Use of the Syrian Hamster Embryo Cell Transformation Assay for Carcinogenicity Prediction of Chemicals Currently Being Tested by the National Toxicology Program in Rodent Bioassays

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The Syrian hamster embryo (SHE) cell transformation assay was used to predict the carcinogenicity of 26 chemicals currently being tested in the rodent bioassay by the National Toxicology Program as part of its program titled “Strategies for Predicting Chemical Carcinogenesis in Rodents.” Of these 26 chemicals, 17 were found to be positive in the SHE cell transformation assay while 9 were negative. Carcinogenicity predictions were made for these chemicals, based upon the SHE cell transformation assay results. Our predictions will be compared with the rodent bioassay results as they become available. — Environ Health Perspect 104(Suppl 5):1075–1084 (1996)

Key words: SHE assay, cell transformation, carcinogenicity prediction, rodent bioassay

Introduction

Since the seminal work of Berwald and Sachs (1), Syrian hamster embryo (SHE) cells have been used to evaluate the potential of a wide variety of chemical and physical agents to induce morphological transformation (2). SHE cells are diploid, genetically stable, of finite lifespan, and capable of metabolizing many chemicals to their ultimate carcinogenic form (3). SHE cells have also been used in a number of laboratories to study mechanisms of carcinogenesis (4). Following carcinogen exposure, SHE cells display a multistage pattern of progression to neoplasia that is similar to the multistage progression of in vivo carcinogenesis (4–6). Because of these factors, SHE cells are an attractive model for determining the neoplastic transformation potential of chemical agents.

More than 472 chemical/physical agents have been tested in the SHE cell transformation assay (2). Of these 472 agents, 213 have in vivo rodent carcinogenicity data available. Of these 213 agents, 177 were rodent carcinogens and 36 were noncarcinogens. For these agents, the SHE cell transformation assay has a concordance with the rodent bioassay of 80% (171/213), a sensitivity of 82% (146/177), and a specificity of 69% (25/36). Recently, we have modified the methodology used to conduct the SHE cell transformation assay, principally by reducing the culture medium pH from 7.5 to 6.7 (7). Using this modified protocol, we have tested over 56 chemicals, including 30 carcinogens and 18 noncarcinogens (8). The SHE cell transformation assay conducted with the reduced pH methodology has an overall concordance with the rodent bioassay of 85% (41/48), a sensitivity of 87% (26/30), and a specificity of 83% (15/18). It is our current position that SHE cell transformation assay data, in combination with other information such as structure-activity relationship analysis, genetic toxicity results, and when available subchronic toxicity data and metabolism considerations, can be used for predicting rodent carcinogenicity.

Twenty-six of the chemicals currently being tested for rodent carcinogenicity by the National Toxicology Program (NTP) were provided to us for predicting the outcome of these bioassays using the SHE cell transformation assay. We submit our predictions based upon SHE cell transformation assay results in the hope that the SHE cell transformation assay will become a method used for improved carcinogenicity prediction and risk assessment.

Methods

A description of the protocol for conduct of the reduced pH SHE cell transformation assay was originally published in 1989 (9). A more detailed description of this protocol is currently in press (7). Briefly, the methods we use are as follows: A cytotoxicity screen is done initially to determine a dose level that produces 50% or greater cytotoxicity, based on reduction in cloning efficiency. This is the top dose tested, with at least four additional doses tested, down to a dose level that causes minimal cytotoxicity. The SHE cell transformation assay is typically done in two individual trials, each consisting of the five test chemical doses and a solvent control (usually dimethyl sulfoxide [DMSO] or culture medium) plus a positive control (benzo[a]pyrene). Each trial consists of 20 culture dishes/test-chemical treatment group, with between 25 and 45 SHE cell colonies per culture dish to generate the approximately 1000 or more colonies necessary for adequate assay sensitivity. The cells are exposed to test chemical for either 24 hr followed by 6 to 7 days of growth or for 7 days, after which the colonies are fixed with methanol and stained with Giemsa and scored for morphological transformation with a stereo microscope. With pooled data from at least two trials, morphological transformation (MT) frequencies (number of MTs/total colonies scored × 100) are determined for each dose level, and a Fisher’s exact test (10) is conducted comparing the transformation frequency of the solvent control pairwise with each test chemical dose group. A trend test (11) is also conducted on the pooled transformation frequency/dose group data. A test chemical is considered positive in the SHE cell transformation assay if it causes a statistically significant (p<0.05) increase in MT (relative to the solvent control) in at least two dose groups, or if it causes a statistically significant increase in MT in one dose with a statistically significant (p<0.05) positive dose–response trend test. If either the 24-hr or the 7-day exposure is positive, the overall SHE cell transformation assay call for a test chemical is positive. Our predictions of carcinogenicity for the chemicals tested in this study are based
Table 1. Syrian hamster embryo (SHE) cell transformation assay results for 26 chemicals currently being tested in the rodent bioassay.

