Some Primary Considerations in the Interpretation of the Dominant-Lethal Assay
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Introduction

Among the various procedures proposed for use in assessing the mutagenic potential of drugs, the dominant-lethal (D-L) assay stands currently as one of the few tests for measuring mutagenic effects on germ cells. Early identification of the D-L assay as a possible member of a test battery relates strongly to its being a mammalian model. Many scientists within the pharmaceutical industry believe that only those tests which utilize a mammalian model should be considered for primary use in drug safety evaluation protocols. The reason for such belief is obvious when one considers that the entire process of drug safety evaluation is oriented on established concepts in pharmacology and toxicology. Mammalian processes of assimilation, absorption, distribution, metabolism and elimination must be permitted to work on the chemical under test in order to provide some basis for extrapolating mutagenicity test data to man (1). Dose levels tested in these models and routes of drug administration should both reflect human use. Also, differences in the qualitative pharmacologic action of drugs must be considered as an essential part of the criteria applied to dose selection. Test reproducibility and dose-effect relationships must be emphasized in mutagenicity studies in order to identify those levels at which any mutagenic action is first detectable and to relate this level to the dose required for therapeutic efficacy.

In this presentation, data have been selected from a number of D-L studies which relate to these points and the difficulties encountered in interpreting D-L test results.

Methods

Random-bred CD-1 mice (Charles River), 8 weeks of age, were used in all experiments except where noted. Generally, 15 males were assigned to each test and control group, and 2 females were caged with each male. Pregnant females were identified by the presence of a mating plug. The number of total and dead implants/pregnant female were determined by autopsy at 12–14 days of pregnancy. Statistical analyses were computerized and all tests of significance were performed on arcsine transformed data. Weekly summations of test data were compared to a control regression computed across the entire 8 weeks of testing (2). These statistical models are discussed in the paper by Dr. David Salsburg (3).

Results and Discussion

Strain Characterization

Continuous surveillance of the mouse...
strains selected for use in the D-L assay is an absolute necessity. Spurious increases in the number of dead implants/pregnant female, or a reduction in the total implants/pregnant female of the control group can have such a marked effect on determinations of dominant lethality that both these parameters must be monitored continuously. Strains that have high levels of fetal wastage due to genetic factors or infectious disease burdens are not well suited to use in the D-L assay.

Control data on 6820 pregnant females (CD-1 strain) are presented in Table 1. All data are expressed as a function of the week of mating following treatment of the male. The control males mated with these females had received physiological saline. This strain has consistently maintained an average level of total implants/pregnant female close to 12.50. The average number of dead implants/pregnant female is 0.89 and the average of living implants/pregnant female is 11.61. When the number of dead implants is compared to the total implants an average value of 7.1% is obtained.

An example of a shift in the reproductive behavior of this strain is shown in Table 2. During the period of November 1, 1972 to March 1, 1973 the number of dead implants/pregnant female rose to a value of 1.02. This was accompanied by a reduction in the number of living implants/pregnant female to 11.43. Total implants/pregnant female was 12.38 and the percent dead implants/total implants was 8.2. Although such a shift may appear slight, this degree of fetal wastage can produce problems in the interpretation of test results and reduce the sensitivity of the test (4). The rapid rise observed in this period suggests the introduction of an infectious disease entity although no overt clinical disease was evident.

Occasionally, a genetically aberrant male is encountered which produces a D-L effect in several stages of spermatogenesis. Table 3 shows such a result with significant responses in weeks 1 through 7. The compound involved normally produces a D-L effect in weeks 5 and 6. Additional analyses revealed a single male had produced this response.

