Mutation Research, 154 (1985) 49–67
Elsevier

MTR 07191

Current status of bioassays in genetic toxicology — the dominant lethal assay

A report of the U.S. Environmental Protection Agency Gene-Tox Program *

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(Received 14 December 1984)
(Accepted 18 December 1984)

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* Work Group Report prepared for the Gene-Tox Program (Office of Toxic Substances, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC). The authors are members of the Gene-Tox Work Group on the Dominant Lethal Assay.

** Operated by Union Carbide Corporation under Contract No. W-7405-eng-26 with the U.S. Department of Energy.

Although the review described in this article has been funded wholly or in part by the U.S. Environmental Protection Agency through interagency agreement DOE 40-1123-80, EPA No. 80-DX0953 to the Oak Ridge National Laboratory, it has not been subjected to the agency's required peer and policy review and, therefore, does not necessarily reflect the views of the agency and no official endorsement should be inferred. The protocols stated, suggested use of the assay in a screening program, and research recommended should not be taken to represent agency policy on these matters.

0165-1110/85/$03.30

Summary

The term dominant lethal may be defined as death of the heterozygote arising through multiple chromosomal breaks. The assay is generally conducted by treating male animals, usually mice or rats, acutely (1 dose), subacutely (5 doses), or over the entire period of spermatogenesis. Animals treated acutely or subacutely are mated at weekly intervals to females for a sufficient number of weeks to cover the period of spermatogenesis. Those treated for the entire spermatogenic cycle are mated for 1 or 2 successive weeks at the termination of treatment. Females usually are killed at 14 days of pregnancy and examined for the number of total implantations in the uterus, the number of implantations classified as early deaths, and, in some cases, the number of corpora lutea. The category of early death is the most significant index of dominant lethality. A total of 249 papers were reviewed and 140 chemicals were evaluated. Of the 140 chemicals, 65 were positive by the criteria used by the Work Group in evaluating each publication. The category of "positive" includes those responses of a borderline nature. 99 chemicals were declared negative. There is considerable overlap of chemicals in both categories, which accounts for the incongruity in the total number of chemicals tested and the number considered positive and negative. A total of 44 animal carcinogens have been tested in the dominant lethal assay, 26 of which were positive and 18 negative for a correlation of 59%. The role of the assay should be that of confirming positive results from lower tier chromosomal aberration-detecting systems (confirming in the sense of indicating the ability of the chemical to penetrate gonadal tissue and to produce cytogenetic damage). The dominant lethal assay should not be used as a risk assessment method.

(I) Introduction

It is now generally acknowledged that chemical agents pose hazards to the genetic machinery of the cell. Numerous methods of assessing gene, chromosomal, and primary DNA damage have been proposed. The dominant lethal assay is one of the few tests in which the mutagenic potential of an agent is examined directly in gametic tissue in an intact mammal. This is of importance in mutagenicity testing since there is little direct evidence that genetic damage to somatic cells is reflective of similar damage in germ cells. The obvious advantage of utilizing in vivo mammalian data to extrapolate and assess safety in man need not be further amplified.

(A) History

A dominant lethal mutation is a genetic lesion that can occur in a gamete and, although not interfering with the gamete's ability to fertilize or be fertilized, is lethal to the resulting embryo (Bateman and Epstein, 1971). It has been shown that the genetic basis of these lethal events can be structural and/or numerical chromosomal anomalies (Brewen et al., 1975; Goldstein, 1977;

Hitotsumachi and Kikuchi, 1977; Kratochvilova, 1978; Matter and Jaeger, 1975).

Brenneke (1937) first described dominant lethal mutations in mammals. In 1953, Kaplan and Lyon used dominant lethal events to assess genetic damage from radiation exposure, and Bateman (1966) proposed the use of dominant lethal mutations in screening for mutagens.

(B) Rationale

Dominant lethal assays may be particularly germane to any discussion of human mutations since many recognizable human mutations are due to dominant autosomal traits (United Nations Scientific Committee, 1966). From evidence by Brewen et al. (1975), it was shown that the incidence of broken chromosomes at the first division correlates with dominant lethality. It is expected that the broken chromosomes are eventually lost at anaphase, resulting in a monosomic embryo that subsequently dies in utero.

The dominant lethal assay theoretically can be used to screen agents that cause nondisjunction as well as those causing chromosomal breakage; in practice, the assay has been used only as a screen for chromosomal breakage in gametic cells. Dominant lethal mutations are self-limiting by definition, because they are usually eliminated in the first generation and are not inherited. By themselves, these mutations are believed not to contribute to the genetic load. However, many mutagens produce both lethal and nonlethal chromosomal breakage. Reciprocal translocations and inversions are examples of the latter type breakage. Hence, the production of dominant lethal mutations could indicate the occurrence of other mutagenic events.

(C) Types of studies

There are two fundamental approaches to performing the dominant lethal test. In the first approach, adult male animals are dosed acutely (a single dose or a few doses within a few days) with sublethal concentrations of the agent. These treated animals are then mated sequentially (by weeks) with groups of untreated females. The results of the weekly matings can then indicate the specific stages of gametogenesis that are damaged and responsible for the resultant embryonic mortality. For example, matings of male mice during week 1 reveal effects on spermatozoa; during weeks 2 and 3, on spermatids; weeks 4 and 5, on spermatocytes; week 6, on differentiating spermatogonia; and after week 7, on stem cells (Bateman and Epstein, 1971). The timings are slightly different in male rats, as weeks 1 and 2 represent effects on mature spermatozoa; weeks 3, 4, and 5, on spermatids; weeks 6, 7, and 8, on spermatocytes; weeks 9 and 10, on differentiating spermatogonia, and after week 11, on stem cells (Jackson, 1966). In these assays, the females are sacrificed, usually between days 13 and 19 of gestation, and the uterine contents examined for evidence of dead implantations.

In the second approach the male animals are dosed over the entire span of spermato- and spermiogenesis (8 weeks in the mouse and 10 weeks in the rat) (Green et al., 1975a,b, 1977). These treated males are then mated with groups of untreated females for 2 weeks. As in the aforementioned protocol, the females are sacrificed, usually between days 13 and 19 of gestation, and examined for evidence of dead implantations.

(D) Selection of papers

There were a total of 450 papers listed under "dominant lethal" by the Environmental Mutagen Information Center (EMIC). Initially, each of these articles was examined briefly to exclude from the analysis those articles (1) that presented data as an abstract or only in descriptive form, (2) that were written in a foreign language for which no English translation was available, (3) that involved physical agents (i.e., X-irradiation) and not chemical agents.

Of the 450 papers, 201 were rejected based on the above criteria. Subsequently, the remaining 249 papers were submitted to the dominant lethal Work Group for evaluation. These articles were divided among the Work Group members. Each paper was evaluated by the individual assigned to that paper, and the results of that evaluation were presented to the entire Work Group for consensus of interpretation and final approval. The deliberations of this final review resulted in an additional 104 papers being eliminated, utilizing the following criteria.

(1) The article did not involve a properly defined chemical agent (mixtures).

(2) The results in the article were uninterpretable because of insufficient data or poor study design.

(3) The article was a review and presented no new data.

(4) The article was a symposium presentation without new data.

(5) The rationale for dosage selection was not presented nor could the panel through available references determine the adequacy of the dosage.

(6) The statistical evaluation utilized was inappropriate as determined by the Work Group statistician.

(7) The objectives of the article were outside the scope of the dominant lethal assay Work Group (e.g., investigating the mechanism of dominant lethality) to the exclusion of what were considered adequate data for evaluation of the effects of a chemical.

(8) The article could not be evaluated by the criteria adopted by the dominant lethal Work Group.

