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Human Carcinogens So Far Identified

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The massive exploitation of natural resources, of which tobacco and asbestos are two conspicuous, though very different examples, and the synthesis of industrial chemicals have generated new hazards and new carcinogens which have been added to older ones. The majority of the over 50 agents that have been firmly identified so far as being human carcinogens belong to the relatively new hazards, that is environmental chemicals or chemical mixtures to which humans have been exposed only during the last century and a half. They are of more importance for cancer occurring in men than in women, and there is no evidence so far that they are related to cancers occurring at some of the most common target sites in either sex. It would be mistaken to believe that complete cancer prevention could be achieved solely by controlling these new, or relatively new, carcinogenic agents, but it would be similarly wrong to deny the importance of trying to control them and of continuing to do so. The experimental approach for the identification of carcinogens has an irreplaceable role to play in preventing the dispersal into our environment of new hazards and in identifying among the chemicals already in use, those that are carcinogenic. That a closer integration between the epidemiological and the experimental approaches may succeed in substantially reducing the size of the unknown region within the spectrum of cancer-causing factors, is today's hope that awaits confirmation. At the same time, advances in the understanding of the mechanisms underlying the different steps of the process leading to the clinical manifestation of cancer may help in the uncovering of agents and risk factors that the approaches used, at least in the way they have been used until now, may not have been apt to identify.

Key words: Human carcinogens — Role of experimental data — Environmental factors — Cancer prevention

The identification of etiological agents of cancer has depended on the availability of at least two forms of evidence: the high incidence of a relatively rare disease in well defined population groups, and the results of experimental carcinogenicity tests.

The identification of cancer-causing agents has therefore been conditioned by the observational nature of the epidemiological approach and its consequent potential for bias, and by the simplified cause-effect relationship and schematism underlying long-term carcinogenicity tests, in which tumors are taken to occur as a direct consequence of the exposure to a single agent. This characteristic of long-term tests descends directly from the experiments of Yamagiwa and Ichikawa in 1915,[1] which marked the beginning of experimental carcinogenesis. These experiments were followed a few years later by those of Tsutsui,[2] who described the induction of benign and malignant skin tumors in mice using a method which was adopted all over the world and remained one of the most widely used for many decades. It was almost inevitable that the search for carcinogenic agents became oriented towards those agents that these two approaches could most easily and reliably identify.

It has been sometimes claimed that man-made carcinogens are more easily identified than natural carcinogens, but the partition between man-made and natural carcinogens is rather artificial; for instance, tobacco is a natural plant, but cigarettes and tobacco smoke can hardly be called natural products. Another example is asbestos, a naturally occurring mineral, but it is only through mining, milling, factory production and the handling of asbestos products that it is disseminated into the environment, leading to direct human exposure.

The confusion between natural and man-made products, together with the idea that prevailed for some time that only man-made products could be carcinogenic, may have been responsible for the fact that the carcinogenicity of ionizing radiation was noted and reported only seven years after the discovery and introduction of X-rays in medicine,[3,4] but that it took another half a century before it was accepted that natural radiation too, particularly that from radon gas, may play a role in the causation of human cancer.[5,6] What is now a much discussed issue — the levels of natural radiation in dwellings — refers to one of the oldest, if not the oldest, environmental carcinogenic factor to which humans have

been exposed. On the other hand, the view that the exposures to natural products may be as relevant and probably even more relevant to human cancer than exposures to man-made products, has recently been vigorously presented.[7]

It has been more a rule than an exception that it takes a very long time before a "new" carcinogenic hazard is recognized and fully appreciated: about 100 years elapsed from the time cigarette smoking became a widespread habit before the carcinogenic hazard was fully recognized and accepted.[8] Although the smoking of tobacco began to spread within Europe not long after the discovery of America, cigarette smoking started to become common only after the production of cigarettes was industrialized. The first cigarette factory was built in Havana, Cuba, in 1853, the second in London in 1856 and the third in Virginia in 1860.[9] The habit of smoking underwent a great expansion during World War I, when soldiers were provided with cigarettes either free or at a subsidized rate.

A massive expansion of industry in general, and of the chemical industry in particular, was taking place at about the same time as the first industrial production of cigarettes. Thus, people have been significantly exposed to etiological agents of cancer firmly identified up to now, with the exception of radiation, combustion products, mycotoxins, and possibly viruses, for a relatively short period of time. Among the relatively recent carcinogens, one could indeed include asbestos and certain metals, since widespread exposure to them began less than two centuries ago.

Experimental and Epidemiological Evidence for Carcinogenicity

One of the first authoritative lists of cancer-causing agents, and probably the best at that time, was prepared by a WHO Expert Committee in 1964.[10] Exposure to sunlight, tobacco smoking, chewing of betel, nass (tobacco mixed with ash, cotton oil or sesame oil and in some areas lime) and tobacco, consumption of alcohol, atmospheric pollution, some medicaments, ionizing radiation and several specific industrial cancer hazards were listed among the recognized etiological factors susceptible to control.

That report, which is still well-worth reading today, gave large credit to experimental carcinogenesis and to the results obtained from long-term carcinogenicity testing. Testing was, in fact, extensively recommended, with the implication that the results obtained could serve as a basis for preventive measures, and would confirm, in some instances, epidemiological observations. The further testing of tobacco smoke was, for instance,

Table I. Industrial Processes Causally Associated with Human Cancer

Exposure	Target organ	
	Human	Animal
Aluminum production	Lung, bladder (lymphoma, esophagus, stomach)[a]	No relevant data
Auramine, manufacture of	Bladder	Mouse, rat: Liver (auramine, technical grade)
Boot and shoe manufacture and repair	Leukemia, nasal sinus (bladder, digestive tract)	No relevant data
Coal gasification	Skin, lung, bladder	No relevant data
Coke production	Skin, lung, kidney	No relevant data
Furniture and cabinet making	Nasal sinus	Inadequate evidence (wood dust)
Hematite mining, underground, with exposure to radon	Lung	Inadequate evidence (hematite) Rat, dog: Lung (radon)
Iron and steel founding	Lung (digestive tract, genito-urinary tract, leukemia)	No relevant data
Isopropyl alcohol manufacture, strong-acid process	Nasal sinus (larynx)	Inadequate evidence (isopropyl oils)
Magenta, manufacture of	Bladder	Inadequate evidence (magenta)
Painters (occupational exposure as)	Lung (esophagus, stomach, bladder)	No relevant data
Rubber industry	Bladder, leukemia (lymphoma, lung, renal tract, digestive tract, skin, liver, larynx, brain, stomach)	Inadequate evidence

a) Suspected target organs in parentheses.

