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Quantitative and qualitative comparison of spontaneous and chemical-induced specific-locus mutation in the *ad-3* region of heterokaryon 12 of *Neurospora crassa*

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Abstract

The data from forward-mutation experiments to obtain specific-locus mutations at two closely linked loci in the adenine-3 (*ad-3*) region of heterokaryon 12 (H-12) of *Neurospora crassa* have been tabulated to determine the relative frequencies and mutational spectra of *ad-3* mutants occurring spontaneously and those induced by 22 different chemical treatments. Previous studies have demonstrated that specific-locus mutations at these two loci result from 5 major genotypic classes, namely two classes of gene/point mutations (*ad-3A*^{R} and *ad-3B*^{R}), and 3 classes of multilocus deletion mutations ([*ad-3A*]^{IR}, [*ad-3B*]^{IR} and [*ad-3A ad-3B*]^{IR}). In addition, prior studies have demonstrated that some chemical mutagens induced *ad-3* mutants exclusively, or almost exclusively, by gene/point mutation and other chemical mutagens by gene/point mutation and multilocus deletion mutation. In the latter cases, there was wide variation in the percentages of *ad-3* mutants in these 5 major genotypic classes. Two comparative methods of analysis that also were used to compare spontaneous and chemical-induced *ad-3* mutational spectra included χ^2-tests on the numbers of *ad-3* mutants resulting in the following two sets of ratios: (1) gene/point mutations and multilocus deletion mutations; and (2) complementing and non-complementing *ad-3B*^{R} mutants. Combination of the *p*-values from χ^2-tests for these two methods of comparison demonstrated that all 22 chemicals induce a spectrum of *ad-3* mutants that is *qualitatively* different from that occurring spontaneously. In addition, these same two methods of comparison have been used to compare the mutagenic effects of each of the 22 chemical treatments with each other. Combination of the data from these two methods of comparison has demonstrated that 93.1% (215/231) of the pairwise combinations of these 22 chemicals were different from each other. The implication of these experimental data on the induction of specific-locus mutations in somatic cells of Neurospora for genetic risk assessment exercises is discussed.

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1. Introduction

In the present paper, the data on spontaneous and chemical-induced specific-locus mutations from a series of earlier experiments (de Serres, 1992b) have been used to examine 3 issues: (1) Does chemical treatment result in comparable increases in the induction of specific-locus mutations at different genetic loci?; (2) is the chemical-induced genetic damage qualitatively different from that which occurs spontaneously?; and (3) Do chemical-induced specific-locus mutations differ from each other?

In an earlier paper (de Serres and Webber, 1996), both non-ionizing and ionizing radiation-induced specific-locus mutations were demonstrated to be quantitatively and qualitatively different from spontaneous mutations at two loci in the *ad-3* region of somatic cells of *Neurospora crassa*. All 7 radiation treatments induced specific-locus mutations both by gene/point mutation (*ad-3A*^{R} and *ad-3B*^{R}), and multilocus deletion mutation ([*ad-3A*]^{IR}, [*ad-3B*]^{IR}, and [*ad-3A ad-3B*]^{IR}), but there were marked differences in numbers of *ad-3* mutants in each of these 5 major genotypic classes, as well as differences among the radiation-induced *ad-3* mutational spectra and the spontaneous *ad-3* mutational spectrum. These results are in agreement with similar comparisons of spontaneous and radiation-induced specific-locus mutations in mammalian in vitro systems (Sankaranarayanan, 1991b). In addition, comparison of the 7 radiation-induced samples of *ad-3* mutants demonstrated that they were qualitatively different from each other.

Comparisons of spontaneous and induced specific-locus mutations in Neurospora can be used to address some of the critical issues in genetic risk assessment concerning: (1) the distribution of induced specific-locus mutations at specific loci; and (2) the nature of induced genetic damage resulting in specific-locus mutations. Information on such parameters is of paramount importance in genetic risk assessment (de Serres, 1992b), because it has been assumed in the doubling dose method of risk assessment exercises (BEIR III, 1980; CCEM, 1983; BEIR V, 1990) that induced genetic damage in somatic and germ cells will result in comparable increases over the spontaneous forward-mutation frequencies at all loci in the genome. In addition, due to the absence of experimental data in these early risk assessment exercises, it also was assumed that the induced spectrum of genetic damage is qualitatively identical to that occurring spontaneously. In a series of recent reviews, current experimental evidence relevant to these issues has been reviewed (Sankaranarayanan, 1991a,b, 1994) to develop a basis for the utilization of data from in vitro and in vivo systems for estimation of the genetic risks of human exposure to ionizing radiation and to chemical mutagens.

