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Comparison between Carcinogenicity and Mutagenicity Based on Chemicals Evaluated in the IARC Monographs

by H. Bartsch* and L. Tomatis*

The qualitative relationship between carcinogenicity and mutagenicity (DNA-damaging activity), based on chemicals which are known to be or suspected of being carcinogenic to man and/or to experimental animals, is analyzed using 532 chemicals evaluated in Volumes 1-25 of the IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. About 40 compounds (industrial processes) were found to be either definitely or probably carcinogenic to man, and 130 chemicals have been adequately tested in rodents and most of them also in various short-term assays. For a comparison between the carcinogenicity of a chemical and its behavior in short-term tests, systems were selected that have a value for predicting carcinogenicity. These were divided into mutagenicity in (A) the *S. typhimurium*/microsome assay, (B) other submammalian systems and (C) cultured mammalian cells; (D) chromosomal abnormalities in mammalian cells; (E) DNA damage and repair; (F) cell transformation (or altered growth properties) *in vitro*.

The following conclusions can be drawn. In the absence of studies in man, long-term animal tests are still today the only ones capable of providing evidence of the carcinogenic effect of a chemical. The development and application of an appropriate combination of short-term tests (despite current limitations) can significantly contribute to the prediction/confirmation of the carcinogenic effects of chemicals in animals/man. Confidence in positive tests results is increased when they are confirmed in multiple short-term tests using nonrepetitive end points and different activation systems. Assays to detect carcinogens which do not act via electrophiles (promoters) need to be developed. The results of a given short-term test should be interpreted in the context of other toxicological data. Increasing demand for quantitative carcinogenicity data requires further examination of whether or not there is a quantitative relationship between the potency of a carcinogen in experimental animals/man, and its genotoxic activity in short-term tests. At present, such a relationship is not sufficiently established for it to be used for the prediction of the carcinogenic potency of new compounds.

There is increasing evidence to suggest that DNA damage (expressed mainly as mutations) is involved in the induction of many cancers; however, the relevance of the various biological end points used in short-term assays to mechanisms of tumor induction is not known precisely. All test procedures must therefore be validated before they can be used to predict the carcinogenicity of chemicals. Ideally, such validations would be based on correlations between responses in short-term tests and data from epidemiological studies in humans.

*Division of Environmental Carcinogenesis, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 8, France.

Chemicals evaluated in Volumes 1–25 of the IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (*1–26*) as either definitely or probably carcinogenic to humans, and which have been tested in various mutagenic and other short-term assays, offer a basis for such an analysis. For the purpose of this discussion, therefore, we used information available through the IARC program on the evaluation of the carcinogenic risk of chemicals to humans, in which monographs are prepared on individual chemicals, groups of chemicals, or industrial processes (*27*). A total of 532 compounds have been evaluated in that program.

Epidemiological studies and/or case reports were available for only about 60 chemicals, groups of

chemicals, industries or industrial processes; for 22 of these, the available evidence was sufficient to support a causal relationship with the occurrence of cancers in humans (Table 1). Among the latter are seven industrial processes (the manufacture of auramine, chromate-producing industries, hematite mining, the manufacture of isopropyl alcohol, nickel refining, boot and shoe manufacture and repair, and the furniture/cabinet-making industry). For these processes, no direct correlation can be made between data for humans and for experimental animals, because the identity of the agent(s) responsible for the carcinogenic effect in humans is unknown. The remaining 15 compounds were also found to be carcinogenic in one or (mostly) several experimental animal species. Results of a recent long-term carcinogenicity test of benzene point to its carcinogenicity in rats (*28*). Results of carcinogenicity tests on arsenic were negative, although there is sufficient evidence that arsenic compounds induce skin and lung cancer in humans.

An additional 18 compounds were considered as probably carcinogenic to humans (Table 2). While the carcinogenicity to humans of the previous group of chemicals and industrial processes could be assessed exclusively on the basis of epidemiological data that provided sufficient evidence of a causal relationship, the carcinogenic risk of this second group of chemicals was evaluated taking into consideration evidence from studies in both humans and experimental animals (*21*). The evidence that chemicals in this group are carcinogenic to humans varies from being almost sufficient (subgroup A) to suggestive (subgroup B).

The remaining compounds for which epidemiological data were available are: chloramphenicol, chlordane/heptachlor, chloroprene, DDT, dieldrin, epichlorohydrin, hematite, hexachlorocyclohexane (BHC and lindane), *N*-phenyl-2-naphthylamine, phenytoin, reserpine, styrene, trichloroethylene, triaziquone, *o*-dichlorobenzene, dichlorobenzidine, phenylbutazone, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin, *o*- and *p*-toluidine and vinylidene chloride. These could not be classified as to their carcinogenicity to humans due to limitations of the available epidemiological data and/or to the fact that only limited evidence of carcinogenicity was provided by data from experimental animals. For those compounds, therefore, no comparison can be made between epidemiological and experimental data.