Table II. Chemicals and Groups of Chemicals Causally Associated with Human Cancer for which Exposure Has Been Mostly Occupational

Exposure	Target organ	
	Human	Animal
4-Aminobiphenyl	Bladder	Mouse: Liver, bladder Rat: Mammary gland, intestinal tract Rabbit, dog: Bladder
Arsenic and arsenic compounds[a]	Skin, lung (liver, hematopoietic system, gastrointestinal tract, kidney)[b]	Mouse, hamster: (lung, respiratory tract)
Asbestos	Lung, pleura, peritoneum, gastrointestinal tract, larynx	Rat: Lung, pleura, peritoneum (mesothelioma) Mouse: Peritoneum Hamster: Pleura, peritoneum
Benzene	Leukemia	Mouse: Lymphoma, lung, Zymbal gland Rat: Various sites, including Zymbal gland, oral cavity
Benzidine	Bladder	Mouse: Liver Rat: Zymbal gland, mammary gland Hamster: Liver Dog: Bladder
Bis(chloromethyl)ether and chloromethyl methyl ether (technical grade)	Lung	Mouse: Lung, local, skin Rat: Lung, nasal cavity Hamster: (respiratory tract)
Chromium compounds, hexavalent[a]	Lung (gastrointestinal tract)	Mouse: Local Rat: Lung
Coal-tars	Skin, lung (bladder)	Mouse: Lung, skin, local Rat: Lung Rabbit: Skin
Coal-tar pitches	Skin, lung, bladder (gastrointestinal tract, leukemia)	Mouse: Skin
Mineral oils, untreated and mildly treated	Skin (respiratory tract, bladder, gastrointestinal tract)	Mouse, rabbit, monkey: Skin
Mustard gas (Sulfur mustard)	Lung, larynx, pharynx	Mouse: (lung, local)
2-Naphthylamine	Bladder (liver)	Mouse: Liver, lung Rat, hamster, dog, primates: Bladder
Nickel and nickel compounds[a]	Nasal sinus, lung (larynx)	Mouse, rat, hamster, rabbit: Local Rat: Lung
Shale-oils	Skin (colon)	Mouse: Lung, local Rabbit: Local
Soots	Skin, lung	Mouse: Skin, local Rat: Lung
Talc containing asbestiform fibres	Lung (pleura)	Inadequate evidence
Vinyl chloride	Liver, lung, brain, lymphatic and hematopoietic system (gastrointestinal tract)	Mouse: Liver, mammary gland, lung Rat: Liver, Zymbal gland Hamster: Liver, skin, forestomach Rabbit: Lung, skin

a) The evaluation of carcinogenicity to humans applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group.
b) Suspected target organs in parentheses.

recommended, in spite of the overwhelming epidemiological evidence for its carcinogenicity in humans. Such an attitude was not much different from that prevailing when Passey[11] in 1922 painted the skin of mice with coal tar, using the method developed by Tsutsui,[2] and obtained results which were regarded as providing final

Table III. Drugs Causally Associated with Human Cancer

Exposure	Target organ	
	Human	Animal
Analgesic mixtures containing phenacetin	Renal pelvis/ureter, bladder	Rat: (kidney, renal pelvis, liver)[b]
Azathioprine	Lymphoma, skin, mesenchymal tumors, hepatobiliary system	Mouse: (lymphoma) Rat: (lymphoma, Zymbal gland)
N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine)	Bladder	Mouse: (lung) Rat: (local)
1,4-Butanediol dimethanesulfonate (Myleran)	Leukemia	Mouse: (leukemia, lymphoma, ovary)
Chlorambucil	Leukemia	Mouse: Lung (ovary, lymphoma) Rat: Lymphoma, leukemia
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	Leukemia	Rat: (lung)
Cyclophosphamide	Bladder, leukemia	Mouse: Leukemia, lung, local, mammary gland Rat: Bladder, mammary gland, leukemia
Diethylstilbestrol	Cervix/vagina, breast, testis (endometrium)	Mouse: Cervix/vagina, uterus, mammary gland, ovary, lymphoma Rat: Mammary gland, pituitary Hamster: Kidney, cervix/vagina, endometrium
Estrogen replacement therapy	Endometrium, breast	As below, for Estrogens, steroidal
Estrogens, nonsteroidal[a]	Cervix/vagina, breast, testis (endometrium)	for diethylstilbestrol, see above for dienestrol Hamster: (kidney) for hexoestrol Hamster: Kidney for chlorotrianisene No adequate data
Estrogens, steroidal[a]	Endometrium, breast	for conjugated estrogens Hamster: (kidney) for estradiol-17β and esters Mouse: Mammary gland, pituitary, uterus, cervix/vagina, testis, lymphoma, bone Rat: Mammary gland, pituitary Hamster: Kidney Guinea-pig: Uterus for estriol Mouse: (mammary gland) Hamster: (kidney) for estrone Mouse: Mammary gland Rat: Mammary gland, pituitary, adrenal Hamster: Kidney for ethinylestradiol Mouse: Pituitary, mammary gland, uterus, cervix/vagina Rat: Liver, pituitary, mammary gland Hamster: Kidney for mestranol Mouse: Pituitary, mammary gland Rat: Mammary gland

Teble III — continued

Exposure	Target organ	
	Human	Animal
Melphalan	Leukemia	Mouse: Lymphosarcoma, lung Rat: Peritoneum
8-Methoxypsoralen (Methoxsalen) plus UV radiation	Skin	Mouse: Skin
MOPP and other combined chemotherapy including alkylating agents	Leukemia	No adequate data
Oral contraceptives, combined[c]	Liver	Similar to above for progestin and estrogen combinations
Oral contraceptives, sequential	Endometrium	for dimethisterone in combination with ethinylestradiol Inadequate evidence
Treosulfan	Leukemia	No data available

a) The evaluation of carcinogenicity to humans applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group.
b) Suspected target organs in parentheses.
c) There is also conclusive evidence that these agents protect against cancer of the ovary and endometrium.

confirmation of the observations made by Percival Pott on humans a century and a half before.

