Carcinogens and Human Health: Part 2

Bruce N. Ames and Lois Swirsky Gold (Perspective, 31 Aug., p. 970) and Philip H. Abelson (Editorial, 21 Sept., p. 1357) question the use and validity of long-term laboratory animal studies to identify potentially hazardous compounds. These laboratory experiments, like other biological assays, are not perfect; yet in the absence of adequate epidemiologic data they are the best available methods for identifying and assessing potential human health risks (*1*).

All of the chemicals known to cause cancer in humans also cause cancer in laboratory animals (*2*). Eight of these chemicals were first shown to induce cancer in laboratory animals (*3*); subsequently, epidemiologic investigations showed that they also induced cancer in humans (*2, 4*). A more recent example, 1,3-butadiene, widely used in the rubber industry, was reported in 1983 to cause multiple cancers in mice at concentrations permitted in the workplace (*5*). Further studies showed that 1,3-butadiene caused cancers at low inhalation exposures; epidemiological studies now suggest that 1,3-butadiene is carcinogenic to humans (*6*).

There are two major sources of information from which to estimate the potential adverse effects of such substances on public health: controlled laboratory studies with experimental animals and in vitro systems; and human epidemiology studies, often based on workplace exposures or inferred from less easily controlled studies of the general population (*7*). Gathering data about humans can confirm past hazards but, unfortunately, this information comes too late to prevent the continuation of disease and is costly and difficult to obtain (*8*).

Accordingly, the United States, the Organization for Economic Cooperation and Development (OECD), the World Health Organization's International Program on Chemical Safety, the International Agency for Research on Cancer (IARC), the Japanese National Institute of Hygienic Sciences, and various industrial laboratories are conducting studies in animals to estimate the risks to humans from chemicals, as well as to provide the basis for risk management and the reduction of potential human health hazards.

The National Cancer Institute (NCI) and, subsequently, the National Toxicology Program have evaluated approximately 400 chemicals for their toxic and carcinogenic effects in laboratory animals (*9*). These toxicology studies are typically carried out in both sexes of two species of rodents divided randomly into sets of 50 to 60 animals per exposure and control groups; three exposure concentrations are gradated down from a top level selected to show some toxicity that should not compromise unduly the animal's well-being or growth and survival. The criteria for selecting substances for comprehensive evaluation have evolved over time. Initially, applied research focused on suspected carcinogens; more recently, chemicals produced in great volumes and to which many people are exposed have been added to the list.

The magnitude of the task of evaluating these chemicals is enormous. In 1990, the Chemical Abstracts Services catalogued the 10 millionth unique chemical in their computer collection. In 1984, the National Research Council of the National Academy of Sciences estimated that information about the potential effects on humans exists for only 20% of the thousands of common chemicals (*10*). The staff of the House Agriculture Subcommittee on Department Operations, Research, and Foreign Agriculture compared information available to the Environmental Protection Agency (EPA) with the data required by law for 1200 active pesticide ingredients and found that for 79 to 84% of registered and commercially used active ingredients oncogenicity studies were inadequate and for 90 to 93% mutagenicity studies were inadequate (*11*). In 1990 the OECD reported that about half of the highest production chemicals had not been subjected to adequate toxicologic evaluation (*12*).

At present, 54 chemicals, mixtures of chemicals, or occupational exposures are considered carcinogenic to humans (*2, 3*). Of the 34 identifiable chemicals or mixtures of chemicals that have been shown to cause cancer in humans, 31 also induce cancer in animals. The remaining three have not been adequately studied in experimental models.

NCI studies have implicated certain pesticides in human cancers (*13*), and epidemiologist R. Doll has stated that the eventual number of occupational carcinogens could be quite large (*14*).

Since one object of laboratory animal experiments is to study the toxic effects of a compound, the effect must be elicited for the experiments to have value: the amount of chemical administered must produce a response. The exposure level used in long-term carcinogenesis experiments, often called the maximum tolerated dose (MTD) (*15*), has been selected to produce some mild toxic effects but not to alter normal growth and development. Unless cancer is the lethal end point, life-spans also must be equivalent to controls.