For a comparison between the carcinogenicity of a chemical in humans and its behavior in mutagenicity and other short-term tests, a number of systems were selected on the basis of data in the literature that indicate their value for predicting carcinogenicity or their ability to detect specific classes of carcinogens (Table 3). The list is not exhaustive, since many assays are still being evaluated in terms of their usefulness, their reproducibility and their

Table 1. Chemicals, groups of chemicals, industries or industrial processes associated with the induction of cancer in humans.[a]

Chemicals and groups of chemicals	Industries and industrial processes
4-Aminobiphenyl	Auramine (manufacture)
Arsenic and arsenic compounds	Boot and shoe manufacture and repair[b]
Asbestos	Furniture and cabinet-making industry[b]
Benzene	Hematite mining (radon?)
Benzidine	Isopropyl alcohol (manufacture by using the strong-acid process
N,*N*-Bis(2-chloroethyl)-2-naphthylamine	Nickel refining
Bis(chloromethyl) ether and technical-grade chloromethyl methyl ether	
Chromium and certain chromium compounds	
Conjugated estrogens[b]	
Cyclophosphamide[b]	
Diethylstilbestrol	
Melphalan	
Mustard gas	
2-Naphthylamine	
Soots, tars and mineral oils	
Vinyl chloride	

[a]Compiled from IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (*1-26*).
[b]Added to IARC (*21*) by subsequent working groups at IARC.

Table 2. Chemicals, groups of chemicals, industries or industrial processes strongly suspected of being associated with the induction of cancer in humans.[a]

Subgroup A: Higher degree of human evidence	Subgroup B: Lower degree of human evidence
Aflatoxins	Acrylonitrile
Cadmium and certain cadmium compounds	Amitrole
Chlorambucil	Auramine
Nickel and certain nickel compounds	Azathioprine[b]
Tris(1-aziridinyl)phosphine sulfide (thio-TEPA)	Beryllium and certain beryllium compounds
	Carbon tetrachloride
	Dimethyl carbamoyl chloride
	Dimethyl sulfate
	Ethylene oxide
	Iron dextran
	Oxymetholone
	Phenacetin
	Polychlorinated biphenyls (PCBs)

[a]Compiled from IARC Monographs on the Carcinogenic Risk of Chemicals to Humans (*1-26*).
[b]Added to IARC (*21*) by a subsequent working group at IARC.

Table 3. Selected short-term tests for the detection of chemical carcinogens or promoting agents.[a]

System (reference)	Genetic/biochemical end point monitored	Metabolic activation system
A. Mutagenesis in *Salmonella typhimurium (29-31)*	Histidine auxotrophs	Postmitochondrial rodent (or human) liver fractions
B. Mutagenesis in other submammalian indicator organisms		
Escherichia coli (32-35)	Arginine and tryptophan auxotrophs Prophage induction, growth inhibition (repair-deficient strains)	Host-mediated assays (urine and feces analysis) *in vivo*
Saccharomyces cerevisiae (36)	Mutations, gene conversion and mitotic recombinations	
Neurospora crassa (37)	Adenine auxotrophs	
Drosophila melanogaster (38)	Recessive lethal mutations	
C. Mutagenesis in cultured mammalian cells		
Chinese hamster ovary (CHO) and lung *(39-43)*	Mutations at HGPRT-locus	Postmitochondrial rodent liver fraction
Mouse lymphoma (L-5178Y) *(44)*	$TK^{+/-}TK^{-/-}$ mutations	Cell-mediated assays (cocultivation of lethally irradiated rat embryo cells or hepatocytes)
Rat liver epithelial cells *(45)*	8-Azaguanine resistance	
D. Chromosome analysis		
Chinese hamster cells and human fibroblasts; human peripheral blood lymphocytes *(46-48)*	Sister chromatid exchanges, chromosomal aberrations	Postmitochondrial rodent liver fraction *in vivo*
E. DNA damage and repair		
Chinese hamster lung (V79) *(49)*	Single-strand breaks in DNA (alkaline elution)	Postmitochondrial rodent liver fraction *in vivo*
Various rodent tissues (treatment *in vivo (59)*		
HeLa cells, rat hepatocytes, human skin fibroblasts *(51-53)*	Unscheduled DNA repair	
DNA synthesis *in vitro (54)*	Decreased fidelity	
F. *In vitro* cell transformation (altered growth properties)		
Early-passage Syrian hamster embryo *(55)* Mouse embryo C3H/10T½ *(56, 57)*	Morphological transformations	None
Newborn Syrian hamster kidney (BHK21) *(58, 59)*	Growth in agar	Postmitochondrial rodent liver fraction

[a]These tests were selected on the basis of data which indicate: their sensitivity in dectecting several classes of carcinogens and of discriminating between carcinogens and noncarcinogens or their unique capability to detect particular classes of carcinogen or promoting agent. This list is not exhaustive and the degree to which these tests have been validated varies widely.

comparability with carcinogenicity data obtained *in vivo* (*59,60*). The tests considered were divided arbitrarily into six categories on the basis of their end points: (A) mutagenicity in the *Salmonella typhimurium* microsome assay; (B) mutagenicity in other submammalian systems, including *Escherichia coli, Saccharomyces cerevisiae, Neurospora crassa* and *Drosophila melanogaster*; (C) mutagenicity in cultured mammalian cells; (D) chromosomal abnormalities in mammalian cells; (E) DNA damage and repair in mammalian cells; (F) cell transformation (or altered growth properties) *in vitro*.

