent with such a model. Gently put, the model is inappropriate. Finally, the confidence intervals as drawn can come only from an experiment without a low response at some low dose, that is, an experiment without a control group.

If the point is that an inappropriate biological model combined with a defective experiment is likely to give nonuseful answers, I agree wholeheartedly. If the point is that that graph provides a serious argument against extrapolation using a reasonable biological model from data gathered in a well-conducted experiment, then serious questions arise in my mind about its pertinence and their logic.

By way of analogy, I find that a picture of a centipede is not proof (or even evidence) that a horse is not a quadruped.

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References

1. D. B. Clayson, *Cancer Bull.* **29**, 161 (1977).

There is a continuing national debate over determining the risks of small amounts of toxic substances by extrapolations of massive exposure studies. The legend of figure 1 of Maugh's 6 October 1978 article on chemical carcinogens states "Most scientists, however, now think that the actual response is indicated by the smooth curve passing through zero dose and zero response." Dose is plotted on a logarithmic scale. In figure 2 response is plotted on a logarithmic scale. How do "most" scientists plan to extrapolate these logarithmic scales to zero?

All models of dose versus response in which the curve increases monotonically *must* include the points (0,0) and (∞, 100 percent), but this does not help to distinguish among them. In probit theory the relationship between log dose and susceptibility is a normal curve. Response is the integral of this normal curve which is "S shaped" but can give the impression of a straight line over narrow dose ranges. Probit analysis requires at least two real points to independently evaluate potency and the standard deviation of a diverse population to this effect.

It is ironic that so much energy is wasted on a debate over what amounts to the proper choice of coordinate axes.

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Mexican Oil Reserves

All of us owe a debt to William D. Metz for his commentary "Mexico: The premier oil discovery in the Western Hemisphere" (News and Comment, 22 Dec. 1978, p. 1261). Recognition in the United States of the Mexican accomplishment is long overdue, and Metz makes his point in a most effective man-