A range of doses is used to compensate for the relatively small number of animals, generally 50 to 100, in a test group (*1, 15, 16*). The maximum doses are sometimes relatively high, but rarely massive. In many cases (for example, 1,3-butadiene, benzene, phenacetin, vinyl chloride, methylene chloride, and ethylene dibromide) studies were conducted at exposure levels near or below that to which humans are actually exposed.

One of the myths surrounding the animal bioassay is that using the MTD can result in unique carcinogenic effects that are not present at lower exposure concentrations. Chemicals that are carcinogenic only at the maximum dose studied are historically rare. In approximately 90% of the compounds studied, supporting evidence for carcinogenicity at the same target site is seen at lower doses.

Regarding toxicity and carcinogenicity, D. G. Hoel *et al.* (*17*) reported that in 73 of 127 positive sex-species-specific experiments carried out by the National Toxicology Program (NTP), there was a statistically significant increase in tumor incidences in both the low- and high-dose groups, and in another 42 there was a numerically elevated carcinogenic response in the low-dose group relative to that in controls. Only 3% of the chemicals were considered to be possibly "high-dose only" carcinogens. For example, when 1,3-butadiene was studied in mice at inhalation exposure levels of 625 and 1250 parts per million (ppm), it was found to be carcinogenic (*5*). It was still carcinogenic at concentrations as low as 6.25 ppm (*18*), an otherwise nontoxic level. Thus, to separate chemicals into different categories of mechanism of action for risk assessment purposes on the basis of "high-dose only" results is premature.

Further, the systemic toxic effects (such as hepatotoxicity) of chemicals, are often not correlated with the site(s) of carcinogenic action (*17*). For example, Monuron, a pesticide, produced liver degeneration and hepatocytomegaly in male mice (toxicity with cell death and no cancer), but no increase in liver tumors. Asbestos causes mesothelioma without evidence of asbestosis. This supports the view that cancer is not merely a consequence of toxicity (*19*).

The study period for carcinogenesis bioassays is typically 2 years, not the lifetime of the animal model, which is closer to 3 to 4 years. In other words, the cancers that appear are those of late middle age, not of the very old. This may be important when we consider that the age-adjusted incidences of human cancers are rising (*20*), about 1% in each of the last 15 years, even as the human

lifespan in the United States is increasing, which makes early identification of hazardous substances more urgent (*21*).

An issue that is widely discussed concerns the evidence necessary to decide that a chemical is carcinogenic in rodents. In a large series of statistical comparisons, some apparently significant differences between chemically exposed and control groups will occur by chance (*16*). The NTP estimates that the false positive rate associated with NTP rodent studies is at most only 7 to 8% (*22*). The false negative rate (those chemicals that exhibit no carcinogenicity during the period of the study but that would eventually be shown to be carcinogenic) is much more difficult to evaluate. Further, each sex of each species is considered a separate experiment and is reported separately by the NTP. This permits others, such as the EPA or the IARC, to evaluate and index independently carcinogenicity.

Studies of cell division and reduction-oxidation reactions as mechanisms in the development of cancer are contributing to our understanding of the carcinogenic processes and are the subject of intense scrutiny in laboratories around the world. However, metabolic effects that occur in living systems and the interaction of many genetic factors appear to be crucial in these processes as well (*23*).

We are, indeed, developing a "new toxicology" based on well-conducted rodent studies supplemented by relevant information on pharmacokinetics, and mechanistic studies involving oncogene activation or suppressor gene inactivation. Scientists, including those at the National Institute of Environmental Health Sciences and elsewhere, have identified what appears to be the same activated *Ki-ras* oncogene in mouse and human lung tumors (*24*). The *H-ras* gene has been shown to have the same amino acid sequence in both humans and rodents (*25*). Similarly, tumor suppressor genes have also been identified across species (*26*).