It was around the time of the publication of the WHO list, under the pressure of the overwhelming epidemiological evidence of the carcinogenicity of tobacco smoke on the one hand, and the difficulty of reproducing the striking findings in humans in animal studies on the other, that an attitude began to prevail which encouraged epidemiologists to lay down certain criteria for assessing causation for chronic diseases in humans which could essentially stand on the epidemiological evidence alone.[12, 13] The attitude prevailing today is that only epidemiological studies can provide unequivocal evidence that an exposure is carcinogenic to humans. This has had as a consequence that the experimental evidence, in particular that obtained in long-term animal tests, has been often regarded as a sort of second-rate type of evidence: it is claimed that chemicals proven to be carcinogenic in animals cannot be considered human carcinogens until there is epidemiological proof. As a matter of fact, it has been sometimes regarded as being less relevant than the results obtained in short-term tests for genetic and related effects,[14] in spite of the fact that in the latter, the end points are not always clearly linked to a carcinogenic mechanism and therefore they may be of uncertain relevance to malignant transformation. In general, though, the piece of information that is lacking is considered to be the most important. For example, in the case of alcoholic beverages, it is said that they should not be classified as carcinogenic to humans because the experimental evidence is missing, notwithstanding the convincing epidemiological evidence.[15]

Within the IARC Monographs Programme, chemicals for which less than sufficient evidence of carcinogenicity in humans is available would not be assigned to Group 1 (agents carcinogenic to humans) notwithstanding the extent of the evidence of carcinogenic activity provided by experimental testing.[16] The availability of relevant epidemiological data may therefore introduce a bias in the compilation of a list of agents recognized as carcinogenic to humans. In particular, it is not always clear what determines whether an exposure will become the object of an epidemiological investigation. Even if there were willingness to do a study, cohort or case control studies may sometimes not be feasible. Epidemiological data also arrive always too late, that is, some people must have already developed cancer at a time when probably a much larger population may have been or continues to be exposed. The IARC recommends therefore that in the absence of adequate human data, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans. The IARC thus attempts to reconcile a scientifically objective analysis of the data with an interpretation of the evidence of carcinogenicity provided by experimental data that is biologically plausible, is public health oriented, and takes into account the principles of primary prevention.

Recognized Carcinogenic Agents

Some 25 years after the report of the WHO Expert Committee of 1964[10] the list of etiological factors of human cancer is considerably longer (IARC Mono-

Table IV. Environmental Agents and Cultural Risk Factors Causally Associated with Human Cancer

Exposure	Target organ	
	Human	Animal
Aflatoxins	Liver (lung)[a]	Mouse: Liver, lung; Rat: Liver, kidney, colon; Hamster, primates, ducks, fish: Liver
Alcoholic beverages	Oral cavity, pharynx, larynx, esophagus, liver (breast)	Inadequate evidence
Betel-quid with tobacco	Oral cavity (pharynx, larynx, esophagus)	Mouse: (skin, local); Hamster: (forestomach, cheek pouch)
Erionite	Pleura, peritoneum	Mouse, rat: Pleura, peritoneum
Radon and its decay products	Lung	Rat, dog: Lung
Tobacco products, smokeless (chewing tobacco, oral snuff)	Oral cavity (pharynx, esophagus)	Inadequate evidence
Tobacco smoke	Lung, bladder, oral cavity, larynx, pharynx, esophagus, pancreas, renal pelvis (stomach, liver, cervix)	Rat, hamster: Respiratory tract
Hepatitis B virus[b]	Liver	
Human T-cell leukemia virus[b]	Leukemia	
Ionizing radiation[b]	Leukemia, skin, various internal organs	Various organs
Ultraviolet radiation[b]	Skin	Skin

a) Suspected target organs in parentheses.
b) Not yet evaluated in IARC Monographs.

graphs 1–48), but still reflects the absolute preponderance of environmental chemical agents, although radiations and some viruses are included. Twelve of the cancer-causing agents of the WHO 1964 list were environmental chemicals or complex chemical mixtures, and so are 53 of the 57 exposures recognized today as carcinogenic to humans. This large majority of chemical agents may lead one to assume that cancer is a disease predominantly related to environmental chemicals. One may then ask if the chemical agents so far identified are actually the most important ones and, consequently, if the tests used for their identification were suitable to identify the important agents responsible for human cancer. These points will be discussed making use of the data base of the IARC Monographs Programme, from which the lists of human carcinogens in Tables I–IV have been mostly, although not exclusively derived.

For convenience, the recognized agents evaluated as being carcinogenic to humans are presented in separate lists of industrial processes (Table I), chemicals and groups of chemicals for which exposures have been mostly occupational (Table II), drugs causally associated with cancer in humans (Table III), and environmental and culturally determined factors (Table IV).

These lists reflect the point made above, that most recognized carcinogens (as well as most of the probable human carcinogens) are chemicals to which humans have begun to be exposed only within the last 150 years. The human species has in fact been confronted with the massive presence of man-made chemicals in the general and working environment and with the expansion of the most carcinogenic cultural habit (tobacco smoking) only since the middle of the last century.

Cancer of Men and of Women and in Different Organs

One limitation of the present lists of recognized carcinogenic agents is their imbalance in relation to cancers in men and women. This can be partly explained by the facts that men have been much more frequently exposed to carcinogenic hazards through their occupation, took up the habit of smoking earlier than did women and, where the habit of drinking alcoholic beverages is common, they drink more than do women. It may also, however, reflect the bias of our society to show concern about matters involving men.

