Can Laboratory Animal Carcinogenicity Studies Predict Cancer in Exposed Children?

David P. Rall

Assistant Surgeon General (Retired), U.S. Public Health Service, Washington, DC

A key to the prevention of childhood cancer is the control of carcinogens to which children are exposed. The first step in this process is to identify those chemicals that are likely to cause cancer in children. The best way to identify carcinogens, today, is the use of the rodent lifetime cancer test—the bioassay. The test has vocal critics, but is adequately reliable if properly used. Perhaps the major criticism concerns the use of the maximum tolerated dose as the highest dose tested. Critics claim that this dose causes cellular killing. The resultant cellular proliferation "fixes" preexisting mutations that can lead to cancer. This occurs but in a small fraction of the tests, and the high dose is necessary to achieve statistical sensitivity. All human carcinogens have been shown, when properly studied, to be carcinogenic in rodents. Many human carcinogens were first shown to cause cancer in rodent tests. Regulators rarely ban chemicals that have been demonstrated to be carcinogenic. Further, most chemicals in use today have not been properly tested. The potential errors in the rodent cancer test seem small when compared to the errors in the economic projections of the effects of restricting chemicals. Although not perfect, the rodent cancer test, when used properly, can help protect our children, and us, from cancer. — Environ Health Perspect 103(Suppl 6):173–175 (1995)

Key words: carcinogenicity, childhood cancer, pesticides, bioassay laboratory, animal tests, maximum tolerated dose (MTD)

The watchword is that prevention is the key to good health and to a smaller national health bill. It also is the key to protecting children from cancer.

The incidence of childhood cancer is rising while the death rate is decreasing. Unlike most adult cancer, much of childhood cancer is fortunately curable, but at a terrible cost in dollars ($100,000s plus) and great pain and suffering.

If the causes of childhood cancer can be identified and then controlled, cancer can often be prevented rather than treated.

Can we use laboratory animal tests to predict that a chemical will cause cancer in an exposed human? This is particularly important because animal tests offer the only realistic opportunity to detect carcinogenic chemicals before people are exposed to them. This concept is the basis for the 1976 Toxic Substances Control Act (TOSCA). But today it is being severely challenged by everybody from Ames and Ableson to *The New York Times*. I am particularly distressed by the *Times*.

Let me, at the outset, make two points. First, predictions of human health effects from laboratory animal tests are not perfect. But I know of no biological system that achieves perfection. Prediction of rat or mouse carcinogenicity from results in the other species is greater than 80% (*1*). Second, there is no other method available today that can predict, with precision, carcinogenic effects before they occur in the population.

In general there are three ways to predict human carcinogenicity for a new chemical. First is structure–activity relationships. These work well when dealing with a chemical from a known series of chemical moieties. A number of new computer-based systems seem quite good. But, since they are largely dependent on previous knowledge of toxicities, they may fail if a chemical from a completely unknown series is studied. For example, once it is known that folic acid antagonists are toxic, structural predictions do well. But, how is the first one of a series predicted?

There was great hope, some years ago, that short-term tests could screen those potentially carcinogenic compounds from all others and that expensive long-term testing could concentrate on those few. It does not look as if this is possible. A series of 75 chemicals that had been well tested in long-term mouse and rat studies were tested in four short-term assays: *Salmonella,* the Ames test, and three others (*2*). The *Salmonella* test predicted better than any other and no combination of tests was better than that for *Salmonella.* If the *Salmonella* test was positive, the rats and mice usually were positive. If the *Salmonella* test was negative, however, almost half the chemicals were carcinogenic. So far these tests can identify chemicals that are very likely to be carcinogenic, but can not surely identify ones that are negative and therefore safe with respect to carcinogenicity. In the best of all worlds, it would be the opposite.

The long-term rodent carcinogenicity test is the third predictive test. This test is not without disadvantages. It is expensive in both time and money. Each complete test costs hundreds of thousands of dollars and 1000+ hours of pathologist time. And it takes at least 9 months to initiate, more than 24 months to conduct, and another 24 months to analyze and write up. In the typical test, male and female inbred mice and rats are used. One group of 50 or more animals is given a high dose, the maximum tolerated dose (MTD), and two groups are given lower doses of the test compound. There is also a control group.