### Table 1. Dominant-lethal assay: historical control, CD-1 strain, March 1971 through March 1973.

| Week | Number pregnant | Embryos | Dead implants | Total implants | Tot. impl. | Dead impl. | Live impl. | % Dead impl. |
|------|----------------|---------|---------------|----------------|------------|------------|-----------|-------------|
|      |                |         |               |                | preg. fem. | preg. fem. | preg. fem. |             |
| 1    | 871            | 9,880   | 745           | 10,625         | 12.20      | 0.86       | 11.34     | 7.0         |
| 2    | 1,009          | 11,499  | 884           | 12,383         | 12.27      | 0.88       | 11.40     | 7.1         |
| 3    | 966            | 11,228  | 823           | 12,051         | 12.48      | 0.85       | 11.62     | 6.8         |
| 4    | 901            | 10,360  | 830           | 11,190         | 12.42      | 0.92       | 11.50     | 7.4         |
| 5    | 863            | 10,100  | 749           | 10,849         | 12.57      | 0.87       | 11.70     | 6.9         |
| 6    | 745            | 8,869   | 667           | 9,536          | 12.80      | 0.90       | 11.90     | 7.0         |
| 7    | 718            | 8,463   | 631           | 9,094          | 12.67      | 0.88       | 11.79     | 6.9         |
| 8    | 747            | 8,680   | 703           | 9,383          | 12.56      | 0.94       | 11.62     | 7.5         |

### Test Reproducibility

A true mutagenic response in the D-L assay can be characterized by a statistically significant increase in dead implants/pregnant female accompanied by a statistically significant reduction in living implants/pregnant female. Additionally, the compound involved should show a dose response relationship during a specific stage in the spermatogenic cycle. If a statistically significant response cannot be demonstrated reproducibly in the same stage of spermatogenesis, then a spurious positive result should be suspected. Table 4 demonstrates the typical response of the mutagen, ethyl methane-sulfonate. In both experiments, the number of dead implants/pregnant female increases markedly during the first two weeks of mating. It should be noted that a significant decrease in the number of living implants per pregnant female occurs in the same two
weeks. A dose–response curve is shown in Figure 1 for the period 7–11 days following mating (5).

Another example of a reproducible D-L effect is depicted in Table 5. The purine analog, 6-mercaptopurine has produced a consistent D-L effect during weeks 5 and 6 of the spermatogenic cycle (6). Again, the parameter of living implants/pregnant female showed a simultaneous and significant reduction.

**Nonreproducible Results**

In contrast to the reproducibility obtained

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**Table 2. Dominant-lethal assay: historical control, CD-1 strain, November 1, 1972—March 1, 1973.**

| Week | Number pregnant | Embryos | Dead implants | Total implants | Tot. impl. Preg. fem. | Dead impl. Preg. fem. | Live impl. Preg. fem. | % Dead impl. |
|------|-----------------|---------|---------------|----------------|----------------------|----------------------|-----------------------|-------------|
| 1    | 148             | 1684    | 128           | 1812           | 12.24                | 0.86                 | 11.38                 | 7.06        |
| 2    | 163             | 1891    | 155           | 2046           | 12.55                | 0.95                 | 11.60                 | 7.58        |
| 3    | 166             | 1853    | 161           | 2014           | 12.13                | 0.97                 | 11.16                 | 7.99        |
| 4    | 156             | 1808    | 172           | 1980           | 12.69                | 1.10                 | 11.59                 | 8.69        |
| 5    | 156             | 1808    | 154           | 1962           | 12.58                | 0.99                 | 11.59                 | 8.59        |
| 6    | 129             | 1505    | 133           | 1638           | 12.70                | 1.03                 | 11.67                 | 8.12        |
| 7    | 122             | 1387    | 115           | 1502           | 12.31                | 0.94                 | 11.37                 | 7.66        |
| 8    | 98              | 1086    | 127           | 1213           | 12.38                | 1.30                 | 11.08                 | 10.47       |

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Table 3. Results of testing with a genetically aberrant male.