| Chemical [CAS no.] | Dose (µg/ml) | RPE, %a | Number of MTF/total colonies | MTF, %c | Fisher’s exact test, p-value |
|---------------------|--------------|---------|-------------------------------|---------|-----------------------------|
| Anthraquinone [84-65-1] | | | | | |
| 24-hr | | | | | |
| Control | 100(43)d | 6/1404 | 0.43 | 0.1951# |
| 0.5 | 108 | 4/1139 | 0.35 | 0.5986 |
| 1 | 105 | 6/1051 | 0.57 | 0.3657 |
| 3 | 111 | 6/1162 | 0.52 | 0.4526 |
| 5 | 93 | 7/1320 | 0.53 | 0.4548 |
| 6 | 88 | 10/1264 | 0.80 | 0.1604 |
| 7 | 95 | 9/1328 | 0.68 | 0.2659 |
| 8 | 100 | 9/1396 | 0.64 | 0.3002 |
| 9 | 100 | 6/1403 | 0.43 | 0.6126 |
| 4.25 | 80 | 5/1362 | 0.37 | 0.5068 |
| 4.5 | 74 | 6/1217 | 0.66 | 0.1363 |
| 5 | 41 | 8/1051 | 0.76 | 0.0860 |
| 5.25 | 32 | 4/1022 | 0.39 | 0.4783 |
| 7-day | | | | | |
| Control | 100(48)d | 5/1636 | 0.31 | 0.1567* |
| r-Butylhydroquinone [11948-33-0] | | | | | |
| 24-hr | | | | | |
| Control | 100(44)d | 4/1243 | 0.32 | 0.1530# |
| 1 | 96 | 8/1189 | 0.67 | 0.1725 |
| 2 | 100 | 10/1227 | 0.82 | 0.0853 |
| 3 | 90 | 5/1038 | 0.48 | 0.3904 |
| 5 | 82 | 7/1427 | 0.49 | 0.3568 |
| 7 | 48 | 2/1317 | 0.15 | 0.3154 |
| 7-day | | | | | |
| Control | 100(43)d | 6/1417 | 0.42 | 0.1030* |
| 3 | 107 | 6/1562 | 0.38 | 0.5457 |
| 4 | 98 | 9/1476 | 0.63 | 0.3075 |
| 5 | 94 | 3/1395 | 0.22 | 0.2746 |
| 6 | 53 | 7/1406 | 0.50 | 0.4943 |
| 7 | 25 | 10/1083 | 0.92 | 0.0973 |
| 1-Chloro-2-propanol + 2-chloro-1-propanol(75:25)[127-00-4] | | | | | |
| 24-hr | | | | | |
| Control | 100(44)d | 3/1330 | 0.23 | 0.0009** |
| 1000 | 93 | 3/1231 | 0.73 | 0.0566 |
| 2000 | 89 | 3/1169 | 0.67 | 0.0807 |
| 3000 | 84 | 14/1113 | 1.26 | 0.0021* |
| 4000 | 80 | 13/1050 | 1.24 | 0.0027* |
| 5000 | 77 | 19/1031 | 1.84 | 0.0000** |
| Cobalt sulfate hydrate [10026-24-1] | | | | | |
| 24-hr | | | | | |
| Control | 100(44)d | 4/1484 | 0.27 | 0.0739* |
| 0.125 | 88 | 18/1276 | 1.41 | 0.0077* |
| 0.250 | 62 | 14/1217 | 1.15 | 0.0049* |
| 0.375 | 70 | 12/1041 | 1.15 | 0.0064* |
| 0.5 | 45 | 12/1095 | 1.10 | 0.0085* |
| 1 | 34 | 12/1148 | 1.05 | 0.0110* |
| Codeine [76-57-3] | | | | | |
| 24-hr | | | | | |
| Control | 100 (45)d | 4/1603 | 0.25 | 0.1012* |
| 1000 | 95 | 5/1480 | 0.34 | 0.4509 |
| 1225 | 96 | 5/1475 | 0.41 | 0.3195 |
| 1450 | 88 | 9/1380 | 0.60 | 0.1293 |
| 1675 | 56 | 5/1240 | 0.40 | 0.3460 |
| 1900 | 39 | 9/1493 | 0.54 | 0.1699 |
| 7-day | | | | | |
| Control | 100(36)d | 4/1155 | 0.35 | 0.4894* |
| 200 | 91 | 5/1045 | 0.57 | 0.3168 |
| 250 | 90 | 3/1033 | 0.67 | 0.0937 |
| 300 | 88 | 5/1017 | 0.59 | 0.3012 |
| 350 | 63 | 4/1080 | 0.37 | 0.5996 |
| 400 | 55 | 6/1117 | 0.54 | 0.3562 |