An additional, but related, issue is whether chemical-induced specific-locus mutations are qualitatively different from each other. We have already demonstrated (de Serres, 1992a) that chemicals can induce specific-locus mutation exclusively, or almost exclusively, by gene/point mutation, but also by gene/point mutation and multilocus deletion mutation. In genetic risk assessment exercises, these differences must be taken into account as well as any possible differences in the frequencies of different types of genetic damage *within* each of these two classes.

The primary objective for the development of a specific-locus forward-mutation assay in the *ad-3* region of a two-component heterokaryon of Neurospora was the potential for determining spontaneous and induced mutation frequencies, mutagenic potency, and forward-mutational spectra at the two closely linked loci *ad-3A* and *ad-3B* (de Serres, 1991, 1992a,b). The development of this forward-mutation assay made it possible to compare such parameters from experiments with somatic cells of Neurospora with comparable experiments on germ cells in the mouse (de Serres and Brockman, 1995; de Serres and Malling, 1995), especially with regard to forward mutation at the two closely linked loci *dilute (d)* and *short-ear (se)* (Russell, 1951).

The rationale to the approach in the present report is that if chemical-induced *ad-3* mutants are qualita-

tively similar to spontaneous *ad-3* mutants and they result from qualitatively similar genetic damage(s), then there will be no quantitative differences between the mutational spectra of induced and spontaneous gene/point mutations and multilocus deletions in the *ad-3* region. Furthermore, if chemical-induced *ad-3* mutants are qualitatively similar to spontaneous *ad-3* mutants at the molecular level, and result from the same array of base-pair substitutions, deletions, etc., then there will be no quantitative differences between the numbers of complementing and non-complementing mutants at the *ad-3B* locus.

The comparisons include: (1) the spontaneous and induced frequencies of *ad-3* mutants; (2) the ratios of gene/point mutations and multilocus deletion mutations ($ad\text{-}3^{R}/[ad\text{-}3]^{IR}$ ratio); and (3) the ratios of complementing and non-complementing $ad\text{-}3B^{R}$ mutants in tests for allelic complementation ($ad\text{-}3B^{R\text{-}C}/ad\text{-}3B^{R\text{-}NC}$ ratio). These comparisons make it possible to examine the issues of whether a mutagenic chemical treatment: (1) resulted in proportional increases in the induction of specific-locus mutations at the *ad-3A* and *ad-3B* loci; and (2) induced comparable *ad-3* mutational spectra in terms of the induction of 5 major genotypic classes of gene/point mutations and multilocus deletion mutations as occurs spontaneously.

These different methods of genetic analysis have demonstrated that each of the 22 chemical-induced spectrum of *ad-3* mutants is *qualitatively different* from the spontaneous spectrum in somatic cells of Neurospora. In addition, the present data demonstrate that 93.1% (215/231) of the pairwise combinations of these 22 chemicals were *qualitatively different* from each other.

2. Materials and methods

2.1. Abbreviations

Abbreviations for the chemical mutagens discussed in this paper are as follows: AF-2, 2(2-furyl)-3-(5-nitro-2-furyl) acrylamide; AHA, 2-amino-N^6-hydroxyadenine; 2AP, 2-aminopurine; DEO, 1,2,7,8-diepoxyoctane; DEP, 1,2,4,5-diepoxypentane; EDB, ethylene dibromide; EI, ethylenimine; ENU, ethylnitrosourea; ETO, ethylene oxide; FANFT, 2-formylamino-4-(5-nitro-2-furyl) thiazole; HC, N^4-hydroxycytidine; 4HAQO, 4-hydroxyaminoquinoline 1-oxide; ICR-170, 2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl)aminopropylamino] acridine dihydrochloride; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NA, nitrous acid; 4NQO, 4-nitroquinoline 1-oxide; PDMT, 1-phenyl-3-dimethyltriazene; PMMT, 1-phenyl-3-monomethyltriazene; PROCARB, procarbazine; SQ18506, *trans*-5-amino-3-[2-(5-nitro-2-furyl)-vinyl-1,2,4-oxadiazole; SP, spontaneous; TEM, triethylenemelamine.