| Week | Number pregnant | Total implants | Tot. impl. | Dead impl. | % Dead | Live impl. |
|------|-----------------|----------------|------------|------------|--------|------------|
|      | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   |
| 1    | 26  | 34  | 319 | 375 | 12.3 | 11.0 | 0.65 | 1.24 | 5.3  | 11.2 | 11.6 | 9.8 |
| 2    | 43  | 46  | 535 | 586 | 12.4 | 12.7 | 1.19 | 1.32 | 9.5  | 10.4 | 11.3 | 11.4 |
| 3    | 39  | 47  | 460 | 612 | 11.8 | 13.0 | 0.62 | 1.26 | 5.2  | 9.6  | 11.2 | 11.8 |
| 4    | 34  | 47  | 435 | 619 | 12.8 | 13.2 | 0.79 | 1.38 | 6.2  | 10.5 | 12.0 | 11.8 |
| 5    | 41  | 47  | 498 | 594 | 12.1 | 12.6 | 1.17 | 1.79 | 9.6  | 14.1 | 11.0 | 10.9 |
| 6    | 28  | 29  | 360 | 338 | 12.9 | 11.7 | 1.07 | 2.28 | 8.3  | 19.5 | 11.8 | 9.4  |
| 7    | 44  | 41  | 563 | 516 | 12.8 | 12.6 | 1.11 | 1.63 | 8.7  | 13.0 | 11.7 | 11.0 |
| 8    | 48  | 27  | 623 | 343 | 13.0 | 12.7 | 0.97 | 1.41 | 7.5  | 11.1 | 12.0 | 11.3 |

* C denotes controls; T denotes treated animals.

**Significance at the 1% level (dead implants/pregnant females).

during the same stage of spermatogenesis with a true mutagen, spurious or false-positive results do not repeat during the same stage of spermatogenesis. An example of this kind of results is shown in Table 6. The compound produced effects on two separate stages of spermatogenesis in the first two experiments. A third experiment performed at the same dose level was negative in both weeks 2 and 4. It should be noted that the parameter of living implants/pregnant female was not significantly reduced.

Tables 7–12 show the kind of inconsistencies which may occur in the dominant-lethal assay with a nonmutagenic substance. The response at week 7 at a dose of 7.5 mg/kg (Table 8) was not reproduced at a level of 75 mg/kg (Table 10). Further, the response

Table 4. Example of a reproducible result in the dominant-lethal assay with ethyl methanesulfonate, 300 mg/kg, oral.

| Week | Number pregnant | Total implants | Tot. impl. | Dead impl. | % Dead | Live impl. |
|------|-----------------|----------------|------------|------------|--------|------------|
|      | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   |
| 1    | 58  | 43  | 704 | 488 | 12.14 | 11.35 | 1.12 | 1.81 | 9.23 | 15.98 | 11.02 | 9.53 |
| 2    | 51  | 54  | 657 | 588 | 12.88 | 10.89 | 1.06 | 3.26 | 8.22 | 29.93 | 11.82 | 7.63 |
| 3    | 56  | 36  | 763 | 457 | 13.62 | 12.69 | 1.09 | 0.86 | 7.99 | 6.78  | 12.54 | 11.83 |
| 4    | 28  | 37  | 379 | 468 | 13.54 | 12.65 | 0.90 | 0.89 | 7.12 | 7.05  | 12.57 | 11.76 |
| 5    | 34  | 29  | 496 | 385 | 14.59 | 13.28 | 1.41 | 0.76 | 9.68 | 5.71  | 13.18 | 12.52 |
| 6    | 40  | 33  | 579 | 475 | 14.48 | 14.39 | 1.30 | 0.97 | 8.98 | 6.74  | 13.17 | 13.42 |
| 7    | 41  | 25  | 550 | 314 | 13.41 | 12.56 | 0.76 | 0.76 | 5.64 | 6.05  | 12.66 | 11.80 |
| 8    | 31  | 37  | 427 | 455 | 13.77 | 12.30 | 1.26 | 0.95 | 9.13 | 7.69  | 12.52 | 11.35 |

* Significance at the 1% level (dead implants/pregnant female).
Table 5. Example of a reproducible result in the dominant-lethal assay with 6-mercaptopurine, 150 mg/kg, IP.