(Continued)
**Table 1. (Continued)**

| Chemical                          | Dose (µg/ml) | RPE, % a | Number of MTb/total colonies | MTF, % c | Fisher’s exact test, p-value |
|----------------------------------|--------------|----------|------------------------------|----------|-----------------------------|
| D & C Yellow no. 11 [8003-22-3]  |              |          |                              |          |                             |
| 24-hr                            | Control      | 100 (45)d | 2/1245                       | 0.16     | 0.0113**                    |
| 0.1                              | 96           | 10/1214  |                              | 0.82     | 0.0171*                     |
| 1                                | 91           | 13/1149  |                              | 1.13     | 0.0023*                     |
| 2.5                              | 93           | 15/1170  |                              | 1.28     | 0.0007*                     |
| 5                                | 76           | 14/1484  |                              | 0.94     | 0.0067*                     |
| 7.5                              | 62           | 15/1366  |                              | 1.10     | 0.0021*                     |
| Diethanolamine [111-42-2]        |              |          |                              |          |                             |
| 24-hr                            | Control      | 100 (42)d | 4/1404                       | 0.29     | 0.0053**                    |
| 2500                             | 102          | 3/1454   | 0.14                         | 0.4974   |
| 3000                             | 90           | 11/1227  | 0.90                         | 0.0337*  |
| 3500                             | 83           | 9/1184   | 0.76                         | 0.0770   |
| 4000                             | 60           | 8/1227   | 0.55                         | 0.1380   |
| 4500                             | 40           | 12/1262  | 0.95                         | 0.0234*  |
| 7-day                             | Control      | 100 (47)d | 4/1309                       | 0.31     | 0.4056*                     |
| 250                              | 89           | 13/1174  | 1.11                         | 0.0139*  |
| 500                              | 77           | 14/1014  | 1.38                         | 0.0034*  |
| 1000                             | 57           | 18/1096  | 1.64                         | 0.0005*  |
| 1500                             | 49           | 13/1078  | 1.21                         | 0.0067*  |
| 2500                             | 36           | 1/961    | 0.10                         | 0.2976   |
| 1,2-Dihydro-2,2,4-trimethylquinoline [147-47-7] |          |          |                              |          |                             |
| 24-hr                            | Control      | 100 (50)d | 3/1439                       | 0.21     | 0.0000**                    |
| 70                               | 98           | 2/1447   | 0.14                         | 0.4974   |
| 80                               | 106          | 4/1559   | 0.26                         | 0.5436   |
| 90                               | 84           | 6/1227   | 0.49                         | 0.1817   |
| 100                              | 72           | 11/1045  | 1.05                         | 0.0061*  |
| 115                              | 30           | 10/941   | 1.06                         | 0.0069*  |
| Emodin [518-82-1]                |              |          |                              |          |                             |
| 24-hr                            | Control      | 100 (41)d | 3/1369                       | 0.22     | 0.0014**                    |
| 2.5                              | 90           | 7/1237   | 0.57                         | 0.1330   |
| 0.25                             | 85           | 7/1138   | 0.62                         | 0.1062   |
| 10                               | 73           | 7/993    | 0.71                         | 0.0711   |
| 13.75                            | 51           | 9/2021   | 0.75                         | 0.0430*  |
| 17.5                             | 37           | 14/1096  | 1.28                         | 0.0016*  |
| Ethylbenzene [100-41-4]          |              |          |                              |          |                             |
| 24-hr                            | Control      | 100 (45)d | 3/1511                       | 0.20     | 0.3595*                     |
| 100                              | 84           | 3/1279   | 0.24                         | 0.5755   |
| 200                              | 76           | 4/1136   | 0.35                         | 0.3480   |
| 300                              | 69           | 4/1039   | 0.39                         | 0.3041   |
| 400                              | 62           | 6/1405   | 0.43                         | 0.2191   |
| 500                              | 51           | 3/1574   | 0.19                         | 0.6361   |
| 7-day                             | Control      | 100 (41)d | 8/3358                       | 0.59     | 0.0076**                    |
| 100                              | 96           | 11/340   | 0.82                         | 0.3127   |
| 125                              | 90           | 8/1252   | 0.64                         | 0.5335   |
| 150                              | 89           | 16/1246  | 1.28                         | 0.0491*  |
| 175                              | 59           | 15/1222  | 1.21                         | 0.0089*  |
| 200                              | 37           | 16/1164  | 1.38                         | 0.0342*  |
| Ethylene glycol monobutyl ether [111-76-2] |          |          |                              |          |                             |
| 24-hr                            | Control      | 100 (53)d | 6/1474                       | 0.41     | 0.1256*                     |
| 500                              | 79           | 7/1179   | 0.59                         | 0.3406   |
| 750                              | 75           | 8/1112   | 0.72                         | 0.2108   |
| 1000                             | 70           | 11/1044  | 1.05                         | 0.0451*  |
| 1250                             | 53           | 15/1222  | 1.23                         | 0.0139*  |
| 1500                             | 42           | 8/1517   | 0.53                         | 0.4163   |