The high dose, the MTD, the highest dose that causes only minimal toxicity, which is necessary for maximum statistical sensitivity, is criticized because it may cause damage which may lead to cellular proliferation, increased mitosis, and eventually carcinogenicity. There are examples in which proliferation is implicated in the carcinogenic response. And these examples are used as if they are typical. In fact, a review of the 195 long-term tests from the National Toxicology Program (NTP) showed that lower dose(s) as well as the highest dose showed a statistically significant increase in cancer rates for 2/3 of the chemicals. In about 1/5 of the chemicals, cancer rates for multiple doses were increased but not statistically

This paper was presented at the Symposium on Preventing Child Exposures to Environmental Hazards: Research and Policy Issues held 18–19 March 1994 in Washington DC. Manuscript received: December 5, 1994; accepted: May 15, 1995.

Address correspondence to Dr. David P. Rall, 5302 Reno Road, N.W., Washington, DC 20015.

significant. Only 6% were truly high dose only positives. For the vast majority of chemicals, the high maximum tolerated dose is not the cause of carcinogenicity (*3*).

Marshall Anderson and his colleagues have studied oncogene activation in spontaneous and chemical-induced mouse and rat tumors. Two industrial chemicals, furan and furfural, are carcinogenic in rat and mouse livers, yet are negative in typical mutagenicity tests. But the pattern of activated oncogenes in the chemically treated animal liver cancers are qualitatively different from those of the controls. New oncogene mutations were observed: a different mutation in H-*ras* and a new K-*ras* (*4*). With methylene chloride, induced liver tumors had the same oncogenes as the controls, but in the treated excess lung tumors, different oncogenes were observed (*5,6*). If such studies can be performed on human tumors presumptively caused by chemical carcinogens, the results can be compared to those in the experimental studies. I would expect that the essential similarity between rodent and human carcinogenicity would be observed. The advent of the widespread use of the polymerase chain reaction (PCR), particularly with fixed tissue, will be enlightening. Imagine the power of human epidemiology if carcinogens left footprints!

A number of studies have shown that carcinogens are active at low doses that do not cause toxicity, e.g., 1,3-butadiene (*7*). Recent studies by Richard Peto and his collaborators are interesting and important. They studied the carcinogenicity of *N*-nitrosodiethylamine and *N*-nitrosodimethylamine in rats at doses that ranged over 2.5 orders of magnitude (0.033–16.896 ppm in drinking water). Younger rats were much more susceptible to the carcinogens than older rats; rats treated beginning at 2 weeks of age were 6 to 8 times as sensitive to the carcinogens as rats treated beginning at 20 weeks. At doses less than 1 ppm in the diet, the response was linear with dose, while above that the slope flattened (*8,9*).

The question is: How well do rodent tests predict for humans? First we must decide on the criteria for a positive test. In each chemical test male and female rats and mice are used, thus, four sub-experiments are embedded in each chemical test. And with dozens of tissues to be categorized for cancer, the possibilities for random false positives loom large. The details of this process are found in every NTP report. Hasemen at NTP has shown that the use of $p < 0.01$ instead of 0.05 greatly decreases the chances of a false positive. Less stringent standards are adequate for rare tumors. More reliance is placed on trend tests. Thus, 0, 1, 2, 3, or 4 of the sub-experiments can be positive. Is a chemical to be called a carcinogen if only one sub-experiment is positive? What about two? Certainly if three or four are positive, a call of carcinogenicity seems justified. A single positive is viewed as only suspicious; two positives suggest a need to look at the detailed results. Many who discuss carcinogens fail to use such a conservative rating system and any significant increase in one subexperiment (one species, one sex, one organ) is called positive. These persons often are the ones who suggest that "everything is carcinogenic," or "too many rodent carcinogens...." The conservative analysis of the results of the NTP tests suggests that no more than 20 to 25% are positive. It should be remembered that many of the chemicals chosen for study were already thought to be carcinogens.