Recently, it has been said that naturally occurring substances have not been adequately tested in NTP protocols. In fact, 25 to 30% of the chemicals evaluated so far in the NTP program, such as benzene, asbestos, and formaldehyde, occur naturally. However, few of the many substances that occur in plants have been tested under current protocols. Moreover, humans are exposed to mixtures of these compounds that include natural anticarcinogens, antioxidants, and fiber (*27*). The NTP welcomes nominations of such compounds for toxicologic evaluation as well as suggestions for innovative test methodology.

Recent research has documented increases in cancer mortality in industrial countries over the past two decades, increases not linked to cigarette smoking, aging, or improved diagnoses. All forms of cancer except lung and stomach cancer increased from 1968 to 1987, mainly in persons over age 55 (*21, 28*). Cancer is a complex of more than 200 diseases with multiple causes, multiple stages, and long latencies. In sifting through probable causes of these cancer patterns in industrialized countries, the role of a number of variables must be carefully assessed, including those linked to industrial chemicals, altered food supply, and lifestyle practices. Given the complexity of these multiple concerns, toxicology studies that use animals as surrogates for humans shall continue to play a major role in resolving these puzzles for cancer and for a host of other diseases (*8, 28*).

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Response: Rall is defending the National Toxicology Program (NTP) Carcinogen Bioassay Program that he directed for many years. Our papers do not argue to discontinue the bioassay program, but rather point out that we know more now than we did when the program was started, that certain serious difficulties should be addressed, and that results from bioassays are being used inappropriately.

Much of Rall's letter discusses occupational exposures to chemicals, which can sometimes be at very high doses. One purpose of the bioassay program has been to test industrial chemicals that workers have been exposed to at high levels. We agree with Rall that it is important to identify chemical carcinogens in the workplace. We have discussed in our own work that permitted worker exposure levels (PEL) for some rodent carcinogens are too close to the doses that induce tumors in test animals (*1*). For high occupational exposures little extrapolation is required from the doses used in rodent bioassays, and therefore assumptions about extrapolation are less important. This contrasts with the large extrapolations from the low doses of human exposures from pesticide residues or water pollution. While more occupational chemical carcinogens are likely to be detected, it seems unlikely that they will contribute to more than a few percent of all human cancer.

To extrapolate from levels at the maximum tolerated dose (MTD) or one-half the MTD, where almost all cancer tests are done, to the low exposure levels for the general population, however, requires information on mechanism such as those S. M. Cohen and L. B. Ellwein have used in their analysis (*2*). The attempt to prevent cancer by regulating low levels of synthetic chemicals by "risk assessment" with the use of worst-case, 1-in-a-million maximum risk scenarios is not scientifically justified. On average, 1-in-a-million maximum risk from a linearized model is 380,000 times below the MTD used in rodent bioassays (*3*). It seems to us unlikely that ingestion of any chemical at that level is of interest. Testing chemicals for carcinogenicity at near-toxic doses in rodents does not provide enough information to predict the excess numbers of human cancers that might occur at low-dose exposures. In addition this cancer prevention strategy is enormously costly and could be counterproductive if it diverts resources from more important risks.

The current regulatory process does not take into account (*4*) (i) that the natural world of chemicals makes up the vast bulk of chemicals humans are exposed to; (ii) that the toxicology of synthetic and natural toxins is not fundamentally different; (iii) that about half of the *natural* chemicals tested chronically in rats and mice at the MTD are carcinogens; and (iv) that testing at the MTD can frequently cause chronic cell killing and consequent cell replacement (a risk factor for cancer that can be limited to high doses) and that ignoring this mitogenesis effect greatly exaggerates many low-dose risks.

