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The Genetic Toxicity Database of the National Toxicology Program: Evaluation of the Relationships between Genetic Toxicity and Carcinogenicity

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The database of the U.S. National Toxicology Program has been developed over approximately two decades, principally focused on substances evaluated for carcinogenicity in rodent bioassays. These assays generally provide data on the relative toxicity and carcinogenicity of chemicals based upon discrete subchronic (13 week) and chronic (104 week) exposures. A major value of these data are that the assay protocols, rodent strains, and technical methodologies have been generally consistent, thus permitting comparisons between assays and chemicals. The genotoxicity data for many of the same chemicals have been developed also using standardized biological systems and protocols. Data for assays including mutagenicity in Salmonella and mouse lymphoma cells, chromosomal aberrations, and sister chromatid exchange in Chinese hamster ovary cells, transformation of Balb/c 3T3 cells, and *in vivo* cytogenetic effects in rodents have been compiled for many chemicals. The results of all of these assays provide a substantial database for evaluating chemical effects and for defining the complex relationships between mutagenicity and carcinogenicity.

Introduction

The genetic toxicity database of the U.S. National Toxicology Program (NTP) has evolved over approximately a 10-year period. The database was an indirect product of efforts to develop and evaluate methods to detect mutagens and identify potential carcinogens. The latter goal was predominant because the duration and cost of identifying carcinogens through animal bioassays mandates that other methods be sought to reduce dependence on the bioassay.

The efforts of other organizations such as the International Program for Chemical Safety (IPCS) and the U.S. Environmental Protection Agency (EPA) to conduct their own evaluations of methods to identify carcinogens led to the realization that a concerted effort to develop *in vitro* genetic toxicity data was mandatory if an evaluation was ultimately to have any influence on the use of *in vitro* or *in vivo* short-term tests (STTs). Results emerging from the IPCS and EPA studies indicated the necessity of developing assay protocols that could yield at least qualitatively reproducible results within and between laboratories. This goal was generallly achieved by the NTP by having two or more laboratories test identical chemicals that were submitted under code. The use of fixed protocols and coded chemicals, however, create the disadvantage that assays cannot be adapted to specific chemical properties such as solubility, volatility, osmolarity, pH, etc. However, the objective value of the data obtained by the assay of coded chemicals was judged to outweigh the other limitations. The principal source of error introduced by the use of coded chemicals is that while a positive response may be definitive, a negative response might be conditional and could be changed by a more appropriate assay protocol. However, since most of the assays evaluated by the NTP were subjected to coded chemicals, it may be assumed that there was a consistent bias for all of the STTs.

Another result of the IPCS and EPA studies was the demonstration that too few of the chemicals had been tested in all systems and that there was an inadequate number of noncarcinogens tested in any STT. In this regard, the NTP rodent bioassay database provided the most appropriate source of chemicals to meet these deficiencies. Some specific aspects of the data need to be emphasized in relationship to the genetic toxicity database.

Over the past two decades, the rodent carcinogenicity studies conducted by the National Institutes of Health have been generally consistent in regard to the assay protocols, strains of rodents, characterization of chemicals, and the use of a biologically active dose, the so-called maximum tolerated dose or MTD. Despite controversy over the selection of the MTD, it provides a method of administering chemicals at a dose that has biological activity, regardless of whether a chemical is highly or weakly toxic. Because of the above factors, it is possible to make comparisons within and between chemicals and bioassay results. There are more than 300 chemicals that have been tested for carcinogenicity in both rats and mice. Because about half of these

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failed to induce a significant carcinogenic response, this database provides a unique resource for operational noncarcinogens. It is always possible that administration of these chemicals by some other route, at a higher dose or longer duration, or to some other strains or species might induce a carcinogenic effect. However, under conditions where many other chemicals were carcinogenic, these chemicals did not induce neoplasia. In addition, for the carcinogenic chemicals, because of the above methodologies, the patterns, sites, and frequencies of tumors induced in one or both species are also important factors available for evaluation. For the majority of the STT data compiled by the NTP, the chemicals evaluated in the rodent bioassay represent the unifying parameter.

Results

The data currently available for "unique" chemicals, that is, chemicals distinguished by a specific Chemical Abstracts Service (CAS) Registry number, in both *in vitro* and *in vivo* systems are shown in Table 1. The data for each chemical and assay system have been published or will be published in the near future. The specific references reporting data are listed in Table 1 under the respective assay system. A number of reasons influenced the selection of these particular chemicals for testing. The usual reason was that the chemical was proposed for, or had been tested in, the NTP rodent bioassay. Other reasons included chemical structure, unusual chemical properties, or interest by other government agencies. The database, therefore, may or may not be representative of the universe of chemicals to which humans may be exposed. The largest proportion of the chemicals are of synthetic origin but a significant percentage are of natural origin. The data for all the *in vitro* systems, except the Balb/c 3T3 transformation system, are compiled in the computer system of the National Institute of Environmental Health Sciences (NIEHS). The data from the different test systems were either entered at the testing laboratory on floppy disk and submitted to the NIEHS computer or submitted as hard copy reports with data entry onto the computer done at NIEHS. A sample of chemical coded with a six-digit aliquot number was issued to the testing laboratory from a central repository. This sample was generally from the same lot of chemical used or selected for the rodent bioassay. (If the sample was used in a rodent bioassay, there was an extensive analysis for purity and identity. If not among chemicals chosen for bioassay, a limited analysis for purity was conducted.) This represents another aspect of the effort to reduce the many sources of variability that can complicate the evaluation of both chemicals and assay systems. Each *in vitro* assay was generally repeated according to the established protocol for the specific test system, which may include repeating positive or negative responses; additional assays may be performed to resolve any problems in interpreting the overall "call" for each aliquot. After the overall result of either positive, negative, or equivocal is made for each aliquot, the identity of the aliquot is released by the repository and the data then can be accessed from the computer by either chemical name, CAS number, or assay system. The majority of the database test results for each of the *in vitro* assays listed in Table 1 have been published; the publications are referenced in the table.

