## Use of Mammal in a Practical Screening Test for Chemical Mutagens in the Human Environment

Chemical mutagens in the environment are a hazard not yet systematically explored. Although certain mutagens, notably ionizing radiations and alkylating agents, have been studied in detail in bacteria[1,2] and *Drosophila*[3,4], relevance to man is not always apparent. Legator[5] has considered the influence of host-mediated drug effects by studying mutagenicity in bacteria in a mammalian environment. Lüning[6], in discussing mutagenicity testing, stated: "The use of mammals in such tests would be both expensive and time-consuming, and might slow down even more the development of new drugs." Bateman[7], however, considered use of mammals in mutagenicity testing to be practical and more meaningful than studies with other systems in the context of human hazards. We present preliminary data on the feasibility of mutagenicity testing in mice based on screening a wide variety of environmental pollutants, including carcinogens, air and water pollutants, pharmaceuticals, food additives and pesticides.

In the dominant lethal test, treated males are sequentially mated with groups of females. The females are subsequently dissected at mid-term of pregnancy to evaluate the incidence of dominant lethal mutations. Such effects conveniently reflect mutagenic activity and represent foetuses killed *in utero* by mutations directly induced in male germ cells. The possibility of systemic drug effects in females is thus excluded.

In general, $L.D._5$ doses were selected for testing. Where

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strong anti-fertility effects were evidenced at these doses, testing was repeated at lower levels. Test materials were prepared freshly and generally injected intraperitoneally in tricaprylin solution or suspension in 0.1 ml volume. Male Swiss mice (CD-1), 8-10 weeks old, were dosed in minimal groups of 5. Groups of 10 male mice, injected with tricaprylin, served as controls for concurrent testing of 7-9 samples. Within 2h of injection, each male was caged with 3 virgin females, 8-10 weeks old, for 7 days. After 7 days, the females were replaced with fresh virgin animals. This breeding schedule was maintained for 8 consecutive weeks, the duration of the spermatogenic cycle in the mouse. All females were autopsied 13 days from mid-week of their mating; pregnancy thus ranged from 9-15 days. In general, fertilizations were maximal during the middle portion of each mating week. At autopsy, animals were scored for total implantations, early deaths, appearing as small black deciduomata, and late deaths. Corpora lutea were counted under a low-power dissecting microscope.

A mutagenic index (M. I.)[8], reflecting the incidence of dominant lethal mutations in an experimental group of animals, is conventionally calculated as follows:

$$\text{M. I.} = \frac{\text{deciduomata} + \text{late deaths}}{\text{total implantations}} \times 100$$

This index, however, is limited in that it is perforce increased by reduction in total implantations alone; additionally, it reflects neither anti-fertility effects nor the distribution of deciduomata in affected animals.

Results are summarized in Table I, where all effects are listed for the third mating week, with the exception of

aflatoxin(week 4) and methyl methane sulfonate (week 2). The average composite control value of M.I., based on 150 females, for the third week was 1.0 per cent, with a range from 0 to 3 per cent. Control M.I. values of 3-10 per cent have been reported for other strains of mice[7,8,9]. The incidence of late deaths was extremely low; one late death appeared in each of 5 out of a total of 45 compounds tested, for the third mating week. In control females for the third week, 3 late deaths were seen. Mutagenic activity was, in general, maximal in weeks 2 and 3.

Among the carcinogens tested, aflatoxin and benzo[a]-pyrene were mutagenic. The aflatoxin sample, a mixture of aflatoxins $B_1$ and $G_1$, displayed a singular response in that activity commencing in week 3 persisted through week 5. Work on this substance, in collaboration with Prof. G. T. Wogan, is continuing. M.I. values of other carcinogens tested were at control levels throughout the 8-week breeding cycle. The ethyleneimine alkylating agents, TEPA, METEPA, THIO-TEPA, and triethylene melamine were all strongly mutagenic in weeks 2 and 3. The effects of methyl methane sulfonate, mutagenic as previously reported[7,8], were maximal in week 2, and declined to control levels by week 3. Organic extracts of atmospheric particulate pollutants and of finished drinking water displayed control M.I. levels. The latter samples consisted of a reconstituted mixture of ethanol and chloroform adsorbate extracts in an aqueous solvent. Butylated hydroxytoluene and the 3 xanthines tested, including caffeine, showed control M.I. values. Caffeine breaks chromosomes in human cell cultures[10], is mutagenic in bacteria[11], and possibly also in rats[12], in whom dominant