The etiological agents of human cancer so far firmly identified are predominantly associated with tumors occurring at certain sites. Nineteen of the 57 factors causally associated with cancer in humans induce lung cancer, 12 bladder cancer, 12 leukemia/lymphoma, and 10 skin cancer (see Tables I–IV). Among the common target organs for firmly identified carcinogenic agents,

Table V. Risk Factors for which an Association with the Occurrence of Human Cancer Has Been Observed but a Causal Relationship Has Not Been Established

Agent	Target organs
Clonorchis sinensis	Liver
Schistosomia haematobium	Urinary bladder
Opisthorchis vivarrini	Liver
Epstein-Barr virus	Rhinopharynx, lymphatic system
Papilloma virus	Cervix uteri
Thirty-six chemicals and three complex mixtures,[a] including	Various sites
Diesel exhaust	Lung
Petroleum refining (occupational exposures)	Leukemia, skin
Dietary factors (?)	Various sites

a) Classified in Group 2A (probably carcinogenic to humans) in volumes 1–48 of the IARC Monographs. A possible association with the occurrence of human cancer also exists for 180 additional chemicals listed in Group 2B.

notably absent are cervix and breast (diethylstilbestrol and other estrogens are the only chemicals which have been shown to be related to breast cancer), the most frequent cancer sites in females, colon-rectum, stomach (until very recently the most frequent site of cancer worldwide),[17] ovary, brain and prostate. Although these absences can be mitigated by the fact that there are indications of possible causal association between certain exposures and cancer at some of these sites, we can hardly ignore the extent of this gap in our understanding of the etiology of many human cancers. Special efforts should be concentrated on exposures for which a probable or possible association has been observed, but no causal link has been established (Table V). Among these are papilloma viruses, which may play a role in the causation of cervical cancer[18, 19] and dietary factors which are likely to play some role in the origin of cancer occurring in the gastro-intestinal tract and other sites, possibly including breast.[20–22]

Economic Issues

A major misunderstanding concerning long-term animal tests is the claim that their exorbitant cost has deprived other fruitful approaches to cancer prevention, as well as basic research, of adequate funding. Waste of money and resources may have occurred, but there is no evidence that a lack of adequate funds for basic research was due to overspending on long-term carcinogenicity tests.

Moreover, if one looks at how much effort has been actually put into the experimental demonstration or confirmation of carcinogenicity of certain agents, one can hardly accept that too much has been spent on testing. For instance, according to the IARC Monographs data base, there have been 36 adequate long-term animal carcinogenicity studies on asbestos (involving a total of 8900 rats and about 1000 mice), 14 on cyclophosphamide (involving 760 rats and about 1200 mice), 9 on 4-aminobiphenyl (involving 70 rats, 3500 mice and 60 dogs), and 16 on 2-naphthylamine (involving 400 rats, about 1500 mice and 70 dogs). Fewer studies and a much smaller number of animals would have been sufficient to prove or disprove the carcinogenicity of asbestos fibers and in fact the majority of these studies aimed at understanding the carcinogenic process. Whatever the total cost of such tests might have been (the cost per animal may differ considerably from country to country) and even taken together with the mechanistic studies, it is definitely small if set against the socio-economic importance of the agents tested. It would be justifiable perhaps to complain that the expense of testing was borne to a greater extent by the common tax-payer than by those who enjoyed the profits of exploitation of the products involved, of which the cost of testing would have represented only a negligible proportion.

Animal Carcinogenicity Studies and Carcinogenicity to Humans

Although it is generally recognized that long-term carcinogenicity tests have played an important role in the identification of proven or probable causes of human cancers, there is at present little consensus on their validity as predictors of human risks. The question usually raised is two-sided: do the results obtained in experimental animals predict a qualitatively and quantitatively similar effect in humans ? The qualitative correlation will be addressed first, while some examples to indicate that a quantitative comparison can be made with some accuracy, at least in certain instances, will be given later.

Qualitative correlations The main objective support for the value of experimental data in predicting a qualitatively similar effect in humans comes from the fact that experimental evidence of carcinogenicity has on several occasions been obtained before the epidemiological evidence. This happened, for instance, in the cases of 4-aminobiphenyl, aflatoxins, diethylstilbestrol, melphalan, 8-methoxypsoralen+UV radiation, mustard gas, radon gas and vinyl chloride.[16]

The qualitative concordance between human and experimental carcinogenicity data may be defined in terms of sensitivity and specificity. Sensitivity determines what proportion of human carcinogens may be detected by long-term carcinogenicity testing.

Of the 53 agents recognized as human carcinogens in the first 48 volumes of the IARC Monographs, 12 are industrial complex exposures and six therapeutic combinations, which cannot be considered here, as they could not be submitted to proper experimental tests. For treosulfan no experimental data on carcinogenicity have been published, and for smokeless tobacco products, all published studies were inadequate for evaluation. Of the remaining 33 chemicals, for 22 (67%) the experimental results provided sufficient evidence of carcinogenicity (i.e., the chemicals were usually carcinogenic in at least two animal species). For 8 chemicals or groups of chemicals, namely analgesic mixtures containing phenacetin, azathioprine, arsenic and arsenic compounds, chlornaphazine, mustard gas, myleran, betel quid with tobacco and 1-(2-chloroethy)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU) the evidence provided by long-term carcinogenicity tests was limited (i.e. evidence of carcinogenicity was available in only one species). The three chemicals with inadequate evidence of carcinogenicity in animals were ethanol, soots and talc containing asbestiform fibers. For alcoholic beverages, one adequate study was available, and did not provide evidence of carcinogenicity.