The remaining 145 articles, which were found acceptable and met the criteria, provide the data base (140 agents tested) for this report.

(II) Test description

(A) Cytogenetic basis of the dominant lethal effect

Dominant lethality is measured by the frequency of embryonic deaths and is believed to arise from chromosomal abnormalities induced in the germ cells of the treated animals. In studies using known mutagens such as MMS, EMS, and TEM, the chromosomes of the resulting embryos have been analyzed, and a good correlation between chromosomal aberrations in early cleavage divisions and dominant lethality has been demonstrated (Brewen et al., 1975; Goldstein, 1977; Hitotsumachi and Kikuchi, 1977; Kratochvilova, 1978; Matter and Jaeger, 1975).

The embryonic deaths generally are found to occur either before or soon after implantation. Death before implantation, a preimplantation loss, is indicated by a reduction in the total number of implantations, and the death at implantation or soon thereafter, a postimplantation loss, is indicated by an increase in the number of dead implantations when compared with controls. The significance of the reduced count in total implantations is to be taken with reservation, because it does not provide a distinction between loss of fertilized eggs before implantation and loss of unfertilized eggs. The former reflects gross chromosomal aberrations that result in embryonic death before implantation. The latter reflects a toxic effect of treatment that reduces the germ cell's ability to fertilize or be fertilized. The increased number of dead implantations is generally accepted as a good indication of dominant lethality. In practice, two types of dead implantations have been noted, early and late. The early dead implantation is referred to as a deciduoma or mole and is the result of an outgrowth of the uterus at the site of the implanting blastocyst that has failed to develop after implantation. The late dead implantation is a dead embryo that has developed to a relatively advanced stage before death. In studies with known mutagens, it is observed that there is an increase of the early embryonic deaths with the dosage of treatment, whereas, the number of late embryonic deaths remains relatively stable irrespective of the treatment (Bateman and Epstein, 1971). Therefore, it is most likely that the early, but not the late, embryonic deaths are true representatives of dominant lethality induced by chemicals. The classification of early versus late implantations may vary from laboratory to laboratory, and, depending on the investigators, the postimplantation loss may represent only the early or both early and late embryonic deaths.

(B) Description of test animals

A variety of organisms such as Drosophila, mouse, rat, hamster, and guinea pig has been used in the dominant lethal test. However, the mouse appears to be the most widely used, with the rat second (other organisms are rarely used). Various strains of outbred, inbred, or hybrid origin mice have been employed; the outbred strains appear to be used most widely. The test animals usually are selected on the basis of availability rather than sensitivity, and studies to determine the relative sensitivity of various strains are still very limited (Generoso, 1969; Generoso et al., 1979).

Females as well as males may be used as test animals, although the majority of tests are conducted in males. Females appear less suitable as

test animals in a system where successful fertilization of the eggs is essential and where embryonic death is evaluated. A chemical administered to the female may interfere with the normal hormonal status of the animal and reduce the reproductive capacity as well as induce embryonic death due to nongenetic causes. A male animal can be analyzed by repeated matings with different females over a period of time, whereas a female can be studied for only one pregnancy.

(III) Interpretation of data

(A) Presentation of data

(1) Dose–response curves

A dose–response relationship was a primary criterion used in the evaluation of test data such as pregnancy rate, incidence of dead implantations, or total implantations. Statistically significant changes in these parameters, but without a dose–response relationship, were considered biologically not significant unless other explanations could be offered (e.g., only one treatment group, an inadequate number of animals due to toxicity, or some other factor that clearly influenced the response).

In interpreting data from the dominant lethal assay, the demonstration of a dose response should be one criterion in concluding that a chemical produced dominant lethality. Inasmuch as the dose response is influenced by a number of factors, some of which may not be considered by the genetic toxicologist prior to conducting an investigation (e.g., slope of dose–effect curve), one should exercise scientific judgment when such a response is not evident (e.g., a consistent one-dose effect over several weeks may well be significant). Epstein et al. (1972) illustrate the concept of dose reversal for a number of chemicals, which is not an uncommon occurrence. In instances when a dose-related response is not evident but there is strong suspicion of activity, the study should be repeated.

(2) Data transformation

Data on the dominant lethal effect can be evaluated using various statistical tests. In one statistical approach, the data may be transformed before analysis. If preimplantation loss is determined by counting corpora lutea, then the preimplantation loss of each female should be transformed to the Freeman–Tukey arc sine before analysis by the Student's t test (Mosteller and Youtz, 1961). The index, dead implantations per pregnant female, should be transformed to the Freeman–Tukey square root before analysis by the t test (Generoso, 1969). The proportion, dead implantations per total implantations, should be transformed to the Freeman–Tukey arc sine before analysis by the t test.

(3) Units of expression of data

Some papers on the dominant lethal test classified dead implantations into early and late postimplantation deaths, while other papers combined these into one category — dead implantations. The Work Group recognizes that early postimplantation deaths are most likely to represent deaths due to genetic alterations, while late deaths are considered a negligible contribution and due primarily to nongenetic factors such as the health of the dam. Therefore, in evaluating the dominant lethal papers, the Work Group decided to combine the categories of early and late deaths, when given separately, so that these classifications will be the same as those for dead implantations. In this way, a degree of uniformity was maintained throughout the evaluation procedure without contributing any significant error.

The main parameter used in evaluation of test data was the average number of dead implantations per pregnant female. However, additional information, such as percentage of animals pregnant and average number of total live implantations, had a bearing on whether the data associated with the parameter were accepted. In the evaluation of the data, the pregnant female was taken as the basic unit of experimentation. However, when data were not presented in this form, the given data were converted whenever possible to per pregnant female.

The following units of expression were found acceptable by the Work Group and recommended for the dominant lethal assay.

(1) Number of pregnant females/number of females mated;

(2) number of fertile males/number of males mated;

(3) average number of corpora lutea per pregnant female (when determined);

(4) average number of implantations per pregnant female;

(5) average number of preimplantation losses per pregnant female (when determined);

(6) average number of dead implantations (early and late) per pregnant female;

(7) proportion of females with one or more dead implantations;

(8) proportion of females with two or more dead implantations;

(9) dead implantations/total implantations.

The Work Group recognizes that in some instances all of the above would be burdensome and recommends reporting data on the following as a minimum.

(1) Number of pregnant females/number of females mated;

(2) number of fertile males/number of males mated;

(3) average number of total implantations per pregnant female;

(4) average number of dead implantations (early and late) per pregnant female;

(5) dead implantations/total implantations.

(B) Criteria for acceptability of data

The Work Group determined that as a minimum the following be considered as criteria for accepting data for the dominant lethal assay:

(1) A rationale should be presented that addresses the selection of the dosage: the maximum tolerated dose (MTD) is defined as the highest nonlethal dose that shows some degree of toxicity.

(2) At least 2, but preferably 3 dosages should be used.

(3) For proper documentation of negative data, results from a concurrent or recently tested positive control should be presented.

(4) When using the acute or subacute dosage regimen, all stages of spermatogenesis should be sampled on a weekly basis to document negative results.

(5) A sample size (number of pregnant females) sufficient to detect a doubling of the background frequency with a 95% probability is required to *conclude* that a result is negative.

(6) Data should be evaluated on a "per pregnant female" basis and analyzed by a parametric statistical procedure on transformed data or a comparable nonparametric procedure.

(C) Statistical evaluation

The Work Group has accepted dominant lethal papers in which any of the following procedures were employed in statistical evaluations: (a) Student's t test, (b) analysis of variance, (c) chi-square test. It was the opinion of the Work Group that of these 3 tests, the chi-square test was applicable only for the fertility index. Comparable nonparametric methods to the former two would be acceptable.