| Week | Number pregnant | Total implants | Tot. impl. Preg. fem. | Dead impl. Preg. fem. | % Dead impl. Preg. fem. | Live impl. Preg. fem. |
|------|-----------------|----------------|-----------------------|-----------------------|------------------------|----------------------|
|      | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    |
| 1    | 26   | 33   | 319  | 411  | 12.3 | 12.5 | 0.65 | 0.67 | 5.3  | 5.4  | 11.6 | 11.8 |
| 2    | 43   | 38   | 535  | 470  | 12.4 | 12.4 | 1.19 | 1.05 | 9.5  | 8.1  | 11.3 | 11.3 |
| 3    | 39   | 43   | 460  | 553  | 11.8 | 12.9 | 0.62 | 0.79 | 5.2  | 6.2  | 11.2 | 12.1 |
| 4    | 34   | 52   | 435  | 665  | 12.8 | 12.8 | 0.79 | 0.96 | 6.2  | 7.5  | 12.0 | 11.8 |
| 5 *  | 41   | 34   | 498  | 408  | 12.1 | 12.0 | 1.17 | 2.24 | 9.6  | 18.6 | 11.0 | 9.8  |
| 6 *  | 28   | 24   | 360  | 284  | 12.9 | 11.8 | 1.07 | 2.50 | 8.3  | 21.1 | 11.8 | 9.3  |
| 7    | 44   | 28   | 563  | 329  | 12.8 | 11.8 | 1.11 | 0.89 | 8.7  | 7.6  | 11.7 | 10.9 |
| 8    | 48   | 37   | 623  | 465  | 13.0 | 12.6 | 0.97 | 1.14 | 7.5  | 9.0  | 12.0 | 11.4 |
| 1    | 41   | 42   | 528  | 511  | 12.9 | 12.2 | 0.71 | 0.98 | 5.5  | 8.0  | 12.2 | 11.2 |
| 2    | 35   | 36   | 438  | 452  | 12.5 | 12.6 | 0.74 | 1.00 | 5.9  | 8.0  | 11.8 | 11.6 |
| 3    | 45   | 50   | 577  | 635  | 12.8 | 12.7 | 0.82 | 0.44 | 6.4  | 3.5  | 12.0 | 12.3 |
| 4    | 44   | 48   | 557  | 606  | 12.7 | 12.6 | 0.89 | 0.65 | 7.0  | 5.1  | 11.8 | 12.0 |
| 5 *  | 40   | 40   | 536  | 487  | 13.4 | 12.2 | 0.83 | 2.10 | 6.2  | 17.3 | 12.6 | 10.1 |
| 6 *  | 38   | 49   | 500  | 610  | 13.2 | 12.4 | 0.97 | 1.73 | 7.4  | 13.9 | 12.2 | 10.7 |
| 7    | 43   | 41   | 560  | 526  | 13.0 | 12.8 | 0.49 | 0.78 | 3.8  | 6.1  | 12.5 | 12.0 |
| 8    | 42   | 38   | 542  | 500  | 12.9 | 13.2 | 0.62 | 0.89 | 4.8  | 6.8  | 12.3 | 12.3 |

* Significance at the 1% level (dead implants/pregnant female).

Table 6. Example of a nonreproducible result in the dominant-lethal assay, experiment 50, dose 12 mg/kg, oral.