2.2. Strains

Heterokaryon 12 (H-12) is a two-component heterokaryon (or dikaryon) heterokaryotic for mutations at the 3 closely linked loci *ad-3A* (adenine-requiring), *ad-3B* (adenine-requiring), and *nic-2* (nicotinamide requiring) (de Serres and Malling, 1971). The *nic-2* marker is located 5–8 map units distal to *ad-3B* (de Serres, 1969). The different genotypic classes of *ad-3* mutants that can be detected with the use of two-component heterokaryons in Neurospora have been listed (de Serres, 1991, 1992a). All spontaneous or induced *ad-3* mutants recovered in experiments with this forward-mutation assay were the result of mutation in somatic cells of Component II of H-12 (strain 74-OR31-16A); thus, all *ad-3* mutants recovered in different experiments on H-12 are isogenic. Strain 74-OR31-16A has the following genetic markers: *al-2* (albino mycelium and conidia), *pan-2* (pantothenate-requiring), and *cot-1* (colonial, temperature-sensitive).

2.3. Terminology

The terms used for the different genotypic classes of *ad-3* mutants have been discussed in previous papers (e.g., de Serres, 1991). In brief, $ad\text{-}3^{R}$ designates *gene/point mutations*, and $(ad\text{-}3)^{IR}$ designates *multilocus deletion mutations*.

2.4. Source of data

The main portion of the data base used in this paper was published in de Serres (1992b). As a result

of studies on X-ray-induced *ad-3* mutants as well as those from other sources, *ad-3* mutants classified as unscorable by us in earlier studies (and omitted from the tabulations of total *ad-3* mutants analyzed) are now known to result from a variety of multiple-locus mutations in the *ad-3* and immediately adjacent genetic regions (see Table 5, de Serres, 1992a). Inclusion of these additional genotypic subclasses of *ad-3* mutants has resulted in some modification of the total numbers of *ad-3* mutants analyzed in individual experiments reported in earlier publications (summarized in de Serres, 1992a). The data utilized in the present study have been derived exclusively from computer printouts of the data base from individual experiments (Smith and de Serres, 1969) that assigns genotypes to the subclasses of *ad-3* mutants (Mutant Characterization program) as described in de Serres (1992a). In addition, 3 of the *ad-3* mutants of spontaneous origin (de Serres et al., 1995) have been reclassified from multilocus deletion mutations covering the *ad-3B* locus ($ad\text{-}3B)^{IR}$ to multiple-locus mutations ($ad\text{-}3B^{R} + RL^{CL}$). The data from each computer printout was entered into a Microsoft® Excel for Windows™ (Microsoft Corporation, Redmond, WA) spreadsheet as described in de Serres and Webber (1996).

2.5. Statistical analysis

Chi-square tests performed by using the SigmaStat™ statistical software program (version 1.03) for Windows (Jandel Scientific, San Rafael, CA).

3. Results

3.1. Evidence for quantitative differences between the spontaneous frequency of ad-3 mutations and the frequencies induced by each of the 22 chemicals

The data presented in Table 1 on the spontaneous frequency of *ad-3* mutants (see de Serres et al., 1995) and the summary data from experiments with 22 different chemical mutagens demonstrate striking *quantitative* differences between the spontaneous and maximal chemical-induced *ad-3* mutation frequencies. These differences range from a modest 15-fold increase (6.1/0.4) in the frequency of *ad-3* mutants after treatment with 2AP to a striking 5321.8-fold increase (2128.7/0.4) after treatment with ICR-170.

3.2. Determination of the numbers and percentages of spontaneous ad-3 mutations in each of the 22 chemical-induced samples

The Neurospora forward-mutation assay in the *ad-3* region of H-12 detects and identifies 3 major genotypic classes of specific-locus mutations: gene/point mutations ($ad\text{-}3^{R}$), multilocus deletion mutations ($[ad\text{-}3]^{IR}$), and unknowns $(ad\text{-}3)^{UNKN}$ (de Serres, 1991, 1992a). The numbers of *ad-3* mutants analyzed (Table 1) are the sum of all 3 major genotypic classes. Only the numbers of *ad-3* mutants in the first two genotypic classes have been included in the present analyses.