(D) Criteria for positive/negative conclusions

In addition to criteria given in Section IIIB, the following should be established to conclude that a result is negative: the number of pregnant females generated should allow detection of a doubling of the spontaneous frequency for a given species and/or strain, with a 95% probability. A 5% or less level of significance should be the criterion for concluding that a result is positive.

(E) Applicability of results to hazard evaluation

The dominant lethal test offers investigators an opportunity to assess mutagenic effects of chemical agents. It is one of the few tests that evaluates the effect of test compounds on germinal tissues, is practical for routine testing procedures, and provides some information on the probability of a chemical affecting a future generation. The test is suited to determine the potential transmissibility of mutagenic effects. In addition, when the dominant lethal test is conducted in the traditional way by mating on a weekly basis over the entire period of spermatogenesis, the stage(s) of spermatogenesis that may be most sensitive to a test compound can be determined as well. The role of the assay should be that of confirming positive results from lower tier chromosomal aberration-detecting systems (confirming in the sense of indicating the ability of the chemical to penetrate gonadal tissue and to produce chromosomal damage). A negative result in any of the mutagenicity tests is not necessarily final proof of the nonmutagenic nature of a compound; the dominant lethal test is no exception.

TABLE 1

CHEMICAL TESTED IN THE DOMINANT LETHAL ASSAY

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
AF-2 (3688-53-7)	−M	+	Soares and Sheridan, 1975
Amethopterin (methotrexate) (59-05-2)	±M	•	Propping et al., 1972
Aminochlorohydrin (D) HCl (34839-14-0)	−R	NR	Jones and Jackson, 1976
Aminochlorohydrin (DL) HCl (34839-12-8)	−R	NR	Jones and Jackson, 1976
Aminochlorohydrin (L) HCl (34839-13-9)	−R	NR	Jones and Jackson, 1976
δ-Aminolevulinic acid HCl (5451-09-2)	−M	NR	Arnold et al., 1975
1-Amino-2-naphthol-3, 6-disulfonic acid·Na salt (42579-07-7)	−R	NR	Palmer et al., 1979
2-Amino-4-nitrophenol (99-57-0)	−R	NR	Burnett et al., 1977
2-Amino-5-nitrophenol (121-88-0)	−R	NR	Burnett et al., 1977
4-Amino-2-nitrophenol (119-34-6)	−R	+L	Burnett et al., 1977
Anovlar (8015-12-1)	−M	NR	Badr and Badr, 1974
Aroclor 1242 (53469-21-9)	−R	NR	Green et al., 1975a
Aroclor 1254 (11097-69-1)	−R	NR	Green et al., 1975a
Azathioprine (446-86-6)	±M	+L	Clark, 1975
Benzonal (744-80-9)	±M	NR	Fonshtein et al., 1976
Butylnitrosourea (869-01-2)	−M	+	Propping et al., 1972
Cadmium chloride (10108-64-2)	−M	+	Gilhavod and Léonard, 1975
Caffeine (58-08-2)	−M	•	Adler, 1969; Epstein, 1970, Epstein et al., 1970a; Aeschbacher et al., 1978
Captan (133-06-2)	+R,M −R	+L	Collins; 1972a
Chloramphenicol (56-75-7)	+M	•	Sram, 1972
Chlordane, AG (57-74-9)	−M ±M	+L	Arnold et al., 1977
Chlordane, technical (12789-03-6)	−M ±M	+L	Arnold et al., 1977
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (13010-47-4)	+R(M,F)	+	Thompson et al., 1975
Chlorohydrin, DL-α (96-24-2)	−R	NR	Jones and Jackson, 1976

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
Cyclamate sodium (139-05-9)	−M(F)	NR	Machemer and Lorke, 1975
Cyclohexylamine (108-91-8)	−M ±M	-	Cattanach and Pollard, 1971; Petersen et al., 1972
Cyclohexylamine sulfate (27817-50-1)	−M M(F)	NR	Lorke and Machemer, 1974; Machemer and Lorke, 1975
Cyclophosphamide (50-18-0)	−M(F) +M M M(F) M M	+	Röhrborn, 1970; Röhrborn, 1970; Fritz et al., 1973; Machemer and Lorke, 1975; Sram, 1976; Zhurkov and Sram, 1978
DDT, technical *p,p'*-DDT (50-29-3)	±M −R	+	Clark, 1974; Palmer et al., 1973
2,4-Diaminoanisole (615-05-4)	−R	NR	Burnett et al., 1977
2,4-Diaminoanisole sulfate (39156-41-7)	−R	+	Sheu and Green, 1979
2,5-Diaminoanisole (5307-02-8)	−R	NR	Burnett et al., 1977
2,5-Diaminoanisole sulfate (42909-29-5)	−R	NR	Sheu and Green, 1979
Dichlorvos (62-73-7)	±M −M(F)	-	Dean and Thorpe, 1970; Dean and Blair, 1976
Dieldrin (60-57-1)	−M	NR	Dean et al., 1975
Diethyladipate (141-28-6)	+M	NR	Singh et al., 1975
Di-2-ethyl-hexyladipate (DEHA) (103-23-1)	+M	NR	Singh et al., 1975
Di-2-ethylhexylphthalate (117-81-7)	+M	+	Singh et al., 1974
Diethylhydroxylamine (3710-84-7)	±R	NR	Legator et al., 1978
Diethylnitrosamine (DEN) (55-18-5)	−M	+	Propping et al., 1972
Diethyl sulfate (64-67-5)	−M	+	Malashenko, 1971; Malashenko and Surkova, 1973
Difolatan (Captafol) (2425-06-1)	±R	NR	Collins, 1972b
Dihydroergotoxin methanesulfonate (8067-24-1)	+M	NR	Roberts and Rand, 1978
Dimethoxyethyl phthalate (117-82-8)	±M	NR	Singh et al., 1974
1,1-Dimethylhydrazine (57-14-7)	−M	+	Brusick and Matheson, 1976a
Dimethylmercury (593-74-8)	±M	NR	Varma et al., 1974a
Dimethylmyleran (+/−) (33447-91-5)	+M	NR	Hemsworth and Wardhaugh, 1977
Dimethylmyleran (*meso*) (33447-90-4)	+M	NR	Hemsworth and Wardhaugh, 1977
Dinitro-*o*-cresol (534-52-1)	−M	NR	Nehez et al., 1978

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
Diquat (2764-72-9)	− M	NR	Pasi et al., 1974; Anderson et al., 1976a
Dimethylnitrosamine (62-75-9)	± M	+	Propping et al., 1972
Dimethyl sulfoxide (67-68-5)	± M	•	Aravindakshan et al., 1975
Docloxythepin succinate (60003-04-5)	− M	NR	Sykora et al., 1979
EMS (ethyl methanesulfonate) (62-50-0)	+ M	+	Ehling et al., 1968;
	M(F)		Generoso and Russell, 1969;
	M(F)		Generoso, 1969;
	M		Soares and Crenshaw, 1974;
	M		Generoso et al., 1974;
	M(F)		Suter and Generoso, 1976;
	M		Arnold et al., 1976a;
	M		Soares, 1976;
	M		Sram, 1976;
	M		Favor and Crenshaw, 1978
Ergotamine tartrate (379-79-3)	+ M	NR	Roberts and Rand, 1978
Ethanol (64-17-5)	+ M	− L	Badr and Badr, 1975
	− M(F)		Machemer and Lorke, 1975
Ethylene oxide (75-21-8)	+ R	•	Embree et al., 1977
Ethylenethiourea (96-45-7)	+ M	+	Schüpbach and Hummler, 1977;
	− M		Teramoto et al., 1977;
	M		Teramoto et al., 1978.
	M		Shirasu et al., 1977
FD&C Red No. 2 (Amaranth) (915-67-3)	− M	NR	Arnold et al., 1976b
Fenitrothion (122-14-5)	− R	NR	Benes et al., 1975
Fluorescent whitening agent No. 1 (6416-68-8)	− M	NR	Müller et al., 1975
Fluorescent whitening agent No. 2 (16090-02-1)	− M	NR	Müller et al., 1975
Fluorescent whitening agent No. 3 (13863-31-5)	− M	NR	Müller et al., 1975
Fluorescent whitening agent No. 4 (27344-41-8)	− M	NR	Müller et al., 1975
Folpet (133-07-3)	− R	NR	Collins, 1972b
	± R		
Fosfesterol tetrasodium (4719-75-9)	+ M	NR	Ehling, 1979
Fotrin (37132-72-2)	+ M	NR	Revazova and Radchenko, 1976
HEMPA (hexamethylphosphoramide) (680-31-9)	+ M	+	Sram et al., 1970
Heptachlor epoxide (1024-57-3)	− M	NR	Arnold et al., 1977
Hexachlorobenzene (118-74-1)	− R	NR	Simon et al., 1979