| Week | Number pregnant | Total implants | Tot. impl. Preg. fem. | Dead impl. Preg. fem. | % Dead impl. | Live impl. Preg. fem. |
|------|-----------------|----------------|-----------------------|-----------------------|--------------|----------------------|
|      | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    | C    | T    |
| 1    | 39   | 35   | 470  | 461  | 12.05 | 13.17 | 0.95 | 0.97 | 7.87 | 7.38 | 11.10 | 12.20 |
| 2 *  | 39   | 45   | 510  | 613  | 13.08 | 13.62 | 0.92 | 1.87 | 7.06 | 13.70 | 12.15 | 11.76 |
| 3    | 35   | 41   | 438  | 557  | 12.51 | 13.59 | 0.97 | 1.15 | 7.76 | 8.44 | 11.54 | 12.44 |
| 4    | 34   | 34   | 436  | 434  | 12.82 | 12.76 | 1.12 | 1.21 | 8.72 | 9.45 | 11.71 | 11.56 |
| 5    | 39   | 35   | 496  | 446  | 12.72 | 12.74 | 1.13 | 0.97 | 8.87 | 7.62 | 11.59 | 11.77 |
| 6    | 40   | 41   | 490  | 486  | 12.25 | 11.85 | 0.75 | 0.90 | 6.12 | 7.61 | 11.50 | 10.95 |
| 7    | 37   | 38   | 464  | 471  | 12.54 | 12.39 | 0.92 | 1.37 | 7.35 | 11.04 | 11.62 | 11.03 |
| 8    | 23   | 25   | 284  | 333  | 12.35 | 13.32 | 1.43 | 1.16 | 11.62 | 8.71 | 10.91 | 12.16 |
| 1    | 38   | 32   | 363  | 305  | 9.55  | 9.53  | 1.03 | 0.66 | 10.74 | 6.89 | 8.53  | 8.88 |
| 2    | 46   | 45   | 540  | 550  | 11.74 | 12.22 | 1.33 | 1.22 | 11.30 | 10.00 | 10.41 | 11.00 |
| 3    | 39   | 48   | 472  | 580  | 12.10 | 12.08 | 0.85 | 1.15 | 6.99 | 9.48 | 11.26 | 10.94 |
| 4 *  | 49   | 32   | 671  | 433  | 13.69 | 13.53 | 0.86 | 1.44 | 6.26 | 10.62 | 12.84 | 12.09 |
| 5    | 28   | 32   | 387  | 467  | 13.82 | 14.59 | 1.32 | 1.16 | 9.56 | 7.92 | 12.50 | 13.44 |
| 6    | 31   | 28   | 429  | 398  | 13.84 | 14.21 | 0.77 | 0.96 | 5.59 | 6.78 | 13.06 | 13.25 |
| 7    | 27   | 29   | 353  | 421  | 13.07 | 14.52 | 0.93 | 0.93 | 7.08 | 6.41 | 12.15 | 13.59 |
| 8    | 31   | 24   | 404  | 339  | 13.03 | 14.12 | 0.81 | 0.67 | 6.19 | 4.72 | 12.23 | 13.46 |

* Significance at the 1% level (dead implants/pregnant female).

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during week 3 at the dose level of 75 mg/kg was not reproduced at 150 mg/kg (Table 12). It should be noted that here again, no significant reduction was observed in either the total or living implants/pregnant female at any dose level during the test. The investigator is thus not mislead when the statistical analysis is performed on a weekly basis and the parameters of total and living implants are examined simultaneously with dead implants/pregnant female. In addition, this compound was not active in any test for mutagenic potential including the host-mediated and in vivo cytogenetic assays.

Table 7. Experimental inconsistency, experiment 42, dose level 7.5 mg/kg, oral.

| Week | Number pregnant | Total implants | Tot. impl. | Dead impl. | % Dead impl. | Total live impl. | Live impl. |
|------|-----------------|----------------|------------|------------|--------------|-----------------|------------|
|      |                 |                | Preg. fem. | Preg. fem. |             | Preg. fem.     | Preg. fem. |
| 1    | 44              | 514            | 383        | 0.98       | 0.88         | 471             | 10.70      |
| 2    | 41              | 508            | 576        | 0.95       | 0.85         | 469             | 11.44      |
| 3    | 48              | 595            | 611        | 1.15       | 1.12         | 540             | 11.25      |
| 4    | 45              | 600            | 499        | 1.18       | 1.00         | 572             | 12.43      |
| 5    | 46              | 624            | 545        | 1.13       | 1.00         | 477             | 11.46      |
| 6    | 39              | 494            | 653        | 1.21       | 1.06         | 447             | 12.49      |
| 7    | 39              | 515            | 542        | 0.72       | 1.57         | 487             | 12.13      |
| 8    | 44              | 572            | 483        | 1.16       | 1.17         | 521             | 11.84      |