How well do the results of laboratory animal tests predict for human carcinogenicity? As noted above, rats and mice predict for each other better than 80%. No such answer is possible for humans, since so few chemicals have been adequately studied in human populations. It is known, however, that all human carcinogens that have been adequately tested have been positive in animal studies, even arsenic. It has been claimed that many animal carcinogens have been shown not to be carcinogenic in humans. What has been shown is that most studies actually have been inconclusive because of small sample sizes and/or because of too low exposures. It is important to remember that the lack of evidence for carcinogenicity is not evidence for the lack of carcinogenicity!

An NAS NRC (*10*) study on pesticide practices examined the quantitative relationship between the dose in rodents that caused cancer and the dose in humans that caused cancer. The dose in humans was about the same as that in the most sensitive species for benzidine, chlornaphthazine, and cigarette smoke. The most sensitive rodent species was more sensitive to diethylstillbestrol (DES) and vinyl chloride. Other more recent studies have shown essentially similar results.

There are an increasing number of cases in which laboratory animal carcinogenicity studies have predicted human carcinogenicity before the fact. These include 4-aminobiphenyl, DES, mustard gas, *bis*-(chloromethyl) ether, estrogens, and vinyl chloride. Recent evidence now suggests that 1,3-butadiene, formaldehyde, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and dioxin also cause cancer in exposed people. Of long-term importance is the confirmation that dioxin is a human carcinogen at extremely low concentrations (*11*). The past regulatory response was that greatly limited dioxin contamination now seems appropriate. Old evidence, more recently unearthed, adds asbestos to that list. In the discovery process in the asbestos lawsuits, it was revealed that scientists at the Trudeau laboratory at Saranac Lake had, in 1942, shown that asbestos causes lung cancer in mice (*12*). But the industrial sponsors of that research prevented that information from becoming public. Remember that Doll published his epic paper in 1955 (*13*). Would this have made any difference? Actually, the Saranac Lake results were discussed at a closed seminar in 1952, which may have lead to Doll's paper.

It has been claimed that regulatory agencies over-respond to reports of rodent carcinogenesis and try to ban any chemical so implicated. Fortunately, the Congressional Office of Technology Assessment studied that claim (*14*). They found that most known rodent carcinogens were not regulated. The report found many regulatory gaps in which many if not most rodent carcinogens were not regulated and few if any were banned.

The most common assumption is that all widely used chemicals and processes have undergone thorough testing. It is not well known that, in fact, a rather small fraction of common chemicals, including food additives, cosmetics, medicines, agricultural chemicals, industrial and other chemicals have been properly tested for carcinogenicity. Another NAS NRC report on toxicity testing estimated that only about 10% of pesticides, 5% of food additives, 18% of medicines, and a low percent of high-volume chemicals had been tested adequately so that a complete hazard assessment was possible (*15*). This finding was confirmed by a congressional study of the House Subcommittee on Agriculture, which found that about 90% of pesticides had not been tested for carcinogenicity. A report by the Organization for Economic and Commercial Development (OECD) confirmed that most of the widely used chemicals had not been adequately tested.

Much attention is paid to the uncertainty in risk estimates, while little is paid to estimates of economic damage. One

example, albeit an old one, is instructive. David Dominick was an Assistant Administrator of U.S. EPA when he was presented with the problem of chlorinated hydrocarbon (a by-product of pesticide manufacture)-contaminated pasture land in Louisiana (*16*). He obtained estimates from agricultural economists of the number of cattle that were so heavily contaminated they would have to be destroyed. The answer was three. The others, fed clean diets, had their contaminant levels lowered enough to be considered marketable. The health risk versus the economic benefit (or damage) analysis, therefore, becomes increasingly murky. And yet there is a great reluctance to regulate strictly any chemical with significant economic importance. Remember the AD Little study of the potential impact if vinyl chloride (VC) were to be strictly regulated (*17*). They predicted economic disaster, the loss of 1.7 to 2.2 million jobs, and a decrease of $65 to 90 billion in the GNP. Yet after VC regulation, there was little impact on the VC or any other industry. Enclosing the production process was costly, but the saving of 5 to 10% of the VC production paid for the effort. And the company that developed the process made money by licensing it. Remember also that the entire asbestos industry was destroyed because of its failure to protect its workers and its repeated denial of health hazards.