The first major genotypic class, $ad\text{-}3^{R}$, has 18 genotypic subclasses of gene/point mutations (including two subclasses of multiple-locus mutations). The second major genotypic class, $(ad\text{-}3)^{IR}$, has 12 genotypic subclasses of multilocus deletion mutations (including 1 subclass of multiple-locus mutations) as listed in Table 6 of de Serres (1992a). In addition, all 13 subclasses of $ad\text{-}3B^{R}$ mutants can be further classified with regard to the results of tests for allelic complementation. Non-complementing $ad\text{-}3B^{R}$ mutants are designated $ad\text{-}3B^{R\text{-}NC}$, whereas those showing allelic complementation (designated $ad\text{-}3B^{R\text{-}C}$) have either non-polarized complementation patterns (designated $ad\text{-}3B^{R\text{-}NP}$), or polarized complementation patterns (designated $ad\text{-}3B^{R\text{-}P}$)(de Serres, 1992a).

The total numbers of *ad-3* mutants recovered in each of the 22 chemical experiments used in the present comparisons are also given in Table 1. This tabulation was developed to present the numbers and percentages of *ad-3* mutants in each treatment series that would be expected to be *ad-3* mutants of spontaneous origin. In experiments with multiple treatment groups, the numbers and percentages of *ad-3* mutants that would be expected to be of spontaneous origin were computed for each treatment. The data were then summarized for the overall experiment. In these cases, the range of forward-mutation frequencies obtained in such experiments is also given.

These data demonstrate that the contribution of *ad-3* mutants of spontaneous origin in each of the samples of chemical-induced *ad-3* mutants is negligible (with the possible exception of PROCARB and 2AP), and would, therefore, have little or no influence on the statistical methods of comparison of spontaneous and chemical-induced *ad-3* mutants employed in the present study.

In addition, the data for EI indicate that 2.8% (5/181) resulted from multilocus deletion mutation. Data presented in a later section (see below) have resulted in this chemical being reclassified (see Table III in de Serres, 1992b) as inducing exclusively, or almost exclusively, gene/point mutations.

3.3. Comparison of the numbers and percentages of ad-3 mutants in each major genotypic class and each major subclass

The numbers and percentages of *ad-3* mutants in each major genotypic class (*ad-3*^{R}, [*ad-3*]^{IR}, and *ad-3*^{UNKN}) produced by exposure to each of the 13 chemicals that induced specific-locus mutations exclusively, or almost exclusively, by gene/point mutation are given in Table 2A–C. In each section of this table, these numbers and percentages also are compared with *ad-3* mutants of spontaneous origin.

The numbers and percentages of *ad-3* mutants in each major genotypic class (*ad-3*^{R}, [*ad-3*]^{IR}, and

Table 1
Comparison of the forward-mutation frequencies of spontaneous and chemical-induced specific-locus mutations at the *ad-3A* and *ad-3B* loci in heterokaryon 12 of *Neurospora crassa*

Expt. no.	Origin [a]	No. of treatments	No. of *ad-3* mutants analyzed [b]	Range of *ad-3* FM frequencies $\times 10^6$ survivor	*ad-3* Mutants of spontaneous origin		References
					No.	%	
Σ [c]	SP	0	172	0.4	172.0	100.0	de Serres et al. (1995)
12-004	NA	4	410	53.5– 135.8	1.9	0.5	Brockman et al. (1969); de Serres et al. (1967)
12-027	MNNG	5	950	17.5–1450.0	2.8	0.3	Malling and de Serres (1970)
12-039	TEM	5	809	22.4– 426.8	5.1	0.6	de Serres and Malling (1995)
12-058	ICR-170	6	1069	2.2–2128.7	6.0	0.6	de Serres and Malling (1994)
12-064	MMS	4	538	13.3– 366.7	7.0	1.3	Malling and de Serres (1973)
12-125	EDB	1	236	19.3	4.7	2.0	de Serres and Malling (1983)
12-163	ENU	1	221	26.0	3.3	1.5	de Serres (1983)
12-180	EI	1	181	29.4	2.4	1.3	Ong and de Serres (1973a)
12-189	PMMT	1	192	81.9	0.9	0.5	Ong and de Serres (1971, 1973b)
12-194	PDMT	3	63	12.7– 45.8	0.8	1.4	Ong and de Serres (1971, 1973b)
12-196	4NQO	1	185	49.3	1.5	0.8	Matter et al. (1972); Ong et al. (1975)
12-197	4HAQO	1	219	54.8	1.5	0.7	Matter et al. (1972); Ong et al. (1975)
12-199	DEP	1	201	87.6	0.7	0.3	Ong and de Serres (1975)
12-217	DEO	1	149	78.2	0.6	0.4	Ong and de Serres (1975)
12-264	SQ18506	1	216	90.0	1.7	0.8	Ong (1977)
12-265	FANFT	1	216	84.0	1.0	0.5	Ong (1977)
12-267	AF-2	2	264	32.4– 42.1	2.8	1.1	Ong and de Serres (1981)
12-650	2AP	1	241	6.1	15.4	6.4	de Serres and Brockman (1991)
12-683	PROCARB	1	217	5.0	16.9	7.8	Brockman and de Serres (1991)
12-733	AHA	1	273	138.3	0.4	0.1	de Serres et al. (1991)
12-758	HC	2	544	37.3– 75.4	4.2	0.8	de Serres and Brockman (1993)
12-780	ETO	2	519	22.0– 35.4	7.6	1.5	de Serres and Brockman (1995)