Table 8. Significance levels, dose level 7.5 mg/kg.

| Week | Significance level | Dead impl. | Live impl. | Tot. impl. |
|------|--------------------|------------|------------|------------|
|      |                    | Preg. fem. | Preg. fem. | Preg. fem. |
| 1    | 0.10+              | 0.10+      | 0.10+      |
| 2    | 0.10+              | 0.10+      | 0.10+      |
| 3    | 0.10+              | 0.10+      | 0.10+      |
| 4    | 0.10+              | 0.10+      | 0.10+      |
| 5    | 0.10+              | 0.10+      | 0.10+      |
| 6    | 0.10+              | 0.10+      | 0.10+      |
| 7    | 0.01*              | 0.10       | 0.10+      |
| 8    | 0.10+              | 0.10+      | 0.10+      |

* Weeks with significance at the 1% level.

Table 9. Experimental inconsistency, experiment 42, dose level, 75 mg/kg, oral.

| Week | Number pregnant | Total implants | Tot. impl. | Dead impl. | % Dead impl. | Total live impl. | Live impl. |
|------|-----------------|----------------|------------|------------|--------------|-----------------|------------|
|      |                 |                | Preg. fem. | Preg. fem. |             | Preg. fem.     | Preg. fem. |
| 1    | 44              | 514            | 487        | 0.98       | 0.67         | 471             | 10.70      |
| 2    | 41              | 508            | 571        | 0.95       | 1.00         | 469             | 11.44      |
| 3    | 48              | 595            | 501        | 1.15       | 1.46         | 540             | 11.25      |
| 4    | 45              | 600            | 539        | 1.18       | 1.17         | 572             | 12.43      |
| 5    | 46              | 624            | 466        | 1.13       | 1.37         | 447             | 11.46      |
| 6    | 39              | 494            | 604        | 1.21       | 0.85         | 487             | 12.49      |
| 7    | 39              | 515            | 608        | 0.72       | 1.09         | 521             | 11.84      |
| 8    | 44              | 572            | 483        | 1.16       | 0.78         | 521             | 11.84      |
Table 10. Significance levels, dose level 75 mg/kg.

| Week | Dead impl. Preg. fem. | Live impl. Preg. fem. | Tot. impl. Preg. fem. |
|------|----------------------|-----------------------|----------------------|
| 1    | 0.10+                | 0.10+                 | 0.10+                |
| 2    | 0.10+                | 0.10+                 | 0.10+                |
| 3    | 0.01*                | 0.10+                 | 0.10+                |
| 4    | 0.10+                | 0.10+                 | 0.10+                |
| 5    | 0.05*                | 0.10+                 | 0.10+                |
| 6    | 0.10+                | 0.10+                 | 0.10+                |
| 7    | 0.10+                | 0.10+                 | 0.10+                |
| 8    | 0.10+                | 0.10+                 | 0.10+                |

* Weeks with significance at the 1% level.

Table 11. Experimental inconsistency, experiment 42, dose level, 150 mg/kg, oral.