The test systems considered either incorporate some aspects of mammalian metabolism, e.g., by adding a microsomal fraction of rodent or human liver *in vitro* or by using metabolically competent rodent cells, or involve activation *in vivo*, as in the host-mediated assay in intact mammalian organisms and in the test in *Drosophila melanogaster* (Table 3). Because of its efficiency, low cost and rapidity, the Salmonella/microsome test has been used most extensively; it therefore also has been most extensively validated, and 30 identified and suspected human carcinogens have been assayed (Tables 4 and 5). Of these 21 (70%) were detected as mutagens. Of the known human carcinogens (Table 4), arsenic compounds (arsenite, As^{III}), asbestos, benzene and diethylstilbestrol were not mutagenic in this test. Sodium arsenite induces point mutations in *E. coli* WP2 strain and caused chromosomal aberrations in cultured human peripheral lymphocytes. Metal carcinogens are normally not mutagenic in the Salmonella test when it is carried out by the standard procedure, although certain metal salts, such as hexavalent chromium compounds, are genotoxic in bacterial and mammalian systems. Several metal carcinogens also decrease the fidelity of DNA polymerase *in vitro* and are active in the

cell transformation test. Diethylstilbestrol weakly stimulates unscheduled DNA synthesis in HeLa cells (*51*), induces mutations in the mouse lymphoma L-5178 ($TK^{+/-}/TK^{-/-}$) system (*44*), but not in Chinese hamster V79 cells in the presence of rat hepatocytes (*61*), and transforms early-passage Syrian hamster embryo cells, but not BHK-21 cells (*31,55*).

Of the possible human carcinogens (Table 5), amitrole, carbon tetrachloride and polychlorinated

Table 4. Identified human carcinogens and their effects in some short-term assays.[a,b]

Human carcinogen	Mutagenicity in *Salmonella* (A)	Mutagenicity in other submammalian assays (B)	Mutagenicity in mammalian cells	Chromosome analysis	DNA damage and repair	Cell transformation
4-Aminobiphenyl	+	+			+	+
Arsenic compounds	–	+		+		
Asbestos	–			+		
Auramine (dye mixture)	+					
Benzene	–			+		
Benzidine	+				+	+
N,N-Bis(2-chloroethyl)-2-naphthylamine	+					
Bis(chloromethyl)ether	+					+
Chromium compounds	+	+	+	+	+	+
Cyclophosphamide[c]	+	+	+	+	+	+
Diethylstilbestrol	–		+;–		+	+;–
Melphalan	+			+		+
Mustard gas	+	+		+	+	
2-Naphthylamine	+					+;–
Soot	+					
Vinyl chloride	+	+	+	+		+

[a]Code (+) correctly identified carcinogen; (–) false-negative response. From IARC Monographs 1-25 (*1-26*) and from references in Table 3; classification refers to test systems grouped in Table 3.

[b]Conjugated estrogens and industrial processes, i.e., hematite mining, manufacture of isopropyl alcohol, nickel refining, boot and shoe manufacture and repair, and furniture and cabinet-making industries, have been omitted, since no results from short-term tests were available.

[c]Classified as a human carcinogen by a working group at IARC, Lyon, October 1980.

Table 5. Possible human carcinogens and their effects in some short-term assays.[a]

Possible human carcinogen	Mutagenicity in *Salmonella* (A)	Mutagenicity in other submammalian systems (B)	Mutagenicity in mammalian cells	Chromosome analysis	DNA damage and repair	Cell transformation
Acrylonitrile	+	+				
Aflatoxins[b]	+	+	+	+	+	+
Amitrole	–	–				+
Auramine (pure)	–	+				–
Azathioprine[c]	+	+		+		
Beryllium compounds	–	–	+	+	+	+
Cadmium compounds					+	
Carbon tetrachloride	–	–				
Chlorambucil	+	+		+		+
Dimethylcarbamoyl chloride	+	+				+
Dimethylsulfate	+					
Ethylene oxide	+	+	–	+		
Iron dextran						
Nickel compounds			+		+	+
Oxymetholone						
Phenacetin	+	–		+		
Polychlorinated bipenyls (PCBs)	–	–		–		–
Tris(1-aziridinyl)phosphine sulfide (thio-TEPA)	+	+		+		

[a]Code (+) correctly identified carcinogen; (–) false-negative response. From IARC Monographs 1-25 (*1-26*) and from references in Table 3; classification refers to test systems grouped in Table 3.

[b]Results in short-term tests refer to aflatoxin B_1 only.

[c]Evaluated and classified as possible human carcinogen by a working group at IARC, Lyon, October 1980.

biphenyls (PCBs), for which there is sufficient evidence of carcinogenicity in experimental animals, were not mutagenic in the Salmonella test. PCBs were negative in all other short-term assays. Phenacetin can be detected as a bacterial mutagen in *S. typhimurium* if hamster liver fractions are used instead of the rat liver preparations generally added in routine testing (*62,63*). Aflatoxin B_1 and cyclophosphamide gave uniformly positive results in all six test systems. Because of the limitations of individual systems, confidence in positive results obtained with new compounds is increased when the results are confirmed in other short-term tests, using either nonrepetitive end points (e.g., those mentioned in Table 3), or different activation systems. When results obtained in several test systems (Table 4 and 5) are combined, it can be seen that 19 our of 34 known or possible human carcinogens were tested in both systems A and B; while 13 and 14 of the 19 were positive in both A and B, respectively, 15 were positive in at least one of the two assays.