[a] See abbreviations in Section 2: Materials and methods.
[b] Includes the numbers of *ad-3* mutants in 3 major genotypic classes: *ad-3*^{R}, (*ad-3*)^{IR}, and *ad-3*^{UNKN}. The first two genotypic classes have been subjected to genetic analysis.
[c] Summation (Σ) of spontaneous *ad-3* mutations in experiments 12-001 through 12-094.

Table 2
Comparison of the percentages in each genotypic class and subclass among spontaneous and chemical-induced *ad-3* mutations: chemicals that induce exclusively, or almost exclusively, gene/point mutations (*ad-3*^{R})

(A) Genotypic class andsubclass	Origin of *ad-3* specific-locus mutations											
	Spontaneous Σ [a]		4NQO [b] (12-0196)		4HAQO (12-197)		PROCARB (12-683)		HC (12-758)		ICR-170 (12-058)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*	173	100.0	185	100.0	219	100.0	219	100.0	544	100.0	1072	100.0
Σ *ad-3*^{R}	145	**83.8**	185	**100.0**	219	**100.0**	210	**95.9**	540	**99.3**	1063	**99.2**
ad-3A^{R}	41	23.7	61	33.0	82	37.4	79	36.1	211	38.8	348	32.5
ad-3B^{R}	104	60.1	124	67.0	137	62.6	131	59.8	329	60.5	715	66.7
Σ (*ad-3*)^{IR}	22	**12.7**	0	**0.0**	0	**0.0**	0	0.0	0	**0.0**	4	**0.4**
(*ad-3A*)^{IR}	6	3.5	0	0.0	0	0.0	0	0.0	0	0.0	3	0.3
(*ad-3B*)^{IR}	12	6.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
(*ad-3A ad-3B*)^{IR}	4	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
(*ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1
(*ad-3A ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Σ *ad-3*^{UNKN}	6	**3.5**	0	**0.0**	0	**0.0**	9	**4.1**	4	**0.7**	5	**0.5**

(B) Genotypic class andsubclass	Origin of *ad-3* specific-locus mutations											
	Spontaneous Σ [a]		AF-2 [b] (12-267)		FANFT (12-265)		SQ18506 (12-264)		NA (12-004)		AHA (12-733)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*	173	100.0	264	100.0	216	100.0	216	100.0	410	100.0	273	100.0
Σ *ad-3*^{R}	145	**83.8**	262	**99.2**	214	**99.1**	214	**99.1**	406	**99.0**	270	**98.9**
ad-3A^{R}	41	23.7	90	34.1	60	27.8	65	30.1	86	21.0	117	42.9
ad-3B^{R}	104	60.1	172	65.2	154	71.3	149	69.0	320	78.0	153	56.0
Σ (*ad-3*)^{IR}	22	**12.7**	2	**0.8**	2	**0.9**	2	**0.9**	1	**0.2**	0	**0.0**
(*ad-3A*)^{IR}	6	3.5	1	0.4	2	0.9	0	0.0	0	0.0	0	0.0
(*ad-3B*)^{IR}	12	6.9	1	0.4	0	0.0	0	0.0	0	0.0	0	0.0
(*ad-3A ad-3B*)^{IR}	4	2.3	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0
(*ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
(*ad-3A ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	2	0.9	0	0.0	0	0.0
Σ *ad-3*^{UNKN}	6	**3.5**	0	**0.0**	0	**0.0**	0	**0.0**	3	**0.7**	3	**1.1**