Table 1. Current status of the NTP genetic toxicity database.

Assay system	Number of unique chemicals	References
In vitro		
Salmonella mutagenesis	1662	(*1-6*)
Chinese hamster ovary cell		(*7-14*)
Chromosome aberrations	606	
Sister chromatid exchange	602	
Mouse lymphoma cell mutagenesis	343	(*23-32*)
BALB/c 3T3 cell transformation	200	(*47-48*)
In vivo		
Drosophila mutagenesis		(*15-22*)
Sex-linked recessive lethal assay	283	
Reciprocal translocation	68	
Rodent bone marrow		(*33-39*)
Chromosomal aberrations	116	(*33-39*)
Sister chromatid exchange	116	
Micronuclei	158	

One other aspect of the program that led to the development of this database deserves commentary because it also relates to ensuring that the data obtained are of the highest quality achievable under such a contract-testing approach. The laboratories participating in the generation of these data operate under the general guidelines for good laboratory practices (GLPS). Because the standardized version of GLPs are not directly applicable to *in vitro* test methods and because repeatability of test results was an integral component of the *in vitro* assay protocols, the GLPs used in this program are a modification of those endorsed by other government agencies. However, the fidelity of data transmission has been ensured by periodic audits of the many testing laboratories. In addition, periodically positive and negative control chemicals were sent as coded aliquots along with other test chemicals to the testing laboratories to ensure the intra- and interlaboratory reproducibility of results.

The efforts to ensure the quality of the genetic toxicity assays and the ability to relate the results of the assays directly to the results of the same chemicals tested in rodent bioassays makes it possible to conduct evaluations in which there can be confidence that the conclusions may have general value. Genetic toxicity data for two groups of chemicals were used in separate evaluations of the capacity of four *in vitro* assays to distinguish between rodent carcinogens and noncarcinogens. The first study included 73 chemicals, and the latter study evaluated an additional 41 chemicals (*40,41*). The predictive value of induced mutagenesis in Salmonella and mouse lymphoma cells and induced chromosomal aberrations and sister chromatid exchanges (SCEs) in CHO cells were similar for both groups of chemicals. The combined data sets were evaluated for the standard parameters of assay performance (sensitivity and specificity) and the Salmonella assay which had the lowest sensitivity (0.48), demonstrated the highest specificity (0.91). Conversely, the mouse lymphoma mutagenesis assay had the highest sensitivity (0.72) but the lowest specificity (0.40). The overall concordance (accuracy) of the four assays were 0.66, 0.61, 0.59, and 0.59 for Salmonella, chromosome aberrations, SCE, and mouse lymphoma cells, respectively. For these chemicals, Salmonella mutagenesis had the best predictive value for carcinogenicity (89%), but a negative response in any of these assays was not predictive for noncarcinogens. It is important to note that there was no complementarity among these four *in vitro* assays and that no combination of these four assays was more predictive of carcinogenic potential than the Salmonella assay alone.

The above results indicate that these assays generally detect similar chemical properties but that other chemical properties can also be related to carcinogenic potential. An extensive evaluation of the relationship between chemical structure, mutagenicity, and carcinogenicity has shown that there is a very high correlation between specific chemical substructures (structural alerts) and carcinogenic potential (*42–46*). These structural alerts thus represent specific risk factors for carcinogenic potential that have high predictive value and are related to general chemical class groups. Chemicals that are carcinogenic but fail to show evidence of potential electrophilic substructures represent a major challenge for future structure–activity analyses. These nongenotoxic or nonmutagenic carcinogens are the focus of extensive research and attempts to develop new assays that may reflect their carcinogenic potential. The search for such assays must include the evaluation of the effects of chemicals that lack mutagenicity (i.e., no structural alerts and are not mutagenic to Salmonella) and are noncarcinogens; a partial group of such agents is listed in Table 2. It is to be emphasized that these noncarcinogens are operationally identified on the basis of exposure of two rodent species for up to 104 weeks to an MTD. Many of these substances are relatively nontoxic and were thus administered to the animals at relatively high doses. This is the most extensive list available of operationally defined noncarcinogens that lack structural alerts for mutagenicity.