Positive results are remarkably common in high-dose screening tests for carcinogens, clastogens (agents that break chromosomes), teratogens, and mutagens (*4*). About half the chemicals tested, whether natural or synthetic, are carcinogens in chronic, high-dose rodent tests. About half the chemicals tested as clastogens in tissue culture tests are positive. A high proportion of positives is also reported for rodent teratogenicity tests: 38% of the 2800 chemicals tested in laboratory animals "have been teratogenic" in the standard, high-dose protocol. It is therefore reasonable to assume that a sizable percentage of both synthetic and natural chemicals will be reproductive toxins at high doses. Mutagens may also be common: of 340 chemicals tested for carcinogenicity in both rats and mice and mutagenicity in *Salmonella*, 46% were mutagens and 70% were either mutagens or carcinogens or both. Mutagens were nearly twice as likely to be carcinogenic as nonmutagens. How much the high frequency of positive results is due to bias in selecting chemicals is not known. Even if selection bias doubled the percentage of positives, which we think is unlikely, the high proportion of positives would still mean that almost eveything natural we eat contains carcinogens, mutagens, teratogens, and clastogens. Thus, testing a random group of natural pesticides and pyrolysis products from cooking should be a high priority for these various tests so that an adequate comparison can be made to synthetic toxins. The NTP selection of chemicals to test has paid almost no attention to natural pesticides and pyrolysis products in our diet.

What chemicals should be tested in the bioassay, given that we are living in a sea of rodent carcinogens (as defined by high-dose tests), the vast proportion of which are likely to be natural? We need to take a broader view of the chemical world and try to identify the greatest potential carcinogenic hazards, whether natural or synthetic; only a tiny fraction of the chemicals humans are exposed to are ever going to be tested in rodent bioassays.

We have recently compared the possible hazards of some rodent carcinogens, using the ratios Human Exposure/Rodent Potency (HERP) and Permitted Exposure/Rodent Potency (PERP). One strategy for choosing chemicals to test is to prioritize chemicals according to how they might rank in terms of possible hazard *if* they were to be identified as rodent carcinogens. A useful first approximation is the analogous ratio of Human Exposure/Rodent Toxicity (HERT). HERT would use readily available LD_{50} values rather than the TD_{50} (carcinogenic potency) values used in HERP. LD_{50} is related to the MTD and the TD_{50} (*5*), and the ranking of human exposures on HERP and HERT will likely be similar. The number of people exposed is also relevant in attempting to prioritize systematically among chemicals. Chemicals with high HERT and population exposure could then be investigated in more detail as to mutagenicity, mitogenicity, pharmacokinetics, and so forth, as discussed by Cohen and Ellwein (*2*) and by Rall. Natural and synthetic chemicals could both be ranked, and if natural chemicals in foods such as chlorogenic acid in coffee, psoralens in celery, or indole carbinol in broccoli turned out to be important, they might be bred out or, for processed foods such as coffee, extracted.

There are alternative strategies to that of testing chemicals one by one that may lead to identifying more important risk factors for human cancer. If the NTP did a series of bioassays each with a particular vitamin or micronutrient deficiency in the rodent chow, we believe they could turn up a series of carcinogenic risks that are of major importance for people. In mice, a marginal folate deficiency is very effective at breaking chromosomes (*6*). More than 30% of the

U.S. population is marginally folate-deficient. There is also epidemiological evidence that folate deficiencies cause birth defects in humans. Accumulating epidemiological evidence indicates that vitamins E and C and betacarotene are major protective factors against both cancer and heart disease, yet a sizable percentage of the public is deficient in these antioxidants. Choline deficiency increases cancer rates in rats (7). In addition, calorie reduction dramatically lowers mitogenesis rates and spontaneous tumor rates in rodents. Protein reduction lowers spontaneous tumor rates in rats. Ad libitum feeding, which encourages overeating, is routinely done in bioassays; overeating increases spontaneous tumor rates, and a variation in food intake is important in tumor incidence (*8*). Human cancers can be due to a variety of factors, such as dietary imbalances, hormones, and chronic infections, that are not likely to be uncovered by screening chemicals in rodents, even if we knew which chemicals to test (*9*).