(C) Genotypic class and subclass	Origin of *ad-3* specific-locus mutations							
	Spontaneous Σ [a]		ENU [b] (12-163)		MNNG (12-027)		EI (12-180)	
	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*	173	100.0	221	100.0	951	100.0	181	100.0
Σ *ad-3*^{R}	145	**83.8**	218	**98.6**	935	**98.3**	175	**96.7**
ad-3A^{R}	41	23.7	76	34.4	180	18.9	60	33.1
ad-3B^{R}	104	60.1	142	64.3	755	79.4	115	63.5
Σ (*ad-3*)^{IR}	22	**12.7**	1	**0.5**	2	**0.2**	5	**2.8**
(*ad-3A*)^{IR}	6	3.5	0	0.0	2	0.2	1	0.6
(*ad-3B*)^{IR}	12	6.9	0	0.0	0	0.0	0	0.0
(*ad-3A ad-3B*)^{IR}	4	2.3	1	0.5	0	0.0	3	1.7
(*ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	0	0.0
(*ad-3A ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	1	0.6
Σ *ad-3*^{UNKN}	6	**3.5**	2	0.9	14	**1.5**	1	**0.6**

[a] Summation (Σ) of spontaneous *ad-3* mutations in experiments 12-001 through 12-094.
[b] See abbreviations in Section 2: Materials and methods.

ad-3^{UNKN}) produced by each of the 9 chemicals that induced specific-locus mutations by gene/point mutation and multilocus deletion mutation are given in Table 3A,B. In both sections of this table, the spontaneous data also are presented or comparison.

3.4. Heterogeneity tests and χ^2-tests on the 13 chemicals that induce ad-3 mutants exclusively, or almost exclusively, from gene/point mutation and ad-3 mutants occurring spontaneously

In the first method of comparison, the numbers of spontaneous and chemical-induced *ad-3* mutants were tabulated (Table 4) to determine the ratios of gene/point mutations (*ad-3A*^{R}, and *ad-3B*^{R}) to multilocus deletion mutations ([*ad-3A*]^{IR}, [*ad-3B*]^{IR} and ([*ad-3A ad-3B*]^{IR}). To permit the use of the χ^2-method of analysis, the 5 genotypic classes of [*ad-3*]^{IR} mutations were reduced to 3 as discussed in de Serres and Webber (1996). This procedure eliminates those genotypic classes with no [*ad-3*]^{IR} mutants in the spontaneous or chemical-induced samples.

A heterogeneity test, with $\alpha = 0.05$, on the data for the numbers of gene/point mutations (*ad-3A*^{R} and *ad-3B*^{R}) and multilocus deletion mutations ([*ad-3A*]^{IR}, [*ad-3B*]^{IR}, and [*ad-3A ad-3B*]^{IR}) induced by

Table 3
Comparison of the percentages in each genotypic class and subclass among spontaneous and chemical-induced *ad-3* mutations: chemicals that induce gene/point mutations (*ad-3*^{R}) and multilocus deletion mutations ([*ad-3*]^{IR})

(A) Genotypic class and subclass	Origin of *ad-3* specific-locus mutations									
	Spontaneous Σ [a]		ETO [b] (12-780)		TEM (12-039)		MMS (12-064)		PMMT (12-189)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*	172	100.0	519	100.0	826	100.0	538	100.0	198	100.0
Σ *ad-3*^{R}	145	**83.7**	503	**96.9**	796	**96.7**	509	**94.6**	185	**93.4**
ad-3A^R	41	23.8	147	28.3	400	48.6	186	34.6	58	29.3
ad-3B^R	104	59.9	356	68.6	396	48.1	323	60.0	127	64.1
Σ (*ad-3*)^{IR}	22	**12.8**	16	**3.1**	27	**3.3**	26	**4.8**	10	**5.1**
(*ad-3A*)^{IR}	6	3.5	4	0.8	16	1.9	3	0.6	3	1.5
(*ad-3B*)^{IR}	12	7.0	3	0.6	2	0.2	14	2.6	1	0.5
(*ad-3A ad-3B*)^{IR}	4	2.3	9	1.7	6	0.7	8	1.5	0	0.0
(*ad-3A ad-3B nic-2*)^{IR}	0	0.0	0	0.0	1	0.1	1	0.2	6	3.0
(*ad-3B nic-2*)^{IR}	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0
Σ *ad-3*^{UNKN}	6	**3.5**	0	**0.0**	3	**0.4**	3	**0.6**	3	**1.5**