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
Hycanthone (3105-97-3)	− M	NR	Ray et al., 1975
Hycanthone methanesulfonate (23255-93-8)	+ R	*	Green et al., 1973
ICR-170 (146-59-8)	− M ± M(F)	+ L	Ehling et al., 1968; Generoso, 1969
Indenopyridine derivative (67110-84-3)	− M	NR	Matter et al., 1979
Imipramine (50-49-7)	− M	NR	Sykora et al., 1979
Isoniazid (54-85-3)	− M	+ L	Röhrborn et al., 1972
Isopropyl methanesulfonate (926-06-7)	+ M M(F) M M M(F) − GP(F)	NR	Moutschen, 1969a; Generoso et al., 1971; Ehling et al., 1972; Generoso et al., 1979; Suter and Generoso, 1976; Caine and Lyon, 1979
Lead subacetate (1335-32-6)	± M	+	Varma et al., 1974b
LSD (50-37-3)	− M	NR	Sram et al., 1974
Lyndiol (8015-14-3)	± M(F)	NR	Badr and Badr, 1974
6-Mercaptopurine (50-44-2)	+ M	+ L	Generoso et al., 1975; Schencking and Frohberg, 1975
Methylhydrazine (60-34-4)	− M,R	+ L	Brusick and Matheson, 1976b
Methyl mercuric chloride (115-09-3)	− M,R	NR	Khera, 1973
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (70-25-7)	+ M − M(F)	+	Ehling et al., 1968; Generoso, 1969
Methylnitrosourea (684-93-5)	− M	+	Propping et al., 1972
Methysergide hydrogen maleate (129-49-7)	+ M	NR	Roberts and Rand, 1978
METEPA (57-39-6)	+ M	*	Epstein et al., 1970c
Mirex (2385-85-5)	− R	+	Khera et al., 1976
Mitomen (302-70-5)	+ M	+	Ehling, 1974
Mitomycin C (50-07-7)	+ M − M	+	Ehling, 1971a; Ehling, 1971b
MMS (methyl methanesulfonate) (66-27-3)	+ M M M M M(F) M M	+	Ehling et al., 1968; Moutschen, 1969b; Partington and Bateman, 1964; Beliles et al., 1973; Machemer and Lorke, 1975; Arnold et al., 1976a; Dean and Johnstone, 1977;

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
MMS (methyl methanesulfonate) (66-27-3) (*continued*)	− M(F) − M		Generoso, 1969; Ehling, 1977
Myleran (busulfan) (55-98-1)	+ M(F)	•	Generoso et al., 1971
Natulan (procarbazine hydrochloride) (366-70-1)	+ M + M	+	Ehling, 1974; Roberts et al., 1979
Nifurpipone (24632-47-1)	− M	NR	Setnikar et al., 1976
Nitrofurantoin (67-20-9)	− M	•	Setnikar et al., 1976
4-Nitro-*o*-phenylene diamine (99-56-9)	± R − R	•	Sheu and Green, 1979; Burnett et al., 1977
2-Nitro-*p*-phenylene diamine (5307-14-2)	− R R	+ L	Sheu and Green, 1979; Burnett et al., 1977
N-Nitrosoethylene thiourea (3715-92-2)	− M	NR	Teramoto et al., 1978
Norethisterone acetate (51-98-9)	± M(F)	NR	Rohrborn and Hansmann, 1974
Oxamniquine (21738-42-1)	− M	NR	Ray et al., 1975
Oxyprothepin decanoate (41931-86-6)	− M	NR	Sykora et al., 1979
Paraquat (4685-14-7)	− M	NR	Pasi et al., 1974; Anderson et al., 1976a
Patulin (149-29-1)	− M	− L	Reddy et al., 1978
Phenylbutazone (50-33-9)	− M M M(F)	NR	Machemer and Hess, 1971; Charles and Léonard, 1978; Machemer and Hess, 1973a
m-Phenylenediamine (108-45-2)	± R − R	NR	Sheu and Green, 1979; Burnett et al., 1977
o-Phenylenediamine (95-54-5)	− R	NR	Burnett et al., 1977
p-Phenylenediamine (106-50-3)	− R	− L	Burnett et al., 1977
n-Phenyl-α-naphthylamine (90-30-2)	± M	NR	Brusick and Matheson, 1976c
Polydimethylsiloxane DC 360 (63148-62-9)	− M	NR	Kennedy et al., 1976
Praziquantel (55268-74-1)	− M	NR	Machemer and Lorke, 1978
Propylene oxide (75-56-9)	− M	+ L	Bootman et al., 1979
n-Propyl methanesulfonate (1912-31-8)	− M(F) + M	NR	Generoso et al., 1971; Ehling et al., 1972
Prothiaden (113-53-1)	− M	NR	Sykora et al., 1979
Puromycin (53-79-2)	− M	NR	Sram, 1972
Ronidazole (7681-76-7)	− M	NR	Hite et al., 1976

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
Rubratoxin B (21794-01-4)	+M	NR	Evans and Harbison, 1977
Rubratoxin B, hydrogenated (31924-91-1)	−M	NR	Evans and Harbison, 1977
Saccharin (81-07-2)	+M	+	Tezabwala and Gothoskar, 1977
Saccharin, sodium (128-44-9)	+M	+	Rao and Qureshi, 1972;
	−M		Machemer and Lorke, 1973b;
	M(F)		Machemer and Lorke, 1975
Sodium bisulfite (7631-90-5)	−M(M,F)	NR	Generoso et al., 1978
Sodium nitrite (7632-00-0)	−M	NR	Teramoto et al., 1978
Sorbitol (50-70-4)	−R	NR	Maxwell and Newell, 1974
Styrene oxide (96-09-3)	−M	+L	Fabry et al., 1978
Triethylenemelamine (TEM) (51-18-3)	+M(F)	+L	Suter and Generoso, 1976;
	M(F)		Cattanach, 1959;
	M		Matter and Generoso, 1974;
	GP		Cox and Lyon, 1975;
	M		Generoso et al., 1977;
	M		Hastings et al., 1976;
	M		Hitotsumachi and Kikuchi, 1977;
	M		Green et al., 1977;
	M		Soares and Sheridan, 1977;
	M		Schreiner and Steelman, 1977;
	M		Staub and Matter, 1977;
	R		Bateman, 1960;
	M		Sheu et al., 1978;
	M(F)		Generoso et al., 1971;
	M		Matter and Jaeger, 1975;
	−GP(F)		Caine and Lyon, 1979
TEPA (545-55-1)	+M		Sram et al., 1970;
	M		Sram, 1972;
	M		Sram and Zudova, 1973;
	M		Epstein et al., 1970c
1,2,3,4-Tetrabromobutane (1529-68-6)	−R	NR	Simon et al., 1978
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (1746-01-6)	−R	+	Khera and Ruddick, 1973
5-Thio-D-glucose (20408-97-3)	−R	NR	Majumdar et al., 1979
Thioproperazine-2-dimethylsulfamido-10-(3-1-methyl-piperazinyl-4) propylphenothiazine (2347-80-0)	−M	NR	Revazova et al., 1975
Thio-TEPA (52-24-4)	+M	+	Malashenko and Surkova, 1974a;
	M		Malashenko and Surkova, 1974b;
	M		Malashenko and Surkova, 1975;
	M		Sram, 1976;
	M		Malashenko et al., 1978