The NTP strategy to analyze mechanisms is a useful change. Increased mitogenesis rates are clearly important in mutagenesis, and we believe that also adding routine measurements of mitogenesis to the 13-week toxicology study and the 2-year bioassay would provide information that would improve dose setting, interpretation of experimental results, and risk assessment. Such information may help to distinguish among rodent carcinogens, for example, between butadiene and sodium saccharin, for which the risk at doses a hundred times below the MTD appears to be vastly different. The work of Cunningham *et al.* at the NTP is a good example of how mechanism studies help to differentiate among chemicals. Their experiments showed that with two pairs of mutagenic isomers (1- versus 2-nitropropane and 2.4- versus 2.6-diaminotoluene), one isomer a carcinogen and the other not, only the carcinogen was mitogenic (*10*). It may be that half the rodent carcinogens are not acting as genotoxins in vivo and that their risk at low doses is zero, but we should look for compounds like butadiene that may be carcinogens at doses as low as 100 times below the MTD (*4*). If there are super carcinogens (*5*), butadiene is a possible example. Butadiene and vinyl chloride are DNA cross-linking agents, and it would be of interest to see whether this property is important in unusual activity at low doses. Studies of mechanisms, including mitogenesis, should help to clarify this. It is clear that the mechanisms of action for all rodent carcinogens are not the same and that one cannot use a simple linearized risk assessment model for all of them.

Rall states that it is a "myth" that testing at the MTD can result in effects that are unique to the high dose and cites the analysis of Hoel *et al.* We think that it is not a myth and that there is accumulating evidence to support mitogenesis effects unique to high doses for particular chemicals analyzed, for example, formaldehyde, melamine, and saccharin. One-half the MTD (which is the "low" dose in a bioassay) is a high dose and can also result in mitogenesis. Our point is that rodent bioassays provide virtually no information about low doses because they are conducted at the MTD and one-half the MTD, both high and close to one another in comparison to low-dose human exposures. It is a rare chemical that is tested across a range of doses. With only two doses and a control in cancer tests, information about dose-response is limited. Even at these two high doses, 44% of the positive sites in NTP bioassays are statistically significant at the MTD, but not at one-half the MTD (among 365 positive sites analyzed in the Carcinogenic Potency Database). Because the NTP bioassays do not measure mitogenesis, Hoel *et al.* (*11*) used an indirect, but inadequate, method to examine the issue. We have discussed the details of this inadequacy (*4, 12*).

Rall cites a recent paper (*13*) that purports to show an overall increase in cancer mortality rates; however, eminent epidemiologists dispute the interpretation (*14*).

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Cold Spring Harbor

Leslie Roberts' quotes of my comments in her News & Comment article "Cold Spring Harbor turns 100" (26 Oct., p. 496) misrepresent what I feel about Cold Spring Harbor's new neuroscience center. I may well have said that such a big jump in the size of the laboratory "is enough to give one sleepless nights" and that it will be "a problem to populate that huge building," but I certainly did not wish to imply that James Watson might fail in this latest endeavor. He has shown in the past an astonishing ability to pick people and make projects flourish, and I have absolutely no doubt that once more he will be successful.

I would also like to comment on the impression given by the article of the financial history of the laboratory. A major crisis occurred just before I became director in 1963. At that time the laboratory was in debt by an amount roughly equal to 50% of its annual budget. When I arrived, the summer program was just beginning and the cash reserve was enough to meet 2 weeks of payroll. That was what gave *me* sleepless nights. By the time Watson became director, in 1968, we had paid off the debt, increased the budget by 30% a year, and built up a reasonable cash reserve. The main problem he faced was to attract good scientists when he could not offer them financial security. The development of the laboratory over the past 22 years will always be seen as a monument to his success.

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Erratum: In the seventh paragraph (column 3) on page 1204 of Marcia Barinaga's Research News article "Biology goes to the movies" (30 Nov.), the diameter of the microtubules observed by Nina and Robert Allen and their colleagues should have been given as 25 nanometers, not 25 angstroms.

Erratum: In the report "Broadly neutralizing antibodies elicited by the hypervariable neutralizing determinant of HIV-1" by K. Javaherian *et al.* (14 Dec., p. 1590), the headings for tables 4 and 5 on page 1592 were incorrectly interchanged.