lethals have been induced following chronic administration in drinking water.[12] Inconsistent results, however, have been reported in Drosophila[13,14]. The pesticides tested yielded M. I. values in the control range. Captan was administered both intraperitoneally and, at higher doses, orally.

Sensitivity of mice to this representative sampling of mutagenic agents is generally restricted to post-meiotic stages of spermatogenesis, consistent with an action on non-replicating DNA. The present testing schedule might therefore be simplified with little sacrifice of screening efficacy. Primary screening might be based on administration of a single $L. D._5$ dose to 5 male mice who would then be mated only during the second and third weeks following injection. The number of animals required would thus be greatly reduced. Controls apart, only 30 females and 5 males would be needed for each drug. Positive findings revealed by this screen could be further explored. Testing could then cover the whole of the spermatogenic cycle, more animals, alternative routes of short and long-term drug administration and a wider dose range.

The diversity of potential chemical mutagens to which man is exposed urges the development of practical screening procedures. The dominant lethal test would appear suitable, especially as 80% of gene mutations in man are attributable to dominant autosomal traits[15]. The test, however, provides no information on point or recessive mutations nor on non-mutagenic sperm damage, which may, nevertheless, modify nucleic acid bases. Modification of the test procedures to evaluate the role of chronic low level exposure to environmental mutagens, either singly or in combination, is currently in progress. Possibly, differences between the reproductive cycles in mouse and man may, additionally, perturb direct extrapolation of risk. Primates aside, however, lower mammals provide the simplest means of relating mutagenic effects to human genetic hazard.

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... OF ENVIRONMENTAL POLLUTANTS: CARCINOGENS, AIR AND WATER POLLUTANTS, PHARMACEUTICALS, FOOD ADDITIVES AND PESTICIDES.

| Compound | Dose mg/kg | No. females bred | No. pregnant (%) | Total no. implants | Avg. no. implants per mouse | Total no. deciduomata | Mutagenic Index |
|---|---|---|---|---|---|---|---|
| CONTROLS: | - | 150 | 116 (77) | 1434 | 12.4 | 12 | 1 |
| CARCINOGENS: | | | | | | | |
| Aflatoxin | 68* | 9 | 7 (78) | 73 | 10.4 | 8 | 11 |
| Benzanthrone | 1000 | 12 | 10 (83) | 125 | 12.5 | 0 | 1 |
| Benzo[a]pyrene | 750 | 18 | 7 (39) | 53 | 7.6 | 5 | 11** |
| Butter yellow | 216 | 21 | 14 (67) | 170 | 12.1 | 2 | 1 |
| Cumene hydroperoxide | 34 | 15 | 13 (87) | 167 | 12.8 | 5 | 3 |
| 1,2,3,4-Diepoxybutane | 17 | 21 | 17 (81) | 206 | 12.1 | 4 | 2** |
| Dimethylhydrazine | 25 | 21 | 14 (67) | 165 | 11.8 | 2 | 1 |
| Dimethylnitrosoamine | 8 | 15 | 11 (73) | 132 | 12.0 | 3 | 2 |
| Hydrazine | 42† | 12 | 12 (100) | 135 | 11.3 | 1 | 1 |
| 3-Methylcholanthrene | 100 | 15 | 12 (80) | 113 | 9.4 | 2 | 2 |
| 4-Nitroquinoline-1-oxide | 5 | 21 | 17 (81) | 204 | 12.0 | 3 | 2 |
| Urethan | 1000 | 15 | 13 (87) | 156 | 12.0 | 2 | 2** |
| ORGANIC EXTRACTS OF ATMOSPHERIC PARTICULATE POLLUTANTS: | | | | | | | |
| Boston 1966 | 333 | 21 | 16 (76) | 200 | 12.5 | 4 | 2 |
| Acid Fraction, New York 1967 | 333 | 21 | 13 (62) | 159 | 12.2 | 0 | 0 |
| Basic Fraction New York 1967 | 666 | 18 | 14 (78) | 163 | 11.6 | 2 | 1 |
| Insoluble Fraction New York 1967 | 333 | 21 | 18 (86) | 222 | 12.3 | 5 | 2 |
| FOODS AND FOOD ADDITIVES: | | | | | | | |
| Butylated hydroxytoluene | 1000 | 9 | 4 (44) | 46 | 11.5 | 0 | 0 |
| Caffeine | 168 | 21 | 12 (57) | 124 | 10.3 | 0 | 0 |
| Theobromine | 380 | 18 | 12 (67) | 132 | 11.0 | 2 | 2 |
| Theophylline | 380 | 12 | 11 (92) | 119 | 10.8 | 3 | 3** |