There is one other important component of the NTP database that can be of great value in validating any new assays purported to identify carcinogens. There are currently about 50 substances that are in some phase of the rodent bioassay. These phases range from ongoing subchronic (i.e., 90 day) toxicity assays for establishing an MTD level for the 2-year assays, to the 2-year studies nearing completion. The carcinogenic potential for these agents is not now known, but it will be established within a 2- to 3-year period. Forty-four of these substances have been used recently to prospectively evaluate the predictive potential of structural alerts and Salmonella mutagenicity for identifying carcinogens (*44*). In addition, because nonmutagenic substances are also included in this group, it was also an opportunity to evaluate the predictive value of subchronic organ-specific toxicity. These same chemicals have been used in two other prediction exercises with computer-based expert systems (*43*). These substances under test represent a unique database that can be used to significantly shorten the time and effort required to evaluate and validate other methods of characterizing potential carcinogens.

Table 2. Nonmutagenic noncarcinogens.

Acetohexamide	Agar
Aldicarb	Anilazine
o-Anthranilic acid	L-Ascorbic acid
Benzoin	Benzyl alcohol
Butylated hydroxytoluene	Caprolactam
Carbromal	2-Chloroethyltrimethylammonium Cl
Chlorpheniramine maleate	3-Chloro-*p*-toluidine
Chlorpropamide	C.I. Acid Orange 10
C.I. Acid Red 14	Coumaphos
Diarylanilide yellow	Diazinon
1,2-Dichlorobenzene	Dichlorodiphenyltrichloroethane
N,N'-Dicyclohexylthiourea	Endrin
Ephedrine sulfate	Erythromycin stearate
Ethionamide	Ethylenediamine tetracetic acid
FD&C Yellow No. 6	Geranyl acetate
Guar gum	Gum arabic
Lindane	Lithocholic acid
Locust bean gum	Malaoxon
Malathion	D-Mannitol
DL-Menthol	Methoxychlor
Methyl methacrylate	Penicillin VK
Pentachloronitrobenzene	Phenformin
Phenol	Phenylephrine HCl
1-Phenyl-3-methyl-5-pyrazolone	*N*-Phenyl-*p*-phenylenediamine
1-Phenyl-2-thiourea	Phthalamide
Phthalic anhydride	Piperonly butoxide
Sodium diethyldithiocarbamate	Sulfisoxazole
3-Sulfolene	Tara gum
2,3,5,6-Tetrachloro-4-nitroanisole	Tetracycline HCl
Tetraethylthiuram disulfide	Tetrakis(hydroxymethyl)phosphonium Cl
Titanium dioxide	Triphenyltin hydroxide
Tolbutamide	Xylenes, commercial mixture
L-Tryptophan	

Discussion

It must be emphasized that the genetic toxicity database of the NTP will only continue to enlarge in specific areas. Limited numbers of new chemicals are being tested for mutagenicity in Salmonella and for chromosomal aberrations *in vitro* and *in vivo*. The concept of identifying one or a few *in vitro* short-term tests with which to screen large numbers of chemicals and to specifically identify potential carcinogens has not been supported by the studies cited above. The NTP attempts to use structural information, Salmonella mutagenicity, *in vivo* cytogenetic effects, and selected subchronic toxicity results as risk factors for potential carcinogens. There is also a continuing effort to develop other *in vitro* and *in vivo* methods that identify other risk factors, particularly for nonmutagenic substances. There are major impediments to the exclusive use of multiple existing *in vitro* systems, beyond the fact that they measure, with varying specificity, some similar chemical properties. They often cannot accommodate the aspects of metabolism, disposition, chronic exposure regimens, and specific chemical–gene interactions that can play critical roles in the multistep or multigene processes of neoplasia. Databases such as those of the NTP are clearly valuable in deducing relationships between structure and biological effects of chemicals. However, databases are not constructively used when applied to retrofitting results. It is unlikely that any existing *in vitro* assays will emerge as useful short-term tests from such retrospective associations. Time and resources will be much better spent searching for new methods of identifying chemical properties that can be etiologically linked to carcinogenesis. Such methods might include those that can identify indirect damage to critical cell functions through free radicals resulting from oxidative metabolism; alteration of normal receptor–ligand functions; disruption of signal transduction pathways; or alteration of regulatory functions for gene transcription. These are not necessarily the most appropriate and certainly are not the only important mechanisms that can be studied. They are, however, mechanisms by which both electrophilic and nonelectrophilic substances could generate heritable phenotypic changes.

There is one other important issue related to the development of databases. An important factor in the conclusions that have been reached about the relationship between *in vitro* short-term tests and rodent carcinogenicity studies has been the availability

of data on rodent noncarcinogens. An equally important factor in efforts to understand the extended relationships between these rodent or *in vitro* data and the risk of induced carcinogenesis in humans is the absence of a database on substances that are probably not carcinogenic to humans. Without the ability to determine the effects of such substances in assays that are supposed to detect carcinogenic potential, there will continue to be uncertainty and controversy about weak and equivocal effects of chemicals. If nothing can be judged to be probably noncarcinogenic for humans, then there will continue to be controversy about weakly positive substances identified as carcinogens.

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