(B) Genotypic class and subclass	Origin of *ad-3* specific-locus mutations											
	Spontaneous Σ [a]		DEP [b] (12-199)		2AP (12-650)		EDB (12-125)		PDMT (12-194)		DEO (12-217)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*	172	100.0	202	100.0	242	100.0	236	100.0	66	100.0	155	100.0
Σ *ad-3*^{R}	145	**83.7**	188	**93.1**	204	**84.3**	190	**80.5**	52	**78.8**	93	**60.0**
ad-3A^R	41	23.8	68	33.7	66	27.3	60	25.4	28	42.4	40	25.8
ad-3B^R	104	59.9	120	59.4	138	57.0	130	55.1	24	36.4	53	34.2
Σ (*ad-3*)^{IR}	22	**12.8**	14	**6.9**	28	**11.6**	41	**17.4**	12	**18.2**	61	**39.4**
(*ad-3A*)^{IR}	6	3.5	4	2.0	16	6.6	9	3.8	1	1.5	9	5.8
(*ad-3B*)^{IR}	12	7.0	1	0.5	11	4.5	6	2.5	1	1.5	22	14.2
(*ad-3A ad-3B*)^{IR}	4	2.3	8	4.0	1	0.4	22	9.3	7	10.6	20	12.9
(*ad-3B nic-2*)^{IR}	0	0.0	1	0.5	0	0.0	0	0.0	1	1.5	3	1.9
(*ad-3A ad-3B nic-2*)^{IR}	0	0.0	0	0.0	0	0.0	4	1.7	2	3.0	7	4.5
Σ *ad-3*^{UNKN}	6	**3.5**	0	**0.0**	10	**4.1**	5	**2.1**	2	**3.2**	1	**0.6**

[a] Summation (Σ) of spontaneous *ad-3* mutations in experiments 12-001 through 12-094.
[b] See abbreviations in Section 2: Materials and methods.

each of the 13 chemicals that induce exclusively, or almost exclusively, gene/point mutations and those occurring spontaneously in each of these 5 genotypic classes gave a χ^2-value of 584.8 with 52 degrees of freedom with $p = < 0.0001$. These statistical analyses demonstrated highly significant heterogeneity in the overall data base.

To determine the basis for this heterogeneity and to determine whether there are significant differences between the spontaneous and chemical-induced *ad-3* mutational spectra, the numbers of *ad-3* mutants in each of these 5 genotypic classes obtained from each of the 13 chemical treatments were compared with those occurring spontaneously (Table 4).

The results of χ^2-tests for pairwise comparisons with the spontaneous distribution of *ad-3* mutants in each of these 5 genotypic classes gave the *p*-values listed in the last column.

The *ad-3*^{R}/[*ad-3*]^{IR} ratios in the 13 chemical-induced samples range from α (for those with no multilocus deletion mutations) to 467.5 (for MNNG-induced 31.43 *ad-3* mutants) as compared with 6.59 for *ad-3* mutants of spontaneous origin. The distribution of the numbers of *ad-3* mutants in each of these 5 genotypic classes was significantly different from the spontaneous distribution for all 13 chemicals. Statistically significant differences in these numbers are indicated in bold face type. The *p*-values for 1 of the 13 chemicals was < 0.001 and the other 12 were < 0.001.

These comparisons provided the first demonstration of quantitative differences between the spontaneous and induced *ad-3* mutational spectra that result from *qualitative differences* between the types of genetic damage resulting in the spontaneous and chemical-induced specific-locus mutations in the *ad-3* region.

3.5. Heterogeneity tests and χ^2-tests on the 9 chemicals that induce ad-3 mutants by gene/point mutation and multilocus deletion mutation and ad-3 mutants occurring spontaneously

A heterogeneity test, with $\alpha = 0.05$, on the data for the numbers of gene/point mutations (*ad-3A*^{R} and *ad-3B*^{R}) and multilocus deletion mutations ([*ad-3A*]^{IR}, [*ad-3B*]^{IR} and [*ad-3A ad-3B*]^{IR}) induced by the 9 chemicals that induce both of these 5 major genotypic classes (Table 5) and those occurring spontaneously gave a χ^2-value of 508.8 with 36