TABLE 1 (continued)

Chemical (CAS No.)	Mutagenicity [a,b]	Carcinogenicity [c]	References
Trenimon (68-76-8)	+ M(M,F) M(F) M(F)	+	Röhrborn, 1970; Machemer and Hess, 1973a; Machemer and Lorke, 1975
Tribromethanol (75-80-9)	− M(F)	NR	Kaufman, 1977
α,α,α-Trifluoro-2-methyl-4'-nitro-*m*-proprionotoluidide (13311-84-7)	− R	NR	Beall, 1974
Triflupromazine (146-54-3)	+ M − M	NR	Petersen and Legator, 1973; Ray et al., 1973
Trimethylphosphate (512-56-1)	+ M	+	Epstein, 1970b
p-Toluenediamine (95-70-5)	− R	NR	Burnett et al., 1977
Trypaflavine (65431-33-6)	± M(F)	NR	Basler et al., 1977
Vinyl chloride (75-01-4)	− M R	+	Anderson et al., 1976b; Short et al., 1977
Vinylidene chloride (75-35-4)	− R	+ L	Short et al., 1977

[a] ±, borderline response; considered positive in the estimation of correlation between mutagenicity and carcinogenicity; +, −, positive response in one experiment and negative in the other experiment, considered positive in the estimation of correlation between mutagenicity and carcinogenicity; +, positive; −, negative.

[b] M, mouse; R, rat; GP, guinea pig; (F), female treated; (M,F), male and female treated in separate experiments; no entry, males treated.

[c] Source: Carcinogen list as reviewed by the EPA Gene-Tox Carcinogenesis Work Group +, positive; + L, positive, limited; − L, negative, limited; NR, not reviewed; *, inconclusive.

(IV) Test performance

(A) Number of chemicals tested

The number of chemicals considered in the dominant lethal assay at the time of this analysis is 140 (Table 1). These chemicals represent a spectrum of classes. It was not possible, however, due to overlap of chemicals among the various classes, to provide substantive information on testing results on a chemical-class basis.

(B) Correlation with mammalian carcinogenicity data

Table 1 summarizes the mutagenicity data available for the dominant lethal assay. Also presented are the results for chemicals that have been tested for carcinogenicity. Inspection of the table shows that of the 44 chemicals declared as carcinogens based on data from animals, 26 produced positive (+) or borderline (±) responses in the dominant lethal assay, for a 59% correlation. Consequently, this test is not viewed as one that could serve as a prescreen for carcinogenicity.

(V) Suggested protocol for testing

A dominant lethal test consists of 3 basic steps: (1) exposure of animals to a test compound, (2) mating of the treated animals to untreated animals of the opposite sex immediately after treatment, and (3) sacrifice and examination of the pregnant females to obtain the number of total, live, and dead implants. The dominant lethality is then assessed on the basis of the number of dead implantations per pregnant female. Since the test is usually conducted by treating males, the following discussion concerns only the male as the test animal.

(A) Treatment conditions

Protocols for the dominant lethal test can be grouped into 3 types. One involves an acute treatment followed by a succession of weekly matings, such as 6–8 weeks in mice and 8–10 weeks in rats. This method allows potential damage induced in the germ cells at a specific stage of development to be expressed through weekly mating. A second protocol involves a prolonged treatment, such as 6–8 weeks in mice and 8–10 weeks in rats, to cover the entire spermatogenic cycle, followed by 1–2 weeks of mating. This method allows potential damage induced in the germ cells at various stages of development to be expressed during the first week of mating but does not show the most sensitive stage. A third protocol involves a short-term treatment (5 daily administrations) followed by a succession of weekly matings. The duration of treatment in this method is too short to cover the entire spermatogenic cycle, yet is often too long to allow precise determination of stage specificity. The first protocol is the method of choice for detecting stage specificity, while the second is best for evaluating prolonged effects of a chemical. The third protocol is used less often than the others; however, there is some evidence that a chemical can produce dominant lethality only after subacute but not after acute administration (Green et al., 1973).

(B) Positive control and negative control

A positive control is often included in testing to ensure that the experimental conditions are optimal for the detection of dominant lethality. This control is necessary especially when the testing is conducted in a new laboratory, or when a new protocol or new species (strain) of animal is employed. In routine testing, a positive control may not always be necessary, but a concurrent negative control is always required because the spontaneous frequency of embryonic deaths is known to vary from species to species, strain to strain, and even from season to season.

(C) Dosage selection and number of doses

In the dominant lethal test, the dosage employed is often selected on the basis of some biological effects reported in the literature or on the usage level for humans, sometimes with no obvious justification. Therefore, a negative response simply may indicate a safe or no-effect level of the test compound without addressing the question of potential mutagenicity. It is essential, therefore, that a compound be tested not only at low but also at the MTD level so that the potential mutagenicity of a compound may be explored to the limit of technical ability. The proper determination of the MTD level is critical, especially in prolonged treatment, because a compound with cumulative toxicity may complicate dosage selection. Ideally, an MTD should induce neither death nor sterility in the treated animals. One way to determine the MTD level is to treat a small number of test animals (e.g., 5 per dose level) for a range of doses (e.g., 4 or 5) for 1 week or longer. The number of doses depends on previous information as regards the toxicity of the agent. The highest nonlethal dose that may slightly affect body weight gain can be selected as the MTD. If a compound has no detectable toxicity, then a dose level of 5 g/kg body weight may be taken as the MTD. Preferably, a minimum of 3 doses, including the MTD, should be used in routine testing.

(D) Route of administration

Chemicals can be and have been administered by a variety of routes: inhalation, gavage, intraperitoneal injection, or intravenous injection. Topical application, however, has not been used as a means of administering chemicals in the dominant lethal assay.

(E) Acceptable spontaneous background frequencies or rates

In determining the acceptable background frequencies or rates, a random analysis of control frequencies in terms of average number of dead implantations per pregnant female has been conducted. This analysis was performed with data from the mouse. Sample sizes of at least 8 females per group were used, with those less than 8 not considered. With respect to the mouse, the average control rate varied from a low of 0.40 to a high of 0.80. It should be pointed out that each strain in this instance was of the random bred type. Control data from approximately 7500 individual females showed 4 out of 100 controls with more than 1 early death and 4 out of 1000 with more than 3

early deaths (Epstein et al., 1972). Although data from individual females were not examined by the Work Group, the average control rate among the various strains did not exceed 1 dead implantation per female.

It would seem appropriate then to consider any control frequency beyond an average of 2 dead implantations per female in the case of the mouse as somewhat suspect and possibly to require a repeat experiment.