(Continued)
### Table 1. (Continued)

| Chemical          | Dose (µg/ml) | RPE, %a | Number of MTb/total colonies | MTF, %c | Fisher’s exact test, p-value |
|-------------------|--------------|---------|------------------------------|---------|-----------------------------|
| **Furfuryl alcohol** [98-00-0] |              |         |                              |         |                             |
| 24-hr             | Control      | 100 (47) | 4/1325                       | 0.30    | 0.2389*                     |
|                   | 5            | 102     | 6/1342                       | 0.45    | 0.3846                      |
|                   | 7.5          | 91      | 7/1212                       | 0.58    | 0.2200                      |
|                   | 10           | 96      | 8/1249                       | 0.64    | 0.1600                      |
|                   | 12.5         | 91      | 6/1212                       | 0.50    | 0.3232                      |
|                   | 15           | 81      | 5/1068                       | 0.47    | 0.3897                      |
|                   | 17.5         | 30      | 8/1433                       | 0.56    | 0.2334                      |
| 7-day             | Control      | 100 (36) | 4/1295                       | 0.31    | 0.3278*                     |
|                   | 25           | 99      | 3/1287                       | 0.23    | 0.5034                      |
|                   | 37.5         | 99      | 5/1277                       | 0.39    | 0.4914                      |
|                   | 50           | 81      | 3/1043                       | 0.29    | 0.6167                      |
|                   | 62.5         | 75      | 3/951                        | 0.32    | 0.6302                      |
|                   | 75           | 40      | 5/1213                       | 0.41    | 0.4598                      |
| **Gallium arsenide** [1303-00-0] |          |         |                              |         |                             |
| 24-hr             | Control      | 100 (48) | 3/1459                       | 0.21    | 0.0000**                    |
|                   | 0.25         | 108     | 21/1579                      | 1.33    | 0.0003*                     |
|                   | 0.50         | 96      | 37/1383                      | 2.68    | 0.0000*                     |
|                   | 0.75         | 83      | 27/1219                      | 2.22    | 0.0000*                     |
|                   | 1.0          | 71      | 35/1040                      | 3.57    | 0.0000*                     |
|                   | 1.25         | 58      | 35/1173                      | 2.98    | 0.0000*                     |
|                   | 1.50         | 29      | 33/1016                      | 3.25    | 0.0000*                     |
| **Isobutyraldehyde** [78-84-2] |          |         |                              |         |                             |
| 24-hr             | Control      | 100 (42) | 4/1184                       | 0.34    | 0.2192*                     |
|                   | 200          | 102     | 8/1195                       | 0.67    | 0.1977                      |
|                   | 300          | 88      | 5/1023                       | 0.49    | 0.4119                      |
|                   | 400          | 86      | 6/1008                       | 0.60    | 0.2614                      |
|                   | 575          | 55      | 6/1080                       | 0.56    | 0.3215                      |
|                   | 750          | 50      | 8/1168                       | 0.69    | 0.1867                      |
| 7-day             | Control      | 100 (34) | 6/1374                       | 0.44    | 0.2833*                     |
|                   | 200          | 95      | 5/1302                       | 0.38    | 0.5364                      |
|                   | 375          | 81      | 5/1112                       | 0.45    | 0.5960                      |
|                   | 500          | 74      | 6/1012                       | 0.58    | 0.4002                      |
|                   | 725          | 55      | 10/1059                      | 0.94    | 0.1003                      |
|                   | 900          | 49      | 4/1258                       | 0.32    | 0.4321                      |
| **Methyleugenol** [93-15-2] |          |         |                              |         |                             |
| 24-hr             | Control      | 100 (44) | 3/1224                       | 0.25    | 0.0521*                     |
|                   | 165          | 98      | 12/1201                      | 1.00    | 0.0156*                     |
|                   | 200          | 95      | 9/1166                       | 0.77    | 0.0615                      |
|                   | 210          | 89      | 14/1448                      | 0.97    | 0.0154*                     |
|                   | 220          | 67      | 12/1522                      | 0.79    | 0.0450*                     |
|                   | 235          | 58      | 15/1427                      | 1.05    | 0.0091*                     |
|                   | 250          | 48      | 13/1370                      | 0.95    | 0.0184*                     |
| **Molybdenum trioxide** [1313-27-5] | |         |                              |         |                             |
| 24-hr             | Control      | 100 (42) | 4/1399                       | 0.29    | 0.0006**                    |
|                   | 50           | 95      | 8/1344                       | 0.60    | 0.1745                      |
|                   | 75           | 83      | 10/1172                      | 0.53    | 0.0486*                     |
|                   | 100          | 74      | 17/1026                      | 1.68    | 0.0003*                     |
|                   | 125          | 57      | 17/1136                      | 1.50    | 0.0008*                     |
|                   | 200          | 33      | 10/970                       | 1.03    | 0.0207*                     |
| **Nitromethane** [75-52-5] |          |         |                              |         |                             |
| 24-hr             | Control      | 100 (50) | 5/1534                       | 0.33    | 0.0010**                    |
|                   | 2000         | 86      | 10/1520                      | 0.76    | 0.0817                      |
|                   | 2500         | 86      | 7/1319                       | 0.53    | 0.2896                      |
|                   | 3000         | 92      | 8/1375                       | 0.58    | 0.2253                      |
|                   | 3500         | 84      | 10/1259                      | 0.79    | 0.0737                      |
|                   | 4000         | 84      | 12/1250                      | 0.96    | 0.0291*                     |
|                   | 5000         | 76      | 14/949                       | 1.48    | 0.0027*                     |
### Table 1. (Continued)