Economic progress is necessary and must be supported and protected. At the same time, care must be used to ensure that the chemicals and processes used do not endanger the health of the workers or the general population. To protect these people and their children, those few critical toxic chemicals and processes must be identified, first by animal tests and if necessary also by epidemiological studies. This information can then be used to devise sensible regulations to protect the people. This is not banning; there are many techniques of risk management such as various restrictions limiting use or by labeling.

Winston Churchill described democracy as a flawed system, yet the very best we had, much better than other systems. Much the same can be said of long-term laboratory animal testing for carcinogen identification, only I do not believe that it is badly flawed. It is a biological system, and like all biological systems, it is not perfect. It does give important information—information that, if used wisely, can prevent the exposure of people to carcinogens, eliminate some disease, and save lives. And there is no alternative.

REFERENCES

1. Haseman JK, Seilkop SK. An examination of the association between maximum-tolerated dose and carcinogenicity in 326 long-term studies in rats and mice. Fundam Appl Toxicol 19:207–213 (1992).
2. Tennant RW, Margolin BH, Shelby M, Zeiger E, Haseman JS, Spalding J, Caspary W, Resnick M, Staesciewicz S, Anderson B, Miner R. Prediction of chemical carcinogenicity in rodents from genetic assays. Science 236:933–944 (1987).
3. Haseman JK, Lockhart A. The relationship between the use of the maximum tolerated dose and study sensitivity for detecting rodent carcinogenicity. Fundam Appl Toxicol 22:382–393 (1994).
4. Reynolds SH, Stoers SJ, Maronpot RR, Anderson MW. Activated oncogenes in B6C3F1 mouse liver tumor: implications for risk assessment. Science 237:1309–1317 (1987).
5. Kari FW, Marinpot RR, Anderson MW. Submission for the OSHA Hearing on the Proposed Occupational Standard for Methylene Chloride. September 16, 1992.
6. Devereux T, Foley J, Marinpot R, Kari F, Anderson M. Protooncogene activation in liver and lung tumors from B6C3F1 mice exposed chronically to methylene chloride. Keystone Symposium on Genomic Instability and Cancer. Cel Kinetics Society, March 1991.
7. Melnick R, Huff J, Chow BJ, Miller RA. Carcinogenicity of 1,3-butadiene in C57BL/C3H F1 mice at low exposure concentrations. Cancer Research 50:6592–6599 (1990).
8. Peto R, Gray R, Brantom P, Grasso P. Effect on 4080 rats of chronic ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine: a detailed dose-response study. Cancer Res 51:64415–6451 (1991).
9. Gray R, Peto R, Brantom P, Grasso P. Chronic nitrosoamine ingestion in 1040 rodents: the effects of the choice of nitrosoamine, the species studied and the age of starting exposure. Cancer Res 51:6470–6491 (1991).
10. NAS NRC Environmental Studies Board. Contemporary Pesticide Practices: Report of the Executive Committee. Washington:National Academy of Sciences, 1975;66–83.
11. Fingerhut MA, Halprin WE, Markow DA, Placell LA, Honchar P, Sweeney AL, Greife PA, Steenland K, Suruda AJ. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. N Engl J Med 324:212–223 (1991).
12. Llienfeld DE. The silence: the asbestos industry and early occupational cancer research—a case study. Am J Public Health 81:791–800 (1991).
13. Doll R. Mortality from lung cancer in asbestos workers. Br J Ind Med 12:81–89 (1955).
14. U.S. Congress. Identifying and Regulating Carcinogens. Washington:Office of Technology Assessment, 1987
15. NAS NRC. Toxicology Testing Needs. Washington:National Academy of Sciences, 1985
16. Dominick DD. HCB tolerances: an example of risk-benefit decision making. In: Decision Making for Regulating Chemicals in the Environment. Washington:National Academy of Sciences, 1975;64.
17. Society of Plastics Industries, Inc. Post-hearing memo to the Occupational Health and Health Administration. August 23, 1974.