Testing Strategy for Complex Mixtures of Carcinogens

Roy E. Albert

ABSTRACT

Of the 50,000 chemicals and mixtures in commercial use, only about 10% have enough data to permit even a partial health risk assessment. A more effective approach is needed than now exists to determine which of these agents is a significant carcinogenic risk to humans. A scheme is proposed here which would use toxicity as a surrogate for carcinogenic potency. This surrogate for carcinogenic potency would be combined with a surrogate for exposure (usage patterns) to yield an estimate of carcinogenic risk. A cut-off for risk would be used equal to a lifetime excess cancer risk of $10^{-5}$. Agents with surrogate estimates greater than $10^{-5}$ would be tested for genotoxicity and, if positive, the chemical would be tested by animal bioassay. Non-genotoxic agents would be tested for promoting activity. Estimates are given of the total cost of the programme.

1 INTRODUCTION

Carcinogenic mixtures can be a major source of public health concern, e.g. cigarette smoke, diesel engine exhaust particulates, petroleum refinery products, dioxin-containing trichlorophenol and its derivatives, halomethanes in drinking water, etc. Complex mixtures can involve collections of dissimilar chemicals, e.g. cigarette smoke, or a collection of congeners of a particular compound, e.g. the chlorinated biphenyls. Testing for the carcinogenicity of mixtures is usually done by conventional bioassay procedures. The major difference between testing pure compounds and mixtures is that additional studies may be added to define the active components of mixtures as a basis for remedial action. For example, the identification of the active components of a complex mixture was particularly effective with petroleum distillates where it was demonstrated that the carcinogens were limited to the high boiling fraction. Effective remedial action followed this discovery.

Regardless of whether the chemical exists in the environment as a single agent or as a mixture, only a small fraction of chemicals in commercial use have been
evaluated for their carcinogenic impact. The purpose of this paper is to describe current carcinogen testing strategies and to suggest improvements.

2 SELECTION OF COMPOUNDS AND MIXTURES FOR TESTING

The universe of chemical substances that require testing is very large. There are about 65,000 chemicals in use including pesticides (~3000), cosmetics (~3000), drugs (~2000), food additives (~9000), chemicals in commerce > $10^6$ lb/year (~13,000) and chemicals in commerce < $10^6$ lb/year and production unknown (~36,000). Essentially all of these are mixtures in that they contain impurities, and many are used as mixtures. Of the approximately 50,000 chemicals in commerce, only about 10% have enough data to permit a partial health hazard assessment and about 80% have no data to permit any health hazard assessment. Of the remaining 17,000 chemicals, including pesticides, cosmetics, drugs, and food additives, about 10–20% have a data base that would permit a partial or complete health hazard assessment and 25–50% have no data base to permit a health hazard assessment (Steering Committee, 1983).

2.1 Current Chemical Selection Practices

In the National Toxicology Program (NTP), chemicals and mixtures are nominated by various groups, including federal agencies, state agencies, the public, labour, and industry. These nominations are evaluated by a Chemical Evaluation Committee at the NTP on the basis of a set of selection principles, chemical properties, usage patterns, and toxicological data.

The nomination process identifies agents which are of concern for a variety of reasons but it does not represent a systematic approach to testing agents on a priority basis according to their potential public health impact as carcinogens.

2.2 Proposed Chemical Selection Strategy

The method proposed here for selecting compounds for testing assumes that all chemicals are carcinogenic and that testing would be done in rank order according to the estimated cancer risk (dose x potency x population size). Exposure levels (dose) and the number of people exposed (occupational and non-occupational considered separately) would be estimated (crudely) from usage patterns. The carcinogenic potency would be estimated according to acute toxicity ($LD_{50}$). Fiering and Wilson (1983) have found, with 113 compounds studied by the National Cancer Institute, that the correlation between carcinogenic potency and $LD_{50}$ has a geometric standard deviation of 5–10. In effect, the proposed chemical selection process would be a rough quantitative carcinogenic risk assessment on the assumption that all agents are carcinogens. Acute toxicity as a surrogate for carcinogenic potency might use cultured cells rather than animals. This proposed
Testing Strategy for Complex Mixtures of Carcinogens

approach would be a systematic, although crude, way to prioritize carcinogenesis testing on the basis of potential carcinogenic impact.

3 CARCINOGENESIS BIOASSAY

The current NTP animal bioassay includes a standardized two-species (F-344 rats and B6C3F1 mice), two-sex, two-year study with 50 animals per group at each of two doses and controls (National Toxicology Program, 1983). Detailed histological examination of virtually all tissues is done on every animal. Each bioassay costs about $400 000, and limited numbers are being done (e.g. 27 new starts in 1982).