(F) Number of test animals

The number of animals (young adults) subjected to treatment is usually small, ranging from several to a few dozen per dose level. Generally, each treated male is mated to several untreated females (mating pairs identified), and the implantations observed in pregnant females are then evaluated for evidence of dominant lethality. Therefore, the number of pregnant females per dose level is the critical parameter in this test. Although this number is determined primarily by the number of males subjected to treatment, it is also determined by such factors as the mating ratio of treated males to untreated females, the reproductive capacity of both male and female strains, and the effect of the chemical on the reproductive capacity of the treated male. Ideally, the number of pregnant females generated should allow detection, with a 95% probability, of a doubling of the spontaneous frequency for a given species or strain of animals. However, a sample size is often selected on the basis of practicality, rather than statistical consideration. When no significant increase over control frequency has been detected, the number of animals per dose level becomes a critical issue, since it determines the multiple of the control frequency that can be ruled out statistically. As a general rule, a sample size should be selected with due statistical consideration. If that approach is not practical, when a negative result is obtained, one should specify the level of induced frequency that can be detected with the sample size employed.

(G) Proper collection of raw data

The number of both live and dead implantations in each pregnant female constitutes the raw data in the dominant lethal test. Therefore, any factors that affect implantations could influence the test results. Examples of these factors are as follows:

(1) Health of the pregnant female. The presence of intercurrent infection may affect implantation; therefore, the implantation data should be collected from healthy mothers.

(2) Time of sacrifice of the females. Daily check for vaginal plugs or the performance of vaginal lavage permits accurate timing of mating and subsequent sacrifice. Vaginal plugs can easily be observed in the mouse. In the rat, vaginal lavage is the recommended technique. If vaginal smears are made or vaginal plugs observed, the day on which sperm or plugs are observed is taken as day 0 of pregnancy, and females are killed on day 14. In routine testing, the females are all sacrificed at a fixed time, approximately 2 weeks following the midweek of cocaging. Therefore, the stage of pregnancy and thus the age of the implantations vary considerably (11–18 days), making the distinction between certain types of implantations somewhat difficult.

The difficulty usually arises when a dosage level causes significant postimplantation loss and the day of sacrifice is such that a number of females are in the early stages of pregnancy (i.e., days 11–12). The distinction between early death and live implantations is more obscure in the early pregnant condition. It may be necessary to eliminate females in the early stage of pregnancy to reduce the margin of error. In some cases, a distinction can be made by dissecting the implantation site and observing the embryo.

(3) The classification of implantations. The implantations are classified as live or dead, and dead implantations are often further classified as early or late. Since the identification of early and late deaths may rely on subjective judgment, well-trained personnel are required to conduct uterine analysis.

(4) Counting of corpora lutea. Inasmuch as postimplantation loss (i.e., early death) is the most significant index of dominant lethality, the assessment of preimplantation loss is not a requirement. If determination of preimplantation loss is a desirable objective, one of two methods can be used. The first method makes use of corpora lutea counts and determines the difference between the number

of corpora lutea and the number of implantations. This technique requires trained and skilled personnel. The rat allows an easier estimation of corpora lutea due to their greater size. The second method is to compare the number of implantations per female in the treated and control groups. Any significant difference may be attributed to preimplantation loss. This approach is used more often for the mouse than for the rat. It should be pointed out that occasionally a reduction in the number of eggs released occurs, and then there is a significant reduction in implantations. In such cases, one may mistakenly implicate the chemical, because the assumption is that comparable numbers of eggs were released in treated and control groups. Fortunately, this confusion does not occur often and can be avoided by performing counts of corpora lutea.

(VI) Conclusions

The dominant lethal assay is a whole animal test system that allows a chemical to be tested in vivo. Furthermore, it is one of the few mutagenicity tests that actually assesses the effect of chemicals on germ cells. This assay has sometimes been regarded as relatively insensitive (i.e., lack of response to certain chemical mutagens); however, the germ cells within the whole animal obviously present a target very different than targets in in vitro systems. Physiological and pharmacological factors must be considered in evaluating results from the dominant lethal assay. It is not enough to highlight response differences between the dominant lethal assay and in vitro assays, but investigators should concentrate on the rationale underlying each response. The failure to obtain a consistent response on repeated testing, particularly when the weekly mating procedure is used, could be viewed as a weakness in this test. This problem is a statistical one brought about by the need to evaluate a large number of individual units (weekly values) for 5 or 6 indexes. The probability of obtaining a positive point increases as the number of points increase. It is not surprising that a number of chemicals have appeared positive but upon repeated testing were found to be negative. Analyses of variance across weeks have been used to evaluate results for all weeks in one spermatogenic cycle, reducing the number of individual statistical tests run. The role of the dominant lethal assay in a mutagenicity testing program should be that of confirming results obtained in other tests that are used to detect chromosomal aberrations. Again, "confirming" is used to denote the ability of the agent to produce chromosomal aberrations in germ cells that contribute to the genome of the offspring. From the analysis of the Work Group, it appears that the dominant lethal assay should not be employed in a carcinogenicity testing program but can be used to detect potential heritable germ cell damage. Although not as valuable as the translocation assay, the dominant lethal assay could provide important information for a subsequent heritable translocation test.

References

Adler, I.-D. (1969) Humangenetik, 7, 137–148.

Aeschbacher, H.U., H. Milon and H.P. Wurzner (1978) Mutation Res., 57, 193–200.

Anderson, D., D.B. McGregor and I.F.H. Purchase (1976a) Mutation Res., 40, 349–358.

Anderson, D., M.C.E. Hodge and I.F.H. Purchase (1976b) Mutation Res., 40, 359–370.

Aravindakshan, M., P.S. Chauhan, A.S. Aiyar and K. Sundaram (1975) Proc. Symp. Mutagenicity, Carcinogenicity and Teratogenicity of Chemicals 1975, Baroda, India, pp. 45–62.

Arnold, D.W., G.L. Kennedy Jr., M.L. Keplinger and J.C. Calandra (1975) Food Cosmet. Toxicol., 13, 63–68.

Arnold, D.W., G.L. Kennedy Jr., M.L. Keplinger and J.C. Calandra (1976a) Toxicol. Appl. Pharmacol., 38, 79–84.

Arnold, D.W., G.L. Kennedy Jr., M.L. Keplinger and J.C. Calandra (1976b) Food Cosmet. Toxicol., 14, 163–165.

Arnold, D.W., G.L. Kennedy Jr., M.L. Keplinger, J.C. Calandra and C.J. Calo (1977) J. Toxicol. Environ. Health, 2, 547–555.

Badr, F.M., and R.S. Badr (1974) Mutation Res., 26, 529–534.

Badr, F.M., and R.S. Badr (1975) Nature (London), 253, 134–136.

Basler, A., M. Brucklacher, F. Nobis and G. Rohrborn (1977) Hum. Genet., 40, 87–92.

Bateman, A.J. (1960) Genet. Res., 1, 381–392.

Bateman, A.J. (1966) Nature (London), 210, 205–206.

Bateman, A.J., and Epstein, S.S. (1971) Dominant lethal mutations in mammals, in: A. Hollaender (Ed.), Chemical Mutagens: Principles and Methods for Their Detection, Vol. 2, Plenum, New York, pp. 541–568.

Beall, J.R. (1974) Toxicol. Appl. Pharmacol., 28, 303–312.

Beliles, R.P., N. Korn and B.W. Benson (1973) Res. Commun. Chem. Pathol. Pharmacol., 5, 713–724.

Benes, V., R.J. Sram and R. Tuscany (1975) J. Hyg. Epidemiol. Microbiol. Immunol., 19, 163–175.

Bootman, J., D.C. Lodge and H.E. Whalley (1979) Mutation Res., 67, 101–112.

Brenneke, H. (1937) Strahlentherapie, 60, 214–238.

Brewen, J.G., H.S. Payne, K.P. Jones and R.J. Preston (1975) Mutation Res., 33, 239–250.

Brusick, D., and D.W. Matheson (1976a) Mutagen and Oncogen Study on 1,1-Dimethylhydrazine, Final Report U.S. NTIS, AD Report (AMRL-TR-76-78), 27 pp.