Negative results obtained in a battery of short-term tests in the absence of animal data are certainly reassuring; however, given the present limitations, it is still necessary to await the results of long-term tests in animals to confirm the absence of a carcinogenic effect, as illustrated by the example of PCBs (Table 5). Cancer induction may occur in multiple steps; some compounds may act, not as complete carcinogens or initiating agents, but as promoters, and are therefore not detectable as electrophilic mutagens. It is therefore essential that assays be developed to detect agents that do not appear to act via electrophilic intermediates but enhance or initiate carcinogenesis by other mechanisms, which today would be missed even in a comprehensive screening program. The possibly multifactorial origin of certain human cancers indicates the need for assays to study the interactions between viruses, carcinogens and tumor-promoting agents (*64*).

Comparison between Data from Long-Term Animal Carcinogenicity Tests and Results of Mutagenicity (Short-Term) Tests

There is a universal consensus that exposure to chemicals causally associated (or strongly suspected of being associated) with the occurrence of cancer in humans must be avoided, although some disagreement might persist on how, and how quickly, this should be done. A different and major problem is the evaluation of the possible carcinogenic hazard to humans of chemicals which have not been studied epidemiologically or noted in case reports. In an attempt to provide better assistance to regulatory bodies, the IARC revised the criteria used with the IARC Monographs Program for assessing the significance of experimental animal data for predicting the possible carcinogenic risk of chemicals to humans (*19-21*). According to these criteria, "sufficient evidence" of carcinogenesis is provided by experimental studies that show an increased incidence of malignant tumors: (a) in multiple species and strains; and/or (b) in multiple experiments (routes or doses); and/or (c) to an unusual degree (with regard to incidence, site, type and/or precocity of onset).

"Limited evidence" of carcinogenicity is provided by experimental data that suffer from certain drawbacks: (a) they were obtained in a single animal species, strain or experiment or in experiments that were restricted by inadequate dosage levels, by inadequate duration of exposure or of period of follow-up or by poor survival; (b) the neoplasms seen occur spontaneously, or are difficult to classify as malignant by histological criteria alone; (c) there is uncertainty about whether the incidence of tumors in test animals was increased in comparison with that in control animals. ("Sufficient evidence" of carcinogenicity and "limited evidence" of carcinogenicity do not represent categories of chemicals, but indicate varying degrees of experimental evidence and do not refer to the potency of the compound as a carcinogen.)

Of the chemicals evaluated in the first 25 volumes of the IARC Monographs, 130 had "sufficient evidence" of carcinogenicity in experimental animals (Table 6). According to the criteria, in the absence of adequate human data, chemicals for which there is sufficient evidence of carcinogenicity in laboratory animals should be regarded, for practical purposes, as if they presented a carcinogenic risk to humans. The use of the expressions "for practical purposes" and "as if they presented a carcinogenic risk" indicates that the correlation between the experimental data and possible human risk was not made on a purely scientific basis, but rather in an attempt to provide regulatory bodies with one of the elements on which priorities in the formulation of preventive measures can be based.

As shown above, there is a good empirical correlation between epidemiological and experimental data, and experimental data may predict a qualitatively similar response in humans; however, this correlation cannot be used to predict quantitative variations in the responses of different species. We are still a long way from the possibility of making scientifically acceptable direct extrapola-

Table 6. Chemicals evaluated in Volumes 1-25 of the IARC Monographs for which there is sufficient evidence of carcinogenicity in experimental animals.[a]