| Chemical [CAS no.] | Dose (µg/ml) | RPE, %a | Number of MTb/total colonies | MTF, %c | Fisher's exact test, p-value |
|-------------------|--------------|---------|-----------------------------|---------|-----------------------------|
| **Oxymetholone** [434-07-1] |              |         |                             |         |                             |
| 24-hr             |              |         |                             |         |                             |
| Control           | 100 (44)af   | 8/1749  | 0.46                         | 0.2375* |
| 7.5               | 91           | 11/1594 | 0.69                         | 0.2534  |
| 10                | 90           | 11/1567 | 0.70                         | 0.2415  |
| 12.5              | 84           | 14/1462 | 0.96                         | 0.0674  |
| 15                | 54           | 9/1679  | 0.54                         | 0.4659  |
| 17.5              | 37           | 10/1370 | 0.73                         | 0.2232  |
| 7-day             |              |         |                             |         |                             |
| Control           | 100 (44)af   | 6/1572  | 0.38                         | 0.0075**|
| 15                | 98           | 15/1542 | 0.97                         | 0.0351* |
| 16.5              | 91           | 20/1432 | 1.40                         | 0.0022* |
| 18                | 91           | 24/1428 | 1.68                         | 0.0003* |
| 20                | 73           | 15/1141 | 1.32                         | 0.0061* |
| 22.5              | 45           | 17/1402 | 1.21                         | 0.0083* |
| **Phenolphthalein** [77-09-8] |              |         |                             |         |                             |
| 24-hr             |              |         |                             |         |                             |
| Control           | 100 (45)af   | 6/1323  | 0.45                         | 0.0021**|
| 15                | 107          | 13/1337 | 0.97                         | 0.0664  |
| 17.5              | 93           | 13/1276 | 1.02                         | 0.0713  |
| 20                | 78           | 17/1054 | 1.61                         | 0.0038* |
| 22.5              | 47           | 18/959  | 1.88                         | 0.0010* |
| 25                | 31           | 10/902  | 1.25                         | 0.0388* |
| **Primactone** [125-33-7] |              |         |                             |         |                             |
| 24-hr             |              |         |                             |         |                             |
| Control           | 100 (39)af   | 6/1315  | 0.46                         | 0.2895* |
| 200               | 103          | 6/1299  | 0.46                         | 0.6047  |
| 250               | 97           | 7/1280  | 0.55                         | 0.4802  |
| 300               | 100          | 9/1323  | 0.45                         | 0.0089  |
| 350               | 100          | 9/1294  | 0.39                         | 0.5109  |
| 400               | 95           | 4/1229  | 0.33                         | 0.4190  |
| 7-day             |              |         |                             |         |                             |
| Control           | 100 (40)af   | 8/1374  | 0.58                         | 0.3861* |
| 200               | 105          | 9/1435  | 0.63                         | 0.5363  |
| 250               | 102          | 11/1394 | 0.79                         | 0.3348  |
| 300               | 104          | 9/1431  | 0.56                         | 0.5662  |
| 350               | 105          | 10/1445 | 0.69                         | 0.4496  |
| 400               | 109          | 11/1499 | 0.73                         | 0.3961  |
| **Pyridine** [110-86-1] |              |         |                             |         |                             |
| 24-hr             |              |         |                             |         |                             |
| Control           | 100 (43)af   | 5/1206  | 0.42                         | 0.0752* |
| 500               | 98           | 6/1169  | 0.51                         | 0.4789  |
| 1650              | 107          | 7/1278  | 0.55                         | 0.4268  |
| 2750              | 105          | 6/1246  | 0.48                         | 0.5221  |
| 3875              | 93           | 9/1114  | 0.81                         | 0.1703  |
| 5600              | 79           | 8/9956  | 0.84                         | 0.1628  |
| 7-day             |              |         |                             |         |                             |
| Control           | 100 (47)af   | 3/1307  | 0.23                         | 0.1381* |
| 3250              | 94           | 5/1211  | 0.41                         | 0.3222  |
| 3500              | 91           | 7/1195  | 0.59                         | 0.1371  |
| 3750              | 81           | 6/1053  | 0.57                         | 0.1595  |
| 4000              | 62           | 7/1320  | 0.53                         | 0.1755  |
| 4250              | 51           | 7/1331  | 0.53                         | 0.1790  |
| **Scopolamine hydrobromide** [6533-68-2] |              |         |                             |         |                             |
| 24-hr             |              |         |                             |         |                             |
| Control           | 100 (44)af   | 2/1405  | 0.14                         | 0.0015**|
| 1000              | 111          | 5/1470  | 0.34                         | 0.2452  |
| 2000              | 91           | 15/1228 | 1.22                         | 0.0004* |
| 3000              | 77           | 14/1068 | 1.31                         | 0.0003* |
| 4000              | 52           | 12/1379 | 0.87                         | 0.0058* |
| 5000              | 43           | 14/1227 | 0.98                         | 0.0022* |