For six of the eight chemicals for which there was limited evidence of carcinogenicity (azathioprine, betel quid with tobacco, chlornaphazine, mustard gas, myleran and methyl-CCNU), the limitations are mainly related to an incomplete or improper testing design and/or reporting of the results, while for one (analgesic mixtures containing phenacetin) the results of the experimental tests provided limited evidence for the mixture although sufficient evidence for phenacetin alone. For arsenic, recent results on its possible mechanism of action (gene amplification) may explain why traditional long-term tests have so far provided only limited evidence for carcinogenicity.[23]

The fact that concordance is imperfect between data in humans and results in experimental animals (namely, for 11 of the 33 human carcinogens tested in experimental animals the evidence is less than sufficient) is often taken as an argument to downgrade the value of results from experimental animals in predicting similar effects in humans. It could however equally well be interpreted to support an opposite view, i.e. that even limited experimental evidence of carcinogenicity provides a serious warning that a chemical could be carcinogenic to humans. If we pool together the results providing sufficient evidence and those providing limited evidence of carcinogenicity then the sensitivity of long-term carcinogenicity studies to detect human carcinogens increases to 91%.

The specificity of long-term animal assays, i.e., the proportion of human non-carcinogens that are negative in animal studies, is difficult to assess since non-carcinogenicity is very difficult to prove. Also, much less effort has been put — for understandable reasons — into studies of non-carcinogenicity. In Supplement 7 to the IARC Monographs,[16] one chemical only, caprolactam, has been classified solely on experimental evidence as "probably not carcinogenic to humans." From the public health point of view, the specificity is much less important than the sensitivity.

Since extrapolation from experimental animal data to humans is just a form of inter-species comparison, it may be relevant to examine the level of consistency between data obtained in different rodent species. Indeed, it has been argued that the concordance between humans and rodents cannot exceed that between mice and rats.[14] The basis for this argument is that rodents are closer to each other in several physiological and biochemical parameters, than they are to humans. It should be noted, however, that the inter-individual variation between humans is extremely wide, in comparison to standard laboratory rodents; it may even exceed the variation between rodent species.[24, 25] When a large number of humans are exposed to a chemical agent, it is therefore likely that some of them are equally sensitive to the carcinogenic action of that chemical as is the most sensitive rodent species.

In a review in 1981, Purchase[26] made a comparison between the carcinogenic activity of 250 chemicals in the mouse and the rat, and found an overall concordance between the two species of 85%, for both the positive and the negative results. A similar percentage was found in a previous survey[27] and a slightly lower percentage of concordance was found within the US National Toxicology Program (NTP) results. A first survey of the latter results made in 1984[28] on 86 chemicals tested showed that 63% of chemicals carcinogenic in the rat were also carcinogenic in the mouse and 74% of those negative in the rat were also negative in the mouse. In a further survey made in 1987[29] on 109 chemicals adequately tested, the percentages were 68% and 78%, respectively.

Of some interest is also that only around 50% of the chemicals tested in the National Toxicology Programme were proven to have a carcinogenic activity even though the chemicals were primarily selected because of a suspicion of carcinogenicity. It is not enough, therefore, to submit a suspect chemical to a long-term carcinogenicity test, even at the maximum tolerated dose (MTD) level, which is included routinely by the NTP in its testing procedures, to make it automatically a carcinogen.

It would appear as simply common sense that a chemical which is shown to be carcinogenic in several species is also likely to be carcinogenic in an additional untested species, and thus also in humans. Few scientists would not consider N-nitroso-dimethylamine, which has been found invariably carcinogenic in all 23 animal species so

far tested,[30] as a potential human carcinogen, even if no final demonstration of its carcinogenic activity in humans is available. After 20 years of experimental studies on the carcinogenicity of N-nitrosamines, the first adequate epidemiological study is still to be published. The marked resistance of rats to 2-naphthylamine-induced cancer (especially bladder cancer) indicates that a failure or difficulty in proving the carcinogenicity of a chemical in certain species cannot be taken as an indication of non-carcinogenicity in humans.[31]

Target organ specificity For most of the chemicals showing both animal and human carcinogenicity, an increased incidence of tumors in the organ(s) that are the target organ(s) in humans has been observed in at least one animal species following at least one route of administration (Tables I–IV). This concordance of target organs could be largely a result of the thorough testing to which chemicals have been subjected when there is epidemiological evidence of carcinogenicity. There is in fact a total absence of concordance of target organs only for two agents, chlornaphazine and methyl-CCNU, both of which were submitted to only a limited number of tests with possibly inadequate test designs.

While a similarity in target organs could be seen as strengthening the concordance between experimental animals and humans, it should not be seen as a requirement. The experimental evidence of carcinogenicity of 2-naphthylamine, bis(chloromethyl) ether (BCME) and cyclophosphamide, for example, was first provided by tumors found in a target organ other than that observed in humans. In the case of BCME, a demand for the induction of tumors in experimental animals similar to those observed in humans actually led to a delay in implementing measures to reduce human exposure.[31] In the case of cyclophosphamide, more than ten years elapsed between the first demonstration of its carcinogenic effect in the lung, liver, testis and mammary gland[32] and the studies demonstrating the induction of tumors of the bladder[33] that was initially thought to be the only target in humans.

Quantitative predictions Results obtained with experimental animals would certainly be more highly appreciated if they could also provide a quantitative prediction of the human risk. The best known recent attempt to compare carcinogenic potency was made by Meselsohn *et al.* in a study promoted by the US National Academy of Sciences,[34] in which the carcinogenic potency of five human carcinogens was compared in humans and in experimental animals. In general, experimental animals appeared to be more susceptible than humans but the effective tumor-producing dose was no more than two orders of magnitude smaller in experimental animals than in humans, and that is within the range of what has at times been seen as an acceptable safety factor. In this study, however, human exposure data were scanty or very crude.

In a more recent survey, Kaldor *et al.*[35] have compared the carcinogenic potency of some cytostatic drugs, for which exposure data in humans are reasonably accurate. The results of this survey indicate that the potency rankings for three nitrogen mustards, cyclophosphamide, chlorambucil and melphalan are very similar in rodents and humans. Methotrexate and actinomycin, which are chemically dissimilar, however, do not fit to such ranking. With the caution imposed by the limited and selected number of chemicals considered, one could at least say that in the only case where the actual exposure levels were measurable in all species with the same accuracy, the carcinogenic response in humans and in two rodent species was closely correlated, even quantitatively.