| Week | Number pregnant | Total implants | Dead impl. | Live impl. | Tot. impl. |
|------|-----------------|----------------|------------|------------|------------|
|      | C | T | C | T | C | T | C | T | C | T | C | T |
| 1    | 44 | 31 | 514 | 371 | 11.68 | 11.97 | 0.98 | 0.97 | 8.37 | 8.09 | 471 | 441 | 10.70 | 11.00 |
| 2    | 41 | 49 | 508 | 607 | 12.39 | 12.39 | 0.95 | 0.94 | 7.68 | 7.58 | 469 | 561 | 11.44 | 11.45 |
| 3    | 48 | 40 | 595 | 485 | 12.40 | 12.12 | 1.15 | 0.98 | 9.24 | 8.04 | 540 | 446 | 11.25 | 11.15 |
| 4    | 45 | 38 | 600 | 483 | 13.33 | 12.71 | 1.18 | 0.95 | 8.83 | 7.45 | 547 | 447 | 12.16 | 11.76 |
| 5    | 46 | 49 | 624 | 671 | 13.57 | 13.69 | 1.13 | 1.10 | 8.33 | 8.05 | 572 | 617 | 12.43 | 12.59 |
| 6    | 39 | 43 | 494 | 544 | 12.67 | 12.65 | 1.21 | 0.79 | 9.51 | 6.25 | 447 | 510 | 11.46 | 11.86 |
| 7    | 39 | 46 | 515 | 620 | 13.21 | 13.48 | 0.72 | 1.07 | 5.44 | 7.90 | 487 | 571 | 12.49 | 12.41 |
| 8    | 44 | 37 | 572 | 478 | 13.00 | 12.92 | 1.16 | 1.27 | 8.92 | 9.83 | 521 | 431 | 11.84 | 11.65 |

Table 12. Significance levels, dose level 150 mg/kg.

| Week | Dead impl. Preg. fem. | Live impl. Preg. fem. | Tot. impl. Preg. fem. |
|------|----------------------|-----------------------|----------------------|
| 1    | 0.10+                | 0.10+                 | 0.10+                |
| 2    | 0.10+                | 0.10+                 | 0.10+                |
| 3    | 0.10+                | 0.10+                 | 0.10+                |
| 4    | 0.10+                | 0.10+                 | 0.10+                |
| 5    | 0.10+                | 0.10+                 | 0.10+                |
| 6    | 0.10+                | 0.10+                 | 0.10+                |
| 7    | 0.10+                | 0.10+                 | 0.10+                |
| 8    | 0.05*                | 0.10+                 | 0.10+                |

* Weeks with significance at 5% level.

Effect of Dose Level

The qualitative pharmacologic action of drugs must be considered when choosing dose levels for D-L experiments. Drugs such as anesthetics and tranquilizers have such pronounced pharmacologic activity that excessive dose levels can produce marked temperature reductions and an inability to mate for several days following a single administration. An example of this kind of overdosage is shown in Figure 2. Here, 10°C degree reductions in body temperature were...
FIGURE 2. Effect of triflupromazine on body temperature.
observed at levels which were tested for mutagenic activity (7, 8). Clearly, such reductions must reduce the overall metabolism of the test animal and therefore influence the metabolism of the drug. Levels of drug used in mutagenicity assessments should be chosen so as not to produce anorexia, sedation, or other exaggerated pharmacological effects (9).

Conclusions

In interpreting D-L data, the need for demonstrating a statistically significant and reproducible effect in the same stage of spermatogenesis cannot be over emphasized. In order to achieve consistent analyses, the degree of variability in important parameters of dead, living and total implants per pregnant female has to be firmly established for each strain of mouse employed. The statistical model utilized should include a transformation to reduce the effect of differing variances which occur in dead and total implants per pregnant female. Also, test results obtained during a specific stage of spermatogenesis should be compared to a control regression computed across the entire 8 weeks of testing. A dose response curve obtained during the active period of dominant lethality will provide additional evidence of compound activity. Data from D-L testing should be correlated and compared to other assessments of mutagenic potential such as the host-mediated and cytogenetic assays before applying the label of mutagen. Finally, the dosage regimen employed should not seriously alter the normal physiological processes of the test animal.

Acknowledgements

The authors gratefully acknowledge the assistance given by Dr. David Salsburg and Mr. Leon Just in the statistical analyses of all data. We also wish to thank Mr. Richard Giddings for computer programming associated with the updating of historical data.

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