(Continued)
**Table 1. (Continued)**

| Chemical                  | Dose (µg/ml) | Number of MTb/total colonies | Fisher’s exact test, p-value |
|---------------------------|--------------|------------------------------|----------------------------|
| Sodium nitrite            |              |                              |                            |
| (7632-00-0)               |              |                              |                            |
| 24-hr                     |              |                              |                            |
| Control                   | 100 (44) d   | 4/1220                       | 0.33                       | 0.0061**                  |
| 375                       | 91           | 12/1115                      | 1.08                       | 0.0252*                   |
| 500                       | 73           | 22/1320                      | 1.67                       | 0.0005*                   |
| 625                       | 68           | 15/1148                      | 1.31                       | 0.0065*                   |
| 750                       | 61           | 17/1112                      | 1.53                       | 0.0018*                   |
| 875                       | 50           | 17/1144                      | 1.49                       | 0.0022*                   |
| Sodium xylenesulfonate    |              |                              |                            |
| (1300-72-7)               |              |                              |                            |
| 24-hr                     |              |                              |                            |
| Control                   | 100 (40) d   | 5/1337                       | 0.37                       | 0.3434*                   |
| 100                       | 98           | 7/1286                       | 0.54                       | 0.3691                    |
| 500                       | 75           | 10/1391                      | 0.72                       | 0.1691                    |
| 1000                      | 60           | 5/1309                       | 0.38                       | 0.6102                    |
| 1500                      | 55           | 5/1329                       | 0.39                       | 0.6020                    |
| 2000                      | 48           | 5/1281                       | 0.39                       | 0.5967                    |
| 7-day                     |              |                              |                            |
| Control                   | 100 (45) d   | 3/1255                       | 0.24                       | 0.4365*                   |
| 100                       | 93           | 5/1160                       | 0.43                       | 0.3209                    |
| 250                       | 89           | 7/1114                       | 0.63                       | 0.1269                    |
| 500                       | 80           | 4/998                        | 0.40                       | 0.3767                    |
| 750                       | 53           | 5/1155                       | 0.43                       | 0.3186                    |
| 875                       | 47           | 4/1239                       | 0.32                       | 0.4930                    |
| Tetrahydrofuran           |              |                              |                            |
| (109-99-9)                |              |                              |                            |
| 24-hr                     |              |                              |                            |
| Control                   | 100 (42) d   | 4/1275                       | 0.31                       | 0.4062*                   |
| 1000                      | 102          | 6/1322                       | 0.45                       | 0.3862                    |
| 2000                      | 102          | 6/1294                       | 0.46                       | 0.3859                    |
| 3000                      | 105          | 5/1393                       | 0.37                       | 0.5365                    |
| 4000                      | 105          | 4/1345                       | 0.30                       | 0.6073                    |
| 5000                      | 112          | 5/1434                       | 0.35                       | 0.5720                    |
| 7-day                     |              |                              |                            |
| Control                   | 100 (42) d   | 3/1432                       | 0.21                       | 0.5165*                   |
| 1000                      | 100          | 3/1438                       | 0.21                       | 0.6584                    |
| 2000                      | 103          | 6/1467                       | 0.41                       | 0.2656                    |
| 3000                      | 96           | 4/1366                       | 0.29                       | 0.4742                    |
| 4000                      | 107          | 4/1537                       | 0.26                       | 0.5387                    |
| 5000                      | 100          | 3/1425                       | 0.21                       | 0.6541                    |
| Vanadium pentoxide        |              |                              |                            |
| (1314-02-1)               |              |                              |                            |
| 24-hr                     |              |                              |                            |
| Control                   | 100 (45) d   | 4/1356                       | 0.30                       | 0.2771*                   |
| 0.05                      | 91           | 6/1259                       | 0.48                       | 0.3324                    |
| 0.125                     | 93           | 8/1221                       | 0.66                       | 0.1469                    |
| 0.375                     | 76           | 8/1023                       | 0.78                       | 0.0865                    |
| 0.625                     | 67           | 9/1337                       | 0.67                       | 0.1277                    |
| 0.75                      | 51           | 5/1342                       | 0.37                       | 0.4941                    |
| 7-day                     |              |                              |                            |
| Control                   | 100 (35) d   | 5/1161                       | 0.43                       | 0.0000**                  |
| 0.125                     | 95           | 15/1124                      | 1.34                       | 0.0170*                   |
| 0.375                     | 91           | 28/1080                      | 2.59                       | 0.0000*                   |
| 0.625                     | 83           | 46/986                       | 4.67                       | 0.0000*                   |
| 0.75                      | 66           | 70/1071                      | 6.54                       | 0.0000*                   |
| 0.875                     | 57           | 91/1150                      | 7.91                       | 0.0000*                   |