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|---|---|---|---|---|---|---|---|
| ORGANIC EXTRACTS OF FINISHED DRINKING WATER: | | | | | | | |
| Maine 1961 | 333 | 21 | 9 (43) | 95 | 10.6 | 1 | 1 |
| Yonkers 1961 | 699 | 21 | 11 (52) | 139 | 12.6 | 1 | 1 |
| PESTICIDES: | | | | | | | |
| Captan | 9 | 21 | 20 (95) | 210 | 10.5 | 2 | 1 |
| Captan | 500¶ | 18 | 15 (83) | 171 | 11.4 | 1 | 1 |
| DDT | 105 | 18 | 13 (72) | 135 | 10.4 | 4 | 3 |
| Maleic hydrazide | 500 | 15 | 11 (73) | 121 | 11.0 | 1 | 1 |
| PHARMACEUTICALS: | | | | | | | |
| Aminopterin | 10 | 12 | 9 (75) | 88 | 9.8 | 0 | 0 |
| Azaribine | 1000¶ | 21 | 14 (67) | 151 | 10.8 | 1 | 1 |
| 5-Bromodeoxyuridine | 500 | 27 | 21 (78) | 248 | 11.8 | 1 | 0 |
| Chloramphenicol | 333 | 21 | 14 (67) | 128 | 9.1 | 2 | 2 |
| Chlorpromazine | 8.3† | 27 | 20 (74) | 237 | 11.9 | 1 | 0 |
| Griseofulvin | 750 | 18 | 14 (78) | 175 | 12.5 | 3 | 2 |
| Hydroxyurea | 500† | 21 | 14 (67) | 160 | 11.4 | 0 | 0 |
| 5-Iododeoxyuridine | 250 | 21 | 16 (76) | 192 | 12.0 | 2 | 1 |
| METEPA | 40 | 21 | 16 (76) | 145 | 9.1 | 39 | 27 |
| Methyl methane sulfonate | 50 | 24 | 19 (79) | 164 | 8.6 | 47 | 29 |
| TEPA | 7 | 18 | 13 (72) | 115 | 8.8 | 44 | 38 |
| THIO-TEPA | 5† | 18 | 15 (83) | 114 | 7.6 | 21 | 18 |
| Triethylene melamine | 0.2 | 21 | 15 (71) | 137 | 9.1 | 36 | 26 |
| MISCELLANEOUS: | | | | | | | |
| Acriflavine | 10.6 | 21 | 14 (67) | 172 | 12.3 | 0 | 0 |
| Acrolein | 1.5 | 15 | 12 (80) | 152 | 12.7 | 7 | 5 |
| Dimethyl sulfate | 23 | 18 | 14 (78) | 150 | 10.7 | 1 | 1 |
| Formaldehyde | 20 | 24 | 19 (79) | 233 | 12.3 | 4 | 2 |
| Methylhydroxylamine | 140† | 21 | 12 (57) | 133 | 11.1 | 0 | 0 |
| Pinacyanole | 1.9 | 12 | 8 (67) | 99 | 12.4 | 1 | 1 |

*Dimethyl sulfoxide suspension †Aqueous solution ¶Oral administration

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