Brusick, D., and D.W. Matheson (1976b) U.S. NTIS, AD Report (AD-A035477), 38 pp.

Brusick, D., and D.W. Matheson (1976c) U.S. NTIS, AD Report (AD-A035476), 30 pp.

Burnett, C., R. Loehr and J. Corbett (1977) J. Toxicol. Environ. Health, 2, 657–662.

Caine, A., and M.F. Lyon (1979) Mutation Res., 59, 231–244.

Cattanach, B.M. (1959) Int. J. Radiat. Biol., 1, 288–292.

Cattanach, B.M., and C.E. Pollard (1971) Mutation Res., 12, 472–474.

Charles, D., and A. Léonard (1978) Toxicol. Lett., 2, 225–230.

Clark, J.M. (1974) Aust. J. Biol. Sci., 27, 427–440.

Clark, J.M. (1975) Mutation Res., 28, 87–99.

Collins, T.F.X. (1972a) Food Cosmet. Toxicol., 10, 353–361.

Collins, T.F.X. (1972b) Food Cosmet. Toxicol., 10, 363–371.

Cox, B.D., and M.F. Lyon (1975) Mutation Res., 30, 293–298.

Dean, B.J., and D. Blair (1976) Mutation Res., 40, 67–72.

Dean, B.J., and E. Thorpe (1970) Arch. Toxicol., 30, 51–59.

Dean, B.J., and A. Johnstone (1977) Mutation Res., 42, 269–278.

Dean, B.J., S.M.A. Doak and M. Somerville (1975) Food Cosmet. Toxicol., 13, 317–323.

Ehling, U.H. (1971a) Mutation Res., 11, 35–44

Ehling, U.H. (1971b) Mutation Res., 13, 433–436.

Ehling, U.H. (1974) Mutation Res., 26, 285–295.

Ehling, U.H. (1977) Arch. Toxicol., 38, 1–11.

Ehling, U.H. (1979) Arch. Toxicol., 42, 171–177.

Ehling, U.H., R.B. Cumming and H.V. Malling (1968) Mutation Res., 5, 417–428.

Ehling, U.H., D.G. Doherty and H.V. Malling (1972) Mutation Res., 15, 175–184.

Embree, J.W., J.P. Lyon and C.H. Hine (1977) Toxicol. Appl. Pharmacol., 40, 261–267.

Epstein, S. (1970) Chemical Mutagenesis in Mammals and Man, pp. 404–419.

Epstein, S.S., W. Bass, E. Arnold and Y. Bishop (1970a) Food Cosmet. Toxicol., 8, 381–401.

Epstein, S.S., W. Bass, E. Arnold and Y. Bishop (1970b) Science, 168, 584–586.

Epstein, S.S., E. Arnold, K. Steinberg, D. Mackintosh, H. Shafner and Y. Bishop (1970c) Toxicol. Appl. Pharmacol., 17, 23–40.

Epstein, S., E. Arnold, J. Andrea, W. Bass and Y. Bishop (1972) Toxicol. Appl. Pharmacol., 23, 288–325.

Evans, M.A., and R.D. Harbison (1977) Toxicol. Appl. Pharmacol., 39, 13–22.

Fabry, L., A. Léonard and M. Roberfroid (1978) Mutation Res., 51, 377–381.

Favor, J., and J.W. Crenshaw (1978) Mutation Res., 53, 21–27.

Fonshtein, L.M., G.N. Zolotareva, E.N. Iskhakova and A.A. Shapiro (1976) Cytol. Genet., 10(2), 1–4.

Fritz, H., D. Mueller and R. Hess (1973) Agents Actions, 3, 35–37.

Generoso, W.M. (1969) Genetics, 61, 461–470.

Generoso, W.M., and W.L. Russell (1969) Mutation Res., 8, 589–598.

Generoso, W.M., S.W. Huff and S.K. Stout (1971) Mutation Res., 11, 411–420.

Generoso, W.M., W.L. Russell, S.W. Huff, S.K. Stout and D.G. Gosslee (1974) Genetics, 77, 741–752.

Generoso, W.M., R.J. Preston and J.G. Brewen (1975) Mutation Res., 28, 437–447.

Generoso, W.M., M. Krishna, R.E. Sotomayor and N.L.A. Cacheiro (1977) Genetics, 85, 65–72.

Generoso, W.M., S.W. Huff and K.T. Cain (1978) Mutation Res., 56, 363–365.

Generoso, W.M., S.W. Huff and K.T. Cain (1979) Genetics, 93, 163–171.

Gilliavod, N., and A. Léonard (1975) Toxicology, 5, 43–47.

Goldstein, L. (1977) Mutation Res., 42, 135–138.

Green, S., J. Carr, F.M. Sauro and M.S. Legator (1973) J. Pharmacol. Exp. Ther., 187, 437–443.

Green, S., F.M. Moreland and W.G. Flamm (1975b) Mutation Res., 31, 340–341.

Green, S., F.M. Sauro and L. Friedman (1975a) Food Cosmet. Toxicol., 13, 507–510.

Green, S., F.M. Moreland and W.G. Flamm (1977) Toxicol. Appl. Pharmacol., 39, 549–552.

Hastings, S.E., K.W. Huffman and M.A. Gallo (1976) Mutation Res., 40, 371–378.

Hemsworth, B.N., and A.A. Wardhaugh (1977) IRCS Libr. Compend., 5, 341.

Hite, M., H. Skeggs, J. Noveroske and H. Peck (1976) Mutation Res., 40, 289–304.

Hitotsumachi, S., and Y. Kikuchi (1977) Mutation Res., 42, 117–124.

Jackson, H. (1966) *Antifertility Compounds in the Male and Female*, Thomas, Springfield, IL, pp. 19–21.

Jones, P., and H. Jackson (1976) Contraception, 13, 639–646.

Kaplan, W.D., and M.F. Lyon (1953) Science, 118, 777–778.

Kaufman, M.H. (1977) J. Reprod. Fertil., 49, 167–168.

Kennedy Jr., G.L., M.L. Keplinger, J.C. Calandra and E.J. Hobbs (1976) J. Toxicol. Environ. Health, 1, 909–920.

Khera, K.S. (1973) Toxicol. Appl. Pharmacol., 24, 167–177.

Khera, K.S., and J.A. Ruddick (1973) Adv. Chem. Ser., 120, 70–84.

Khera, K.S., D.C. Villeneuve, G. Terry, L. Panopio, L. Nash and G. Trivett (1976) Food Cosmet. Toxicol., 14, 25–29.

Kratochvilova, J. (1978) Mutation Res., 54, 47–54.

Legator, M., R.E. Kouri, A.S. Parmar, S. Zimmering, C. Putnam, R. Latt, J. Heicklen, J.F. Meagher, J. Weaver and N. Kelly (1978) Mutagenic Testing of Diethylhydroxylamine, Nitroethane and Diethylamine Hydrogen Sulfite, Report (CAES492-78), 62 pp.

Lorke, D., and L. Machemer (1974) Toxicology, 2, 231–237.

Machemer, L., and R. Hess (1971) Experientia, 27, 1050–1052.

Machemer, L., and R. Hess (1973a) Experientia, 29, 190–192.

Machemer, L., and D. Lorke (1973b) Humangenetik, 19, 193–198.

Machemer, L., and D. Lorke (1975) Mutation Res., 29, 209–214.

Machemer, L., and D. Lorke (1978) Arch. Toxicol., 39, 187–197.