Short-term Tests

Until recently, the predictive value of short-term tests has only been assessed in comparison to rodent carcinogenicity studies because of the limitations of the data available on humans. Their predictive value, which may be as high as 90% when applied within particular classes of chemicals,[36] was recently shown to be around 60% when referring to chemicals belonging to a variety of chemical classes.[37] The type of chemicals used in the comparison can markedly affect the figure of concordance obtained.[38] The recent decline in the degree of concordance between carcinogenicity in laboratory animals and the results of short-term tests is attributed to the growing proportion of chemicals which may act through mechanisms other than those involving mutagenic/electrophilic intermediates, among those that are submitted to a validation assay.[39] This is probably true, but it does not make the predictive value of short-term tests any better, since we do not know at present if all, or even the majority of, human carcinogens act necessarily through a mechanism involving a direct genotoxic effect.

IARC Monographs Supplements 6 and 7[40, 16] give an — albeit limited — opportunity to study the sensitivity of different short-term tests to detect human carcinogens (Table VI). The sensitivity of tests with different endpoints (DNA damage, gene mutation, mitotic recombination, sister-chromatid exchange, micronuclei, chromosome aberrations, or morphological transformation) is between 67 and 85%; if tests with different phylogenetic orders are combined with regard to endpoints, the sensitivities vary between 54 and 82%.

The Problem of Low Exposure Levels

One of the problems that has so far been impossible to solve is that of establishing carcinogenic effects at levels of exposure which are much lower than those involved in certain occupations or therapeutic regimes. An instance

Table VI. Sensitivity of Different Short-term Tests to Detect Human Carcinogens (IARC Monographs Suppl. 7, Suppl. 6)[a]

Genetic endpoint	Prokaryotes	Lower eukaryotes	Insects	Mammalian cells *in vitro*	Human cells *in vitro*	Mammalian cells *in vivo*	Human cells *in vivo*	Overall sensitivity
DNA damage	10/14			15/17	9/14	9/10		78%
Gene mutations	14/20	11/14	8/14	12/19				67%
Mitotic recombination		11/13						85%
Sister chromatid exchange				13/15	10/15	8/10	5/11	78%[b]
Chromosomal aberrations				14/16	10/14		8/13	80%[b]
Micronuclei						9/13		69%
Transformation				14/16		11/14		83%
Overall sensitivity	71%	81%	57%	82%	67%	79%	54%	

a) The numerator gives the number of positive tests and the denominator the number of chemicals tested. Only the results where at least ten chemicals were tested in tests of different phylogenetic orders are given in this table.
b) Tests with human cells are excluded from these percentages as they represent more a test of exposure than a test of genotoxicity.

where low levels of exposure of the general population to an occupational carcinogen could be documented occurred in Japan with regard to asbestos fibers. Asbestos is produced in Japan in only very small quantities, and it began to be imported in the 1950s; the frequency of asbestiform bodies in the lungs of the general population began to increase sharply shortly thereafter.[41] Similar evidence has not been found in other instances. As a rather simple example, no direct population exposure data are available on bitumens, which contain several chemicals with well documented esperimental evidence of carcinogenicity.[42] Although it seems unlikely, we have no way of knowing if the sixty million tons of bitumens used annually contribute in any way to our carcinogenic load. Methods are being developed for ascertaining low levels of exposure and for assessing exposures at the individual level based on the measurement of specific metabolites and/or DNA adducts in urine, tissues or blood and on the use of specific monoclonal or polyclonal antibodies.[43, 44] A better definition of environmental exposures may therefore become possible.

Inertia probably explains in part the gap that remains between the routine implementation of carcinogenicity tests as well as of most epidemiological surveys and the expansion of knowledge of mechanisms of carcinogenesis. The validity of experimental tests in demonstrating the carcinogenicity of many chemicals is beyond doubt, but they cannot be expected to provide evidence, at least when used in the traditional manner, for the carcinogenicity of every causative factor of human cancer. The development of tests capable of demonstrating the promoting or modulating activity of certain exposures,[45–48] as well as the use of short-term tests, may help to complete and refine results obtained in animal tests and contribute to making them more efficient as well as more specific. Similarly, the methodology that is being developed for distinguishing between so-called spontaneous and induced tumors[49, 50] may also help to clarify some of the results of long-term carcinogenicity tests that have been until now difficult to interpret (e.g. an increased incidence in treated animals of tumors commonly occurring in untreated animals, or a slight increase of tumors occurring at various sites in treated animals). Obviously, better understanding of mechanisms will help in improving the design of all forms of tests. It has been recently demonstrated, for instance, in an experiment *in vitro* that the carcinogenicity of arsenic may be linked to its ability to induce gene amplification,[23] which appears to be related to the degree of progression to malignancy.

Conclusion

There are at present over 50 agents for which a causal relationship with human cancer has been demonstrated. The majority of these agents are environmental chemicals to which humans have been exposed for a relatively short period of time, that is no longer than a century and a half. It would be mistaken to believe that complete cancer prevention could be achieved solely by controlling these new, or relatively new, carcinogenic agents, but it would be similarly wrong to deny the importance of trying to control them and of continuing to do so. The industrial exploitation of natural resources, of which tobacco and asbestos are conspicuous, though very different, examples, and the synthesis of new chemicals have indeed generated new hazards and new carcinogens which have been added to older ones. These new hazards

contributed to deep social injustices which have only recently been largely overcome in most industrial countries, but certainly not in all countries of the world. Certain developing countries are, in terms of occupational hazards, in a situation similar to that of the industrialized countries 40–50 years ago. Similarly, developing countries are today faced with the threat of a massive penetration, supported by the tobacco corporations, of the habit of smoking, which occurred many decades ago in industrialized countries.