The bioassay programme stresses standardization and meticulous quality control. The bioassay design suffers from attempting to encompass two conflicting objectives: the identification of agents as carcinogens, which requires emphasis on high doses, and the characterization of dose–response relationships for quantitative risk assessment, which emphasizes low doses. No systematic efforts are being made under the NTP programme to test for tumour promoting or initiating activity. At the present rate of testing, it would take about 200 years to study a 10% sample of the chemicals currently in use at a cost of about $3 billion; even then, with the current chemical selection process, we would not know whether important carcinogens were missed.

3.1 Proposed Carcinogen Bioassay Strategy

Beginning with chemicals having the highest estimated risk levels, as described above, genotoxicity tests would be done for mutagenicity and chromosome damage. Agents which are positive for genotoxicity would be put through a qualitative animal carcinogenicity screen involving two species (rats and mice), both sexes, 30 animals per dose group, and a single supra-maximum tolerated dose at about 20% excess mortality; the animals would be dosed for 1 year and followed for 1.5 years. Only histologically confirmed grossly visible tumours would be scored. If positive, a four-dose NTP-type lifetime dose–response study would be done which would also score only for histologically confirmed grossly visible tumours. If the screen were negative, the agent would be tested as a skin (mouse) and liver (rat) initiator.

For non-genotoxic agents, a single high-dose, single-sex, and single-strain screening study would be done for skin (mouse) and liver (rat) promotion. If positive, full-scale lifetime dose–response bioassays would be done for both promoting activity and complete carcinogenesis by NTP-type bioassay with the exception that the scoring would be done on the basis of histologically confirmed, grossly visible tumours.

This two-step animal bioassay approach using a qualitative screen followed by a full-scale quantitative dose–response bioassay would avoid the problem of
interpreting equivocally positive results by focusing on those agents which are decisively carcinogenic; the approach emphasizes good dose–response data for quantitative risk assessment. One drawback to the proposed approach would be the increased time required for the two sequential studies compared with the current single NTP bioassay. However, with the relatively abbreviated screening bioassay and the much reduced load of histopathological examinations, the total time should not be much greater than that currently required for an NTP bioassay (about 5 years). Another limitation to the proposed approach is that it would probably miss some weak carcinogens; however, it would systematically identify agents which have only initiating or promoting activity.

4 MAGNITUDE OF THE ANIMAL TESTING PROGRAMME UNDER THE PROPOSED STRATEGY: SPECULATIVE ESTIMATES

Assume that about half (30 000) of the 65 000 chemicals are actually used and involve significant exposure. Of these, assume 50% (15 000) would be above a testing cut-off level of $10^{-5}$ lifetime cancer risk and would require genotoxicity testing. Assume 10% (1500) are genotoxic and require qualitative animal screening bioassays for carcinogenicity. Of the 1500, assume one-third (500) are positive in the carcinogenesis animal screening bioassay; 500 would then be tested

Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. of tests</th>
<th>Current dollar cost/test</th>
<th>Total current dollar cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotoxic agents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qualitative screening bioassay for carcinogenicity</td>
<td>1500</td>
<td>100 000</td>
<td>150 M</td>
</tr>
<tr>
<td>Quantitative dose–response bioassay</td>
<td>500</td>
<td>500 000</td>
<td>250 M</td>
</tr>
<tr>
<td>Initiation bioassay</td>
<td>1000</td>
<td>100 000</td>
<td>100 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500 M</td>
</tr>
<tr>
<td><strong>Non-genotoxic agents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioassay for promotion</td>
<td>2700</td>
<td>100 000</td>
<td>270 M</td>
</tr>
<tr>
<td>Quantitative dose–response bioassay</td>
<td>270</td>
<td>500 000</td>
<td>130 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>400 M</td>
</tr>
<tr>
<td><strong>Total cost:</strong></td>
<td></td>
<td></td>
<td>$900 million</td>
</tr>
</tbody>
</table>
by a full-scale bioassay for dose–response data and the remaining 1000 would be tested for initiating activity.

This leaves 13 500 non-genotoxic agents above the $10^{-5}$ lifetime cancer risk level. Using a non-genotoxic testing cut-off of $10^{-3}$ lifetime risk (which is roughly equivalent to a no-observed-effect level with a safety factor of 100), perhaps about 20% or 2700 chemicals would have to be tested by a qualitative screen for promotion; of these, perhaps 10% or 270 compounds would be positive and require full-scale dose–response studies.

At $100,000 each for the qualitative animal carcinogenesis bioassay screen, the initiation bioassay, and the promotion bioassay, and $500,000 for the full-scale dose–response bioassay, the total cost for the animal testing under the proposed testing strategy, as shown in Table 1, would be in the domain of $1 billion.

5 REFERENCES


National Toxicology Program (1983). *Fiscal Year Annual Plan*.