Majumdar, S.K., L.D. Ringer and L. McFadden (1979) J. Hered., 70, 75–77.
Malashenko, A.M. (1971) Sov. Genet., 7(1), 84–91.
Malashenko, A.M., and N.I. Surkova (1973) Lab. Anim. Drug Test Symp. Int. Comm. Lab. Anim. 5th, 1972, pp. 97–103.
Malashenko, A.M., and N.I. Surkova (1974a) Sov. Genet., 10, 51–58.
Malashenko, A.M., and N.I. Surkova (1974b) Sov. Genet., 10, 1004–1008.
Malashenko, A.M., and N.I. Surkova (1975) Sov. Genet., 11, 211–214.
Malashenko, A.M., K.H. Semenov, G.P. Selezneva and N.I. Surkova (1978) Sov. Genet., 14, 35–42.
Matter, B.E., and W.M. Generoso (1974) Genetics, 77, 753–763.
Matter, B.E., and I. Jaeger (1975) Mutation Res., 33, 251–260.
Matter, B.E., I. Jaeger, W. Suter, T. Tsuchimoto and H. Deysseroth (1979) Mutation Res., 66, 113–127.
Maxwell, W.A., and G.W. Newell (1974) in: L. Prakash, F. Sherman, M.W. Miller, C.W. Lawrence and H.W. Taber (Eds.), Proc. Publ. Rochester, 6th Int. Conf. Environ. Toxicol., Thomas, Springfield, IL, pp. 223–252.
Mosteller, F., and C. Youtz (1961) Biometrika, 48, 433–440.
Moutschen, J. (1969a) Experientia, 25, 1337–1338.
Moutschen, J. (1969b) Mutation Res., 8, 581–588.
Muller, D., H. Fritz, M. Langauer and F.F. Strasser (1975) in: F. Coulston and F. Korte (Eds), Environ. Qual. Safety Suppl., 4, 247–263.
Nehez, M., A. Selypes, A. Paldy and Gy. Berencsi (1978) Ecotoxicol. Environ. Safety, 2, 401–405.
Palmer, K.A., S. Green and M.S. Legator (1973) Food Cosmet. Toxicol., 11, 53–62.
Palmer, K.A., C.W. Sheu and S. Green (1979) Food Cosmet. Toxicol., 17, 5–9.
Partington, M., and A.J. Bateman (1964) Heredity, 19, 191–200.
Pasi, A., J.W. Embree, Jr., G.H. Eisenlord and C.H. Hine (1974) Mutation Res., 26, 171–175.
Petersen, K., and M.S. Legator (1973) Mutation Res., 17, 87–92.
Petersen, K.W., M.S. Legator and F.H.J. Figge (1972) Mutation Res., 14, 126–129.
Propping, P., G. Röhrborn and W. Buselmaier (1972) Mol. Gen. Genet., 117, 197–209.
Rao, M.S., and A.B. Qureshi (1972) Indian J. Med. Res., 60, 599–603.
Ray, V.A., H.E. Holden, J.H. Ellis and M.L. Hyneck (1973) Mutation Res., 18, 301–309.
Ray, V.A., H.E. Holden, J.H. Ellis, Jr. and M.L. Hyneck (1975) J. Toxicol. Environ. Health, 1, 211–227.
Reddy, C.S., P.K. Chan and A.W. Hayes (1978) Toxicology, 11, 219–223.
Revazova, Yu.A., and L.U. Radchenko (1976) Sov. Genet., 12, 246–248.
Revazova, Yu.A., G.N. Zolotareva, A.A. Shapiro, S.K. Abilev, V.S. Zhurkov and L.M. Fonshtein (1975) Cytol. Genet., 9(5), 14–17.
Roberts, G.T., and M.J. Rand (1978) Mutation Res., 50, 317–325.
Roberts, G.T., F.M. Johnson, H.V. Malling and R.K. Sharma (1979) Arch. Toxicol., 41, 287–294.
Röhrborn, G. (1970) in: F. Vogel and G. Röhrborn (Eds.), Chemical Mutagenesis in Mammals and Man, Springer, New York, pp. 294–316.
Röhrborn, G. and I. Hansmann (1974) Mutation Res., 26, 535–544.
Röhrborn, G., P. Propping and W. Buselmaier (1972) Mutation Res., 16, 189–194.
Schencking, M. Schulze and H. Frohberg (1975) Arch. Toxicol., 34, 71–75.
Schreiner, C.A., and J.R. Steelman (1977) Toxicol. Appl. Pharmacol., 42, 487–495.
Schüpbach, M., and H. Hummler (1977) Mutation Res., 56, 111–120.
Setnikar, I., M.J. Magistretti and M. Veronese (1976) Proc. Eur. Soc. Toxicol., 17, 405–412
Sheu, C.W., and S. Green (1979) Mutation Res., 68, 85–98.
Sheu, C.W., F.M. Moreland, E.J. Oswald, S. Green and W.G. Flamm (1978) Mutation Res., 50, 241–250.
Shirasu, Y., M. Moriya, K. Kato, F. Lienard, H. Tezuka, S. Teramoto and T. Kada (1977) in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Symp., 1976, 4, 267–285
Short, R.D., J.L. Minor, J.M. Winston and C.-C. Lee (1977) J. Toxicol. Environ. Health, 3, 965–968.
Simon, G.S., R.G. Tardiff and J.F. Borzelleca (1978) Toxicol. Appl. Pharmacol., 44, 661–664.
Simon, G.S., R.G. Tardiff and J.F. Borzelleca (1979) Toxicol. Appl. Pharmacol., 47(2), 415–419.
Singh, A.R., W.H. Lawrence and J. Autian (1974) Toxicol. Appl. Pharmacol., 29, 35–46.
Singh, A.R., W.H. Lawrence and J. Autian (1975) Toxicol. Appl. Pharmacol., 32, 566–576.
Soares, E.R. (1976) Mutation Res., 37, 245–252.
Soares, E.R. and J.W. Crenshaw (1974) Mutation Res., 26, 385–389.
Soares, E.R., and W. Sheridan (1975) Mutation Res., 31, 325–240.
Soares, E.R., and W. Sheridan (1977) Mutation Res., 43, 247–254
Sram, R.J. (1972) Folia Biol. (Prague), 18, 367–373.
Sram, R.J. (1976) Mutation Res., 41, 25–42.
Sram, R.J., and Z. Zudova (1973) Folia Biol. (Prague), 19, 58–67.
Sram, R.J., V. Benes and Z. Zudova (1970) Folia Biol. (Prague), 16, 407–416.
Sram, R.J., Z. Zudova and P. Goetz (1974) Mutation Res., 26, 517–522.
Staub, J.E., and B.E. Matter (1977) Arch. Genet., 50, 29–41.
Suter, K.E., and W.M. Generoso (1976) Mutation Res., 34, 259–270.
Sykora, I., D. Gandalovicova and K. Rezabek (1979) Mutation Res., 66, 291–299.
Teramoto, S., M. Moriya, K. Kato, H. Tezuka, S. Nakamura, A. Shingu and Y. Shirasu (1977) Mutation Res., 56, 121–129.
Teramoto, S., A. Shingu and Y. Shirasu (1978) Mutation Res., 56, 335–340.
Tezabwala, B.U., and S.V. Gothoskar (1977) Indian J. Cancer, 14, 232–234.

Thompson, D.J., J.A. Molello, R.J. Strebing and I.L. Dyke (1975) Toxicol. Appl. Pharmacol., 34, 456–466.

United Nations Scientific Committee (1966) Report on the Effects of Atomic Radiation, United Nations, New York, 99 pp.

Varma, M.H., E.L. Dage and S.R. Joshi (1974a) J. Environ. Syst., 4/2, 135–143.

Varma, M.M., S.R. Joshi and A.O. Adeyemi (1974b) Experientia, 30, 486–487.

Zhurkov, V.S. and R.J. Sram (1978) Sov. Genet., 14, 580–583.