RPE, relative plating efficiency; MT, morphological transformed colony; MTF, morphological transformation frequency. *Relative plating efficiency = (test group plating efficiency × solvent control plating efficiency) × 100. **Morphologically transformed colony. †Morphological transformation frequency = (number of MT colonies + total number of colonies) × 100. *Actual target cell plating efficiency in = (number of colonies/dish + number of cells seeded) × 100. *MTF values are significantly greater than control MTF values at p<0.05 as determined by the Fisher’s exact test. *MTF values are not trend test positive at p<0.05 as determined by an unstratified binomial exact permutation trend test (Cytel Software Corp., Cambridge, MA). **MTF values are trend test positive at p<0.05 as determined by an unstratified binomial exact permutation trend test (Cytel Software Corp., Cambridge, MA).
Table 2. Carcinogenicity predictions for 26 chemicals currently being tested in the rodent bioassay.

| Chemical, mol. wt., CAS no., purity | NTP rodent bioassay | SHE assay results | Carcinogenicity prediction |
|------------------------------------|---------------------|------------------|---------------------------|
| Ellagic acid                        | Feed                |                 | Noncarcinogen             |
| 204.2                              | 1875 1875           | -                |                           |
| 84-65-1                             | 7500 7500           | 24-hr            |
| 99%                                |                     | All doses (-)    |
|                                    |                     | Trend test (-)   |
|                                    |                     | 7-day            |
|                                    |                     | All doses (-)    |
|                                    |                     | Trend test (-)   |
| Anthraquinone                      | Feed                |                 | Noncarcinogen             |
| 208.2                              | 1875 1875           | -                |                           |
| 84-65-1                             | 7500 7500           | 24-hr            |
| 99%                                |                     | All doses (-)    |
| t-Butylhydroquinone                | Feed                |                 | Noncarcinogen             |
| 166.2                              | 182 210             | -                |                           |
| 1948-33-0                           | 563 545             | 24-hr            |
| 99%                                |                     | All doses (-)    |
|                                   |                     | Trend test (-)   |
| 1-Chloro-2-propanol + 2-chloro-1-propanol (75:25 mix) | Water | 34 35 152 159 | + N/A + | Carcinogen |
|                                    |                     | 24-hr            |
|                                    |                     | 3 of 5 doses (+) |
|                                    |                     | Trend test (+)   |
| Cobalt sulfate hydrate             | Inhalation          | 0.47 0.54 0.98 1.00 | + N/A + | Carcinogen |
|                                    |                     | 24-hr            |
|                                    |                     | 5 of 5 doses (+) |
|                                    |                     | Trend test (-)   |
| Codeine                            | Feed                | 58 67 338 327   | -              |
| 299.4                              |                     | 24-hr            |
| 76-57-3                             |                     | All doses (-)    |
| 99.8%                              |                     | Trend test (-)   |
|                                    |                     | 7-day            |
|                                    |                     | All doses (-)    |
|                                    |                     | Trend test (-)   |
| D&C Yellow no. 11                  | Feed                | 182 210 NA NA    | + N/A +        | Carcinogen |
| 273.3                              |                     | 24-hr            |
| 9003-22-3                           |                     | 5 of 5 doses (+) |
| -99%                               |                     | Trend test (+)   |
| Diethanolamine                     | Skin paint          | 64 32 160 160   | + + +          | Carcinogen |
| 105.1                              |                     | 24-hr            |
| 111-42-2                            |                     | 2 of 5 doses (+) |
| 98.8%                              |                     | Trend test (+)   |
|                                    |                     | 7-day            |
|                                    |                     | 4 of 5 doses (+) |
|                                    |                     | Trend test (+)   |
| 1,2-Dihydro-2,2,4-trimethyquinol (monomer) | Skin paint | 100 100 10 10 | + N/A + | Carcinogen |
| 173.3                              |                     | 24-hr            |
| 147-47-7                            |                     | 2 of 5 doses (+) |
| >97%                               |                     | Trend test (+)   |
| Emodin                             | Feed                | 45 53 68 65     | + + +          | Carcinogen |
| 270.2                              |                     | 24-hr            |
| 518-82-1                            |                     | 2 of 5 doses (+) |
| 94.5%                              |                     | Trend test (+)   |