Compound	IARC Monograph Volume	Page no.
Actinomycins	10	29
o-Aminoazotolune	8	61
2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole	7	143
Aramite	5	39
Azaserine	10	73
Benz(a)anthracene	3	45
Benzo(b)fluoranthene	3	69
Benzo(b)pyrene	3	91
Benzyl violet 4B	16	153
Beryllium oxide	1	17
	23	143
Beryllium phosphate	1	17
	23	146
Beryllium sulfate	1	17
	23	146
β-Butyrolactone	11	225
Cadmium chloride	2	74
	11	39
Cadmium oxide	2	74
	11	39
Cadmium sulfate	2	74
	11	39
Cadmium sulfide	2	74
	11	39
Calcium chromate	2	100
	23	212
Chlordecone (Kepone)	20	67
Chloroform	20	401
Citrus red no. 2	8	101
Cycasin	1	157
	10	121
Daunomycin	10	145
N,N'-Diacetylbenzidine	16	293
4,4'-Diaminodiphenyl ether	16	301
2,4-Diaminotoluene	16	83
Dibenz(a,h)acridine	3	247
Dibenz(a,j)acridine	3	254
Dibenz(a,h)anthracene	3	178
7*H*-Dibenzo(c,g)carbazole	3	260
Dibenzo(a,e)pyrene	3	207
Dibenzo(a,h)pyrene	3	207
Dibenzo(a,i)pyrene	3	215
1,2-Dibromo-3-chloropropane	15	139
	20	83
3,3'-Dichlorobenzidine	4	49
3,3'-Dichloro-4,4'-diaminodiphenyl ether	16	309
1,2-Dichloroethane	20	429
Diepoxybutane	11	115
1,2-Diethylhydrazine	4	153
Diethyl sulfate	4	277
Dihydrosafrole	1	170
	10	233
3,3'-Dimethoxybenzidine(*o*-dianisidine)	4	41
Dimethylaminoazobenzene	8	125
Trans-2[(dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole	7	147
3,3'-Dimethylbenzidine (*o*-tolidine)	1	87
1,1-Dimethylhydrazine	4	137
1,2-Dimethylhydrazine	4	145
1,4-Dioxane	11	247
Estradiol-17β and its esters	6	99
	21	279
Estrone and its esters	6	123
	21	343
Ethinylestradiol	6	77
	21	233
Ethylene dibromide	15	195
Ethylenethiourea	7	45
Ethyl methanesulfonate	7	245
2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole	7	151
Glycidaldehyde	11	175
Hexachlorobenzene	20	155
Hexamethylphosphoramide	15	211
Hydrazine	4	127
Indeno(1,2,3-cd)pyrene	3	229
Isosafrole	1	169
	10	232
Lasiocarpine	10	281
Lead acetate	1	40
	23	327
Lead chromate	23	208
Lead phosphate	1	40
	23	327
Lead subacetate	1	40
	23	327
Melphalan	9	167
Mestranol	6	87
	21	257
Methoxsalen + ultraviolet light	24	101
2-Methylaziridine	9	61
Methylazoxymethanol and its acetate	1	164
	10	131
4,4'-Methylene bis(2-chloroaniline)	4	65
4,4'-Methylene bis(2-methylaniline)	4	73
Methyl iodide	15	245
Methyl methanesulfonate	7	253
N-Methyl-N_1-nitro-N_1-nitrosoguanidine	4	183
Methylthiouracil	7	53
Mirex	5	203
	20	283
Mitomycin C	10	171
Monocrotaline	10	291
5-(Morpholinomethyl)-3[(5-nitro-furfurylidene)-amino]-2-oxazolidinone	7	161
Nafenopin	24	125
Nickel subsulfide	2	126
	11	75
Niridazole	13	123
5-Nitroacenaphthene	16	319
1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone	7	181
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide	1	181
	7	185
Nitrogen mustard and its hydrochloride	9	193
Nitrogen mustard *N*-oxide and its hydrochloride	9	209
N-Nitrosodi-*n*-butylamine	4	197
	17	51
N-Nitrosodiethanolamine	17	77

Table 6. *(continued)*

Compound	IARC Monograph Volume	Page no.
N-Nitrosodiethylamine	1	107
	17	83
N-Nitrosodimethylamine	1	95
	17	125
N-Nitrosodi-*n*-propylamine	17	177
N-Nitroso-*N*-ethylurea	1	135
	17	191
N-Nitrosomethylethylamine	17	221
N-Nitroso-*N*-methylurea	1	125
	17	227
N-Nitroso-*N*-methylurethane	4	211
N-Nitrosomethylvinylamine	17	257
N-Nitrosomorpholine	17	263
N'-Nitrosonornicotine	17	281
N-Nitrosopiperidine	17	287
N-Nitrosopyrrolidine	17	313
N-Nitrososarcosine	17	327
Oil orange SS	8	165
Panfuran-S	24	77
Phenazopyridine and its hydrochloride	24	163
Phenoxybenzamine and its hydrochloride	24	185
Ponceau MX	8	189
Ponceau 3R	8	199
1,3-Propane sultone	4	253
β-Propiolactone	4	259
Propylthiouracil	7	67
Safrole	1	69
	10	231
Sintered calcium chromate	23	302
Sintered chromium trioxide	23	302
Sodium saccharin	22	113
Sterigmatocystin	1	175
	10	245
Streptozotocin	4	221
	17	337
Strontium chromate	23	215
Testosterone and its esters	6	209
	21	519
Thioacetamide	7	77
Thiourea	7	95
Toxaphene (polychlorinated camphenes)	20	327
Tris(2,3-dibromopropyl)phosphate	20	575
Trypan blue (commercial grade)	8	267
Uracil mustard	9	235
Urethane	7	111
Zinc beryllium silicate	23	146
Zinc chromate	23	215

[a]Excluding those chemicals associated with cancer induction in humans listed in Tables 1 and 2.

tion from experimental data to the human situation.

We are even further from an extrapolation to human risk from experimental situations, such as those occurring with short-term tests, which do not have the production of tumors as their end point. The number of chemicals which have been definitely recognized or are suspected of being carcinogenic to humans is too small (Tables 4 and 5) to provide a basis for validation of short-term tests. At present, objective judgment of the value of mutagenicity tests for predicting the carcinogenicity of chemicals must perforce be based on comparison with the much larger number of chemicals shown to be carcinogenic (or noncarcinogenic) in experimental animals.