The agents so far identified are of more importance to cancer occurring in men than in women. No chemical has yet been identified that is specifically carcinogenic to the cervix, nor, with the exception of diethylstilbestrol and other estrogens, to the breast. Among the other major sites that are conspicuously absent from the common target sites for the cancer-causing agents so far identified are colon-rectum, stomach, ovary, brain and prostate. Both the epidemiological and experimental approaches may have been subject to similar biases or may have shared similar characteristics (as well as inadequacies) in the identification of the etiologic agents of human cancer that have favored the identification of environmental chemicals.

The carcinogenicity tests, as presently used, still have an irreplaceable role to play to prevent the dispersal into our environment of new hazardous chemicals, as well as in the identification, among the chemicals already in use, of those that are carcinogenic. Their role in the quantification of risks is questionable, but advances in our understanding of the mechanisms of carcinogenesis, may make them more helpful than they have been so far in evaluating risks from low levels of exposure and in allowing direct interspecies extrapolations. Similarly, advances in the understanding of the mechanisms underlying the different steps of the process leading to the clinical manifestation of cancer may help in the uncovering of agents or risk factors that the approaches used, at least in the way they have been used until now, may not have been apt to identify.

That a closer integration between the experimental and epidemiological approaches may also be able to reduce substantially the size of the unknown region within the spectrum of the cancer-causing agents, is one of today's hopes that awaits confirmation.

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REFERENCES

1) Yamagiwa, K. and Ichikawa, K. Über die künstliche Erzeugung von Papillom. *Verh. Jpn. Pathol. Ges.*, **5,** 142–148 (1915).
2) Tsutsui, H. Über das künstlich erzeugte Cancroid bei der Maus. *Gann,* **12,** 17–21 (1918).
3) Frieben, A. Demonstration eines Cancroids des rechten Handrückens das sich nach langdauernder Einwinkung von Röntgenstrahlen entwickelt hat. *Fortschr. Roentgenstr.,* **6,** 106–111 (1902).
4) Sick, H. Karzinom der Haut das aus dem Boden eines Röntgenulcus entstanden ist. *Münch. Med. Wochenschr.,* **50,** 1445 (1902).
5) Rajewsky, B., Schraub, A. and Kahlau, G. Experimentelle Geschwulsterzeugung durch Einatmung von Radiumemanation. *Naturwissenschaften,* **31,** 170–171 (1943).
6) IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, Vol. 43. "Man-made Mineral Fibres and Radon" (1988). International Agency for Research on Cancer, Lyon.
7) Ames, B. N., Magaw, R. and Gold, L. S. Ranking possible carcinogenic hazards. *Science,* **236,** 271–280 (1987).
8) Doll, R. and Hill, A. B. Smoking and carcinoma of the lung. *Br. Med. J.,* **ii,** 739–748 (1950).
9) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 38. "Tobacco Smoking" (1986). International Agency for Research on Cancer, Lyon.
10) World Health Organization. Prevention of Cancer, Tech. Rep. Series No. 276, Geneva, Switzerland (1964).
11) Passey, R. D. Experimental soot cancer. *Br. Med. J.,* **ii,** 1112–1113 (1922).
12) Bradford Hill, A. "Principles of Medical Statistics," pp. 313–323 (1971). The Lancet Limited, London.
13) Doll, R. Prevention of cancer: pointers from epidemiology (1967). The Nuffield Provincial Hospital Trust and Rock Carling Fellowship, London.
14) Lave, L. B., Ennever, F. K., Rosenkranz, H. S. and Omenn, G. S. Information value of the rodent bioassay. *Nature,* **336,** 431–433 (1988).
15) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 44. "Alcoholic Beverages" (1988). International Agency for Research on Cancer, Lyon.
16) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7 (1987). International Agency for Research on Cancer, Lyon.
17) Parkin, D. M., Läärä, E. and Muir, C. S. Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int. J. Cancer,* **41,** 184–197 (1987).
18) Zur Hausen, H. Papilloma virus in human cancer. *In*