(Continued)
| Chemical, mol. wt., CAS no., purity | Route | High dose, mg/kg/day | NTP rodent bioassay | SHE assay results | Carcinogenicity prediction |
|-----------------------------------|-------|----------------------|---------------------|------------------|---------------------------|
| Ethylbenzene 106.2 100-41-4 99% | Inhalation | 511 586 1063 1083 | 24-hr + | + | 7-day 2 of 5 doses (+) Trend test (+) | Carcinogen |
| Ethylene glycol monobutyl ether (monomer) 118.2 111-76-2 99% | Inhalation | 95 109 394 402 | 24-hr + | N/A | | Carcinogen |
| Furfuryl alcohol 98.1 98-00-0 98% | Inhalation | 20 23 42 43 | 24-hr | — | — | Noncarcinogen |
| Gallium arsenide 144.6 1303-00-0 99.9% | Inhalation | 0.16 0.18 0.33 0.33 | 24-hr | + | N/A | | Carcinogen |
| Isobutyraldehyde 72.1 78-84-2 >98% | Inhalation | 925 1061 1925 1963 | 7-day | All doses (−) Trend test (−) | Noncarcinogen |
| Methylmaleonitrile 178.2 93-15-2 99% | Gavage | 150 150 75 75 | 24-hr | + | N/A | | Carcinogen |
| Molybdenum trioxide 144.0 1313-27-5 99% | Inhalation | 16 18 33 33 | 24-hr | + | N/A | | Carcinogen |
| Nitromethane 61.0 75-52-5 98% | Inhalation | 147 169 612 624 | 24-hr | + | N/A | | Carcinogen |
| Oxymetholone 332.5 434-07-1 >95% | Gavage | 150 100 NA NA | 7-day 5 of 5 doses (+) Trend test (+) | + | + | Carcinogen |

(Continued)
Table 2. (Continued)

| Chemical, mol. wt., CAS no., purity | NTP rodent bioassay | SHE assay results | Carcinogenicity prediction |
|------------------------------------|---------------------|------------------|---------------------------|
| Phenolphthalein 318.3 77-09-8 ~98% | Feed 1817 2100 1351 1308 + N/A + 24-hr 3 of 5 doses (+) Trend test (+) | Carcinogen |
| Primaclone 218.3 125-33-7 >99% | Feed 91 105 146 142 — — — 24-hr All doses (-) Trend test (-) | Noncarcinogen |
| Pyridine 79.1 110-86-1 >99% | Water 21 21 152 80 — — — 24-hr All doses (-) Trend test (-) | Noncarcinogen |
| Scopolamine hydrobromide trihydrate 438.3 6533-88-2 ~85% | Gavage 25 25 25 25 + N/A + 24-hr 4 of 5 doses (+) Trend test (+) | Carcinogen |
| Sodium nitrite 69.0 7632-00-0 100% | Water 155 160 456 478 + N/A + 24-hr 5 of 5 doses (+) Trend test (+) | Carcinogen |
| Sodium xylenesulfonate 208.2 1300-72-7 93% | Skin painting 240 240 727 727 — — — 24-hr All doses (-) Trend test (-) | Noncarcinogen |
| Tetrahydrofuran 72.1 109-99-9 >98% | Inhalation 833 955 1733 1767 — — — 24-hr All doses (-) Trend test (-) | Noncarcinogen |
| Vanadium pentoxide 181.9 1314-82-1 98% | Inhalation 0.31 0.36 1.96 2.0 — + + 24-hr All doses (-) Trend test (-) | Carcinogen |
exclusively on the SHE cell transformation assay results.

**Results and Discussion**

As part of the NTP program “Strategies for Predicting Chemical Carcinogenesis in Rodents,” we tested in the SHE cell transformation assay 26 of the chemicals being evaluated by the NTP in the rodent bioassay. Overall, 17 of the 26 chemicals gave a positive response in the SHE cell transformation assay, while 9 chemicals gave a negative response (Table 1). Initially, a 24-h test chemical exposure SHE cell transformation assay was conducted on each of the 26 chemicals. Using this exposure regimen, 14 chemicals were positive (Table 1). For those chemicals that were negative with a 24-h exposure SHE cell transformation assay, an additional 7-day exposure SHE cell transformation assay was performed. Of the 12 chemicals that were negative in the 24-h exposure SHE cell transformation assay, 3 were positive in the 7-day exposure assay, resulting in an overall SHE cell transformation assay call of positive for these three chemicals. We have previously seen this response (24-h negative, 7-day positive) with several chemicals in the SHE cell transformation assay (8). Evidently, chemicals that give a negative 24-h exposure SHE cell transformation assay result and a positive 7-day exposure result must be continuously present in the culture medium for the induction of morphological transformation. As discussed previously (8), this pattern (24-h negative, 7-day positive) indicates a reversible transformation effect that may result from a promotionlike mechanism of action compared to a 24-h positive SHE cell transformation assay result, which reflects a stable, transforming event.

In addition to SHE results, Table 2 includes rodent bioassay predictions for the 26 chemicals based exclusively on SHE cell transformation assay results (Table 2, column 4). The predictions presented in this report are an attempt to demonstrate the usefulness and validity of the SHE assay in chemical carcinogenicity prediction and risk assessment. Our predictions will be compared with the rodent bioassay results when they become available.

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