The selection of such chemicals (or classes of chemicals) for validation studies is biased by the fact that it is limited to those for which carcinogenicity data are available. Moreover, the number of chemicals for which there is adequate evidence of noncarcinogenicity is very small. Thus, the empirically established predictive value of short-term tests (*31,62,65*) is clearly influenced by the quality of the animal data used as a standard for the validation. The level of correlation between results from mutagenicity or other screening tests and those from animal bioassays can thus most reliably be examined by testing chemicals for which there is sufficient evidence of carcinogenicity in animals (Table 6, excluding those listed in Tables 1 and 2). Of these, two-thirds (85) have been tested in the Salmonella/microsome mutagenicity test, and 79% (67/85) were found to be mutagenic. Those which were not mutagenic in the Salmonella/microsome plate test were actinomycins, benzyl violet 4B, beryllium sulfate, chloroform, 1,2-dimethylhydrazine, 1,4-dioxane, ethinylestradiol, lead acetate, hexamethylphosphoramide, nafenopin, *N*-nitrososarcosine (tested in the host-mediated assay using Salmonella), 17β-estradiol, estrone, safrole, sodium saccharin, thioacetamide, thiourea and urethane.

Some of the reasons for production of false-negative results by certain carcinogens in bacterial mutagenicity tests have been discussed in detail (*66*). In the case of 1,2-dimethylhydrazine, such results may be attributable to inadequacies in the *in vitro* metabolic activation system currently used, since this compound was mutagenic in the host-mediated assay (*67*). Similarly, those chemicals such as mitomycin C, which produce mutations in eukaryotic organisms only, e.g., by interference with functions that are not present in prokaryotes, would also be missed in bacterial mutagenicity tests. Certain classes of compounds, however, may not be detectable as mutagens, even with improvements in *in vitro* activation systems of increased sensitivity of genetic indicator organisms; these appear to include sex hormones, thyroid-active compounds, tumor promoters and physically acting agents. As emphasized above, it is essential to develop short-term assays that can detect agents which are definitely carcinogenic in animals but which probably do not act via electrophilic intermediates.

Quantitative Correlations between the Carcinogenic and Mutagenic Activity

Published studies that have examined a possible quantitative correlation between carcinogenicity *in vivo* and mutagenicity *in vitro* include those of Meselson and Russel (*68*), who calculated the carcinogenic potency of 14 chemicals as the TD_{50} (the daily dose of a carcinogen which gives a 50% incidence of cancer in rodents after two years' exposure). Mutagenic activity was determined from results in the Salmonella/microsome test, using the most sensitive bacterial strain. In a double logarithmic plot of mutagenic and carcinogenic activity, most of the compounds showed a linear correlation, with the notable exception of several *N*-nitroso compounds. Clive et al. (*44*) reported correlation studies on 25 chemicals. Carcinogenic activity in rats and mice was expressed as the frequency of tumor-bearing animals per μmole of compound administered per kilogram body weight. This was compared with mutagenic activity in the L5178Y $TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma system in the presence of rat liver fractions, expressed as number of $TK^{-/-}$ mutants per cell per μmole-hr/mL. An approximately linear relationship was obtained over a 10^5-fold range in activity.

Hsieh et al. (*69*) compared the rat liver microsome-mediated mutagenicities of aflatoxin B_1 and several structural analogues with their potency as hepatocarcinogens in several animal species. A good parallelism was found although the carcinogenicity indices were not calculated. Nagao et al. (*70*) tested 31 *N*-nitrosamines, either structurally or metabolically related to *N*-*n*-butyl-*N*-(4-hydroxy-*n*-butyl)nitrosamine or to *N*,*N*-di-*n*-butylnitrosamine, in the Salmonella/microsome mutagenicity assay, using a testing procedure whereby the compound and a 9000*g* supernatant from PCB-treated rats were preincubated 20 min in the presence of *S. typhimurium* strains TA 100 and TA 1535 and then plated. The authors concluded that the mutagenicities of these compounds were not related quantitatively to their potencies as carcinogens.

Langenbach et al. (*43*) assayed a series of β-oxidized derivatives of *N*-nitrosodi-*n*-propylamine for mutagenicity in two systems: (1) liquid incubation assays in the presence of *S. typhimurium* TA 1535 and hamster liver homogenate, and (2) Chinese V79 hamster cells cocultivated with freshly isolated hamster hepatocytes. The mutagenic activity of the four nitroso compounds correlated better with their carcinogenic activity in the hamster in assay (2) than in assay (1). In another study, several hydrazine derivatives were tested both in the Salmonella/microsome assay in the presence of rat liver fractions, and for the induction of DNA damage in liver or lung tissue *in vivo* by using an alkaline elution assay (*71*). The authors concluded that the ability of the 12 compounds to induce lung tumors in mice was better reflected by the assay for DNA damage.