"Accomplishments in Cancer Research 1986," ed. J. G. Fortner and J. E. Rhoads, pp. 74–81 (1987). J. B. Lippincott Co., Philadelphia.
19) Munoz, N., Bosch, X. and Kaldor, J. M. Does human papilloma virus cause cervical cancer? The state of the epidemiological evidence. *Br. J. Cancer,* **57,** 1–5 (1988).
20) Hayashi, Y., Nagao, M., Sugimura, T., Takayama, S., Tomatis, L., Wattenberg, L. W. and Wogan, G. N. "Diet, Nutrition and Cancer" (1985). Japan Scientific Societies Press, Tokyo.
21) Sugimura, T. Past, present and future of mutagens in cooked food. *Environ. Health Perspect.,* **67,** 5–10 (1986).
22) Byers, T. Diet and cancer. *Cancer,* **62,** 1713–1724 (1988).
23) Lee, T-C., Tanaka, N., Lamb, P. W., Gilmer, T. M. and Barrett, J. C. Induction of gene amplification by arsenic. *Science,* **241,** 79–81 (1988).
24) Sabadie, N., Malaveille, C., Camus, A. M. and Bartsch, H. Comparison of the hydroxylation of benzo[*a*]pyrene with the metabolism of vinyl chloride, N-nitrosomorpholine and N-nitroso-N-methylpiperazine to mutagens by humans and rat liver microsomal fractions. *Cancer Res.,* **40,** 119–126 (1980).
25) Sabadie, N., Richter-Reichhelm, H. B., Saracci, R., Mohr, U. and Bartsch, H. Inter-individual differences in oxidative benzo[*a*]pyrene metabolism by normal and tumorous surgical lung specimens from 105 lung cancer patients. *Int. J. Cancer,* **27,** 417–425 (1981).
26) Purchase, I. F. Interspecies comparisons of carcinogenicity. *Br. J. Cancer,* **41,** 454–468 (1981).
27) Tomatis, L., Partensky, C. and Monotesano, R. The predictive value of mouse liver tumour induction in carcinogenicity testing: a literature survey. *Int. J. Cancer,* **12,** 1–20 (1973).
28) Haseman, J. K., Crawford, D. D., Huff, J. E., Boorman, G. A. and McConnell, E. E. Results from 86 two-year carcinogenicity studies conducted by the National Toxicology Program. *J. Toxicol. Environ. Health,* **14,** 621–639 (1984).
29) Huff, J. E., McConnell, E. E., Haseman, J. K., Boorman, G. A., Eustis, S. L., Schwartz, B. A., Rao, G. N., Jameson, C. K., Hart, L. G. and Rall, D. P. Carcinogenesis studies: results of 398 experiments on 104 chemicals from the US National Toxicology Program. *Ann. NY Acad. Sci.,* **534,** 1–30 (1988).
30) Bogovski, P. and Bogovski, S. Animal species in which N-nitroso compounds induce cancer. *Int. J. Cancer,* **27,** 471–474 (1981).
31) Tomatis, L. The value of long-term testing for the implementation of primary prevention. *In* "Origins of Human Cancer," ed. A. Hiatt, J. D. Watson and J. D. Winsten, pp. 1339–1357 (1978). Cold Spring Harbor Laboratory, New York.
32) Tokuoka, S. Induction of tumor in mice with N,N-bis(2-chloroethyl)-N′,O-propylenephosphoric acid ester diamide (cyclophosphamide). *Gann,* **56,** 537–541 (1965).
33) Schmähl, D. and Habs, M. Carcinogenic action of low-dose cyclophosphamide given orally to Sprague-Dawley rats in a lifetime experiment. *Int. J. Cancer,* **23,** 706–712 (1979).
34) National Academy of Sciences. Pest control: an assessment of present and alternative technologies. Volume 1: Contemporary Pest Control Practices and Prospects: The Report of the Executive Committee. Washington, DC (1975)
35) Kaldor, J., Day, N. D. and Hemminki, K. Quantifying the carcinogenicity of antineoplastic drugs. *Eur. J. Cancer Clin. Oncol.,* **24,** 703–711 (1988).
36) McCann, J., Choi, E., Yamasaki, E. and Ames, B. N. Detection of carcinogens as mutagens in the *Salmonella/* microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci. USA,* **72,** 5135–5139 (1975).
37) Tennant, R. W., Margolin, B. H., Shelby, M. D., Zeiger, E., Haseman, J. K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B. and Minor, R. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science,* **236,** 933–941 (1987).
38) Zeiger, E. Carcinogenicity of mutagens: predictive capability of the *Salmonella* mutagenesis assay for rodent carcinogenicity. *Cancer Res.,* **47,** 1287–1296 (1987).
39) Bartsch, H. and Malaveille, C. Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series. *Cell Biol. Toxicol.,* **5,** 115–127 (1989).
40) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 6 (1987). International Agency for Research on Cancer, Lyon.
41) Shishido, S., Iwai, K. and Tukagoshi, K. Incidence of ferruginous bodies in the lungs during a 45-year period and mineralogical analysis of the core fibres and uncoated fibres. *In* "Non-occupational Exposure to Mineral Fibres," ed. J. Bignon, J. Peto and R. Saracci, IARC Scientific Publications Series No. 90, pp. 229–238 (1989). International Agency for Research on Cancer, Lyon.
42) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 35. "Polynuclear Aromatic Compounds, Part 4. Bitumens, Coal-tars and Derived Products, Shale-oils and Soots" (1985). International Agency for Research on Cancer, Lyon.
43) Wild, C. P., Chapot, B., Scherer, E., Den Engelse, L. and Montesano, R. The application of antibody methodologies to the detection of aflatoxin in human body fluids. *In* "Methods for Detection of DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention," ed. H. Bartsch, K. Hemminki and I. K. O'Neill, IARC Scientific Publications No. 89, pp. 67–74 (1988). International Agency for Research on Cancer, Lyon.
44) Montesano, R., Bresil, H., Degan, P., Martel-Planche, G., Serres, M. and Wild, C. P. Detection in human cells of alkylated macromolecules attributable to nitrosamine exposure. *In* "Methods for Detection of DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention," ed. H. Bartsch, K. Hemminki and I. K.

O'Neill, IARC Scientific Publications No. 89, pp. 75–82 (1988). International Agency for Research on Cancer, Lyon.

45) Ito, N., Tsuda, H., Tatematsu, M., Inoue, T., Tagawa, Y., Aoki, T., Uwagawa, S., Kagawa, M., Ogiso, T., Masui, T., Imaida, K., Fukushima, S. and Asamoto, M. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placenta form positive foci in rats — an approach for a new medium-term bioassay system. *Carcinogenesis,* **9,** 387–394 (1988).

46) Ito, N., Imaida, K., Tsuda, H., Shibata, M., Aoki, T., de Camargo, J. L. V. and Fukushima, S. Wide-spectrum intiation models: possible applications to medium-term multiple organ bioassays for carcinogenesis modifiers. *Jpn. J. Cancer Res.,* **79,** 413–417 (1988).

47) Semple, E., Hays, M. A., Purchase, T. H., Haines, L. and Farber, E. Mitogenic activity in platelet-poor plasma from rats with persistent liver nodules or liver cancer. *Biochem. Biophys. Res. Commun.,* **148,** 449–455 (1987).

48) Yamasaki, H. Multistage carcinogenesis: implications for risk estimation. *Cancer Metastasis Rev.,* **7,** 5–18 (1988).

49) Wiseman, R. W., Stowers, S. J., Miller, E. C., Anderson, M. W. and Miller, J. A. Activating mutations of the c-Ha-*ras* protooncogene in chemically induced hepatomas of the male B6C3 F1 mouse. *Proc. Natl. Acad. Sci. USA,* **83,** 5825–5829 (1986).

50) You, M., Candrian, U., Maronpot, R. R., Stoner, G. D. and Anderson, M. W. Activation of the Ki-*ras* protooncogene in spontaneously occurring and chemically induced lung tumours of the strain A mice. *Proc. Natl. Acad. Sci. USA,* **86,** 3070–3074 (1989).