Coombs et al. (*72*) measured the liver microsome-mediated mutagenicity of 35 polycyclic hydrocarbons (derivatives of cyclopentaphenanthrene and chrysene) using Aroclor-pretreated rats and *S. typhimurium* TA 100 strain. These results were compared with data on carcinogenicity obtained from skin painting experiments in mice and expressed as Iball index: (percentage tumor incidence × 100) mean latent period in days. The authors reported little quantitative correspondence between carcinogenic potency and mutagenic activity. Huberman and Sachs (*73*), however, using a cell-mediated mutagenicity assay with Chinese hamster V79 cells cocultivated with lethally irradiated rat embryo cells for metabolic activation, found that the carcinogenicity of 10 polycyclic hydrocarbons paralleled their mutagenicity, as measured by 8-azaguanine or ouabain resistance.

The discrepancies observed between studies in which metabolic activation was provided by cell-free systems and those in which cellular metabolic activation systems were used may be due in part to the fact that certain ultimate reactive mutagenic metabolites produced by rat liver microsomal systems *in vitro* may be different from those which are generated in cells (*74–76*). This observation may explain the lack of correlation between the mutagenicities of five hydrocarbons assayed in the presence of a rat liver microsomal system and their carcinogenicities (expressed as Iball indices) on mouse skin (Table 7) (*77*), which was particularly evident for the benz(a)anthracene (BA) series. Mutagenic activity decreased in the order BA > 7-methyl-BA > 7,12-dimethyl-BA, while carcinogenicity increased in that order.

Wislocki et al. (*78*) also reported no quantitative agreement between the mutagenicity in *S. typhimurium* TA 100 in the presence of activating systems of hydroxymethyl and other derivatives of 7,12-dimethylbenz(a)anthracene and their tumor-initiating activity in mouse skin (two-stage tumorigenesis model).

Bartsch et al.(*77*) found, however, a very close positive association between the liver microsome-mediated mutagenicities of dihydrodiols that can yield bay-region diol-epoxides and the carcinogenic potencies of the parent hydrocarbons. These data are consistent with the assumption that, under the assay conditions utilized, liver microsomes *in vitro*

Table 7. Relationship between the mutagenicity of polycyclic aromatic hydrocarbons and of certain related dihydrodiols in microsome-mediated assays with *Salmonella typhimurium* TA 100 and the extents of reaction with DNA and of tumor initiation and carcinogenesis in mouse skin treated with polycyclic hydrocarbons.[a]

	Polycyclic hydrocarbon			Related dihydrodiol[b]	
	Mutagenicity, *his*$^+$ revertants/nmole[c]	Extent of reaction with DNA in mouse skin, pmole/mg DNA[d]	Tumor initiation on mouse skin, tumors/μmole	Carcinogenicity[e]	Mutagenicity, *his*$^+$ revertants/nmole[c]
Benz(a)anthracene	6	2	0.9	5	8.5
7-Methylbenz(a)anthracene	5	25	1.7	45	33
7,12-Dimethylbenz(a) anthracene	2.4	42	819	95	80
3-Methylcholanthrene	17	26	102	90	35
Benzo(a)pyrene	29	25	25	70	101

[a]From Bartsch et al. (*77*).

[b]The *trans*-dihydrodiols expected to be the metabolic precursors of "bay region" vicinal diol-epoxides were used in each case. These were the 3,4-diols derived from benz(a)anthracene and 7-methylbenz(a)anthracene and 7,12-dimethylbenz(a)anthracene, the 9,10-diol derived from 3-methylcholanthrene and the 7,8-diol derived from benzo(a)pyrene.

[c]Mutations to *his*$^+$ were estimated in *Salmonella typhimurium* TA 100, and the values have been taken from the ascending linear portion of the dose response curves.

[d]Estimated from Sephadex LH20 column elution profiles of hydrolyzates of DNA obtained from the skin of C57BL mice treated *in vivo* with a ^{3}H-labeled polycyclic hydrocarbon (1 μmole/mouse) for 19 hr.

[e]Iball indices for skin tumor formation in mice.

produce predominantly simple, mutagenic oxides, whereas cultured cells or cells *in vivo* can carry out a three-step activation process involving the sequential formation of epoxides, diols and diol-epoxides. The latter are now assumed to be the ultimate carcinogenic metabolites of polycyclic hydrocarbons (*79,80*). However, liver microsomes incubated with the appropriate diol precursor catalyse the formation of vicinal diol-epoxides.

Differences in the pathways leading to intermediates that are mutagenic to *S. typhimurium in vitro* and the electrophilic metabolites known to bind to cellular macromolecules *in vivo* have also become apparent for certain aromatic amines, e.g., 2-acetylaminofluorene (AAF). Reactive esters like AAF-*N*-sulfate and *N*-acetoxy-2-aminofluorene, which are formed *in vivo* and *in vitro*, appear not to be involved in bacterial mutagenesis when *N*-hydroxy-AAF is incubated with rat liver postmitochondrial supernatant and *S. typhimurium* strains (*81*). Such differences could profoundly influence any quantitative correlation between the bacterial mutagenicity and the carcinogenicity of certain aromatic amines.

In order to eliminate the vagaries of metabolic activation, ultimate reactive compounds that do not require enzymic activation and which are structurally related were compared qualitatively and quantitatively in several short-term tests (*82,83*). Reactive esters derived from *N*-hydroxy-2-aminofluorene were assayed for electrophilicity by reaction with methionine, for mutagenicity in *S. typhimurium* strains and in Chinese V79 hamster cells or for the induction of unscheduled DNA repair in cultured human fibroblasts (measured by incorporation of ^{3}H-thymidine, followed by autoradiography). Overall, the data showed a general, qualitative correlation between induction of DNA repair, electrophilicity and carcinogenic activity of these esters. However, quantitative correlations among these activities were poor: the large difference observed in the carcinogenic potency of *N*-myristoyloxy-2-acetylaminofluorene (the most active carcinogen) and that of *N*-acetoxy-2-acetyl-aminofluorene (the least active carcinogen) was not reflected by the biological parameters measured in the *in vitro* systems.

In another study of direct-acting carcinogens (*62*), 10 monofunctional alkylating agents (including carcinogenic *N*-nitrosamides, alkylmethane sulfonates, epoxides, β-propiolactone and 1,3-propane sultone) were assayed for mutagenicity in two *S. typhimurium* strains, TA 1535 and TA 100, and in two test procedures, plate and liquid assays. The mutagenic activities in TA 100 and TA 1535 strains (plate assays) were then compared with the carcinogenic activities of these alkylating agents, expressed as TD_{50} values (Table 8). Although the TD_{50} values for the 10 compounds varied with the mode of administration and animal species, there was no obvious proportionality between carcinogenicity in rodents and mutagenicity in either Salmo-

Table 8. Comparison of carcinogenic activity (TD_{50}) and mutagenicity in *Salmonella typhimurium* TA 100 and TA 1535 of 10 direct-acting alkylating agents.[a]

Compound	Mutagenicity in *S. typhimurium*[b]		Range of TD_{50} in rodents, mg/kg[c]
	TA 1535	TA 100	
N-Nitrosoethylurea	7450	2790	< 0.3 - 40
N-Nitrosomethylurea	660	550	< 5.4 - 155
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	5.7	4.2	1.1 - 262
N-Nitrosomethylurethane	12	9	6.9 - d < 119
Ethylmethane sulfonate	19100	14200	d
1,3-Propane sultone	40	40	< 3.5 - 1345
β-Propiolactone	310	250	104 - 619
Methylmethane sulfonate	ND[e]	680	1082 - 1399
Epichlorohydrin	1130	1130	13718
Glycidaldehyde	19	15	1422 - 16865

[a]Literature data (*62*, *85*).

[b]Expressed as concentration of the test compound (in μmole/L) to produce 500 revertants/plate.

[c]Total dose of carcinogen required to reduce by one-half the probability of the animals being tumor-free throughout a standard lifetime. The formula proposed by Hooper et al. (*86*) and data cited in the IARC Monographs (*1*, *4*, *7*, *11*, *17*) were used for the calculation of TD_{50} values:
$TD_{50} = Dt^3 (\ln 2)/\ln \{ [1-(n_c/N_c)]/[1 - (n_e/N_e)]\}$, where D = total intake of carcinogen; t = experimental time/natural lifetime; n_c = number of tumor-bearing animals (TBA) among controls; N_c = total number of controls; n_e = TBA among experimental animals; N_e = total number of experimental animals. Ranges of TD_{50} values in different rodent species (rats, mice and hamsters) and after different modes of administration.

[d]$TD_{50} > 175$, noncarcinogenic.

[e]Not detected.

nella strain. For example, on the basis of the TD_{50} values, *N*-nitrosoethylurea was the most potent carcinogen studied, but it was only weakly active as a mutagen; glycidaldehyde was one of the most mutagenic compounds, but it was only weakly carcinogenic. These data on a limited number of compounds indicate that a quantitative relationship between the carcinogenesis and mutagenesis of these direct-acting carcinogens in the two Salmonella strains tested cannot be established with enough precision to allow a confident prediction of the carcinogenic potency of new compounds of this class.

Conclusions

Although in the absence of adequate studies in humans long-term animal tests are still today the only ones capable of providing conclusive evidence of the carcinogenic effect of a chemical, the development and application of an appropriate combination of mutagenicity or other short-term tests to screen the human environment, in order to identify both man-made and naturally occurring carcinogens or mutagens, and to quantify their adverse biological effects, is of particular importance. The achievement of this goal will depend heavily on progress made in elucidating the mechanisms of carcinogenesis. Increasing demand for quantitative carcinogenicity data should stimulate further examination of whether there is a quantitative relationship between the potency of a carcinogen in experimental animals and in humans, and its genotoxic activity in short-term tests. Because mutagenic and carcinogenic activities vary over a range of millions (*62*,*66*,*84*), it has been argued that even if only a rough correlation could be established between these two biological activities, it would aid in the assessment of risk of chemicals. However, a recent study of 101 chemicals (*62*) revealed that about 90% of the chemicals showed mutagenic activity ranging over only four orders of magnitude. Thus, an approximate correlation would be of limited practical value. The conflicting results of experimental data published so far with regard to a possible quantitative correlation between the potency of a chemical carcinogen in animals and its activity in short-term mutagenicity tests do not yet sufficiently establish such a relationship for all classes of carcinogens to allow its general use for the confident prediction of carcinogen potency of new compounds.

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