Table 4
Comparison of the numbers of *ad-3* mutants among spontaneous and chemical-induced specific-locus mutations: chemicals that induce *ad-3* mutants exclusively or, almost exclusively, by gene/point mutation (*ad-3*^{R})

Expt. no.	Origin [a]	Gene/point mutations		Multilocus deletion mutations			*ad-3*^{R}/[*ad-3*]^{IR} ratio	*p*-value
		ad-3A^R	*ad-3B*^{R}	(*ad-3A*)^{IR}	(*ad-3B*)^{IR}	(*ad-3A ad-3B*)^{IR}		
Σ [b]	SP	41	104	6	12	4	6.59	N.A.
12-180	EI	60	115	1	0	4	35.0	**< 0.001**
12-265	FANFT	60	154	2	0	0	107.0	**≪ 0.001**
12-264	SQ18506	65	149	0	0	2	117.5	**≪ 0.001**
12-267	AF-2	90	172	1	1	0	131.0	**≪ 0.001**
12-163	ENU	76	142	0	0	1	218.0	**≪ 0.001**
12-058	ICR-170	348	715	3	1	0	265.8	**≪ 0.001**
12-004	NA	86	320	0	0	1	406.0	**≪ 0.001**
12-027	MNNG	180	755	2	0	0	467.5	**≪ 0.001**
12-196	4NQO	61	124	0	0	0	α	**≪ 0.001**
12-197	4HAQO	82	137	0	0	0	α	**≪ 0.001**
12-683	PROCARB	79	131	0	0	0	α	**≪ 0.001**
12-733	AHA	117	153	0	0	0	α	**≪ 0.001**
12-758	HC	211	329	0	0	0	α	**≪ 0.001**

[a] See abbreviations in Section 2: Materials and methods.
[b] Summation (Σ) of spontaneous *ad-3* mutants in experiments 12-001 through 12-094.

degrees of freedom and a p-value of < 0.0001. These heterogeneity tests demonstrate significant quantitative differences among the numbers of *ad-3* mutants in these 5 major genotypic classes, namely two genotypic classes of gene/point mutations and 3 genotypic classes of multilocus deletion mutations.

To determine the basis for this heterogeneity and to determine whether there are significant differences between the numbers of spontaneous and chemical-induced *ad-3* mutants in these 5 major genotypic classes, each of the 9 chemical-induced *ad-3* mutational spectra was compared with that occurring spontaneously.

The 9 chemicals that induced *ad-3* mutants by both gene/point mutation and multilocus deletion mutation are listed in Table 5. The *ad-3*^{R}/[*ad-3*]^{IR} ratios in the 9 chemical-induced samples range from 1.52 to 31.43 as compared with 6.59 for *ad-3* mutants of spontaneous origin. The results of χ^2-tests for pairwise comparisons with the spontaneous distribution of *ad-3* mutants in each of these 5 genotypic classes gave the p-values listed in the last column. The *ad-3*^{R} and [*ad-3*]^{IR} distribution for only 1 of these 9 chemically induced samples, namely 2AP, was not significantly different from the spontaneous distribution ($p = 0.159$). The p-values of the remaining 9 chemicals in bold face type were 0.016 or lower.

These comparisons provided the second demonstration of quantitative differences between the spontaneous and induced *ad-3* mutational spectra that result from *qualitative differences* between the types of genetic damage resulting in the spontaneous and chemical-induced specific-locus mutations in the *ad-3* region.

3.6. Heterogeneity tests on the numbers of ad-3B^{R} mutants showing allelic complementation among spontaneous and chemical-induced ad-3^{R} mutants

Previous studies on allelic complementation and specific revertibility of *ad-3B*^{R} mutants have demonstrated (see de Serres, 1992a,b) that if chemical-induced *ad-3* mutants are qualitatively similar to spontaneous *ad-3* mutants at the molecular level, and result from the same array of base-pair substitution or frame-shift mutations, or intralocal deletions, etc., then, there will be no quantitative differences between the numbers of complementing and non-complementing mutants at the *ad-3B* locus.

A heterogeneity test, with $\alpha = 0.05$, on the numbers (Table 6) of *ad-3B*^{R} mutants showing allelic complementation and of non-complementing mutants among a spontaneous sample and 22 chemical-induced samples gave a χ^2-value of 938.5 with 22 degrees of freedom and a p-value of < 0.0001. A heterogeneity test on the 22 chemicals alone gave a χ^2-value of 936.9 with 21 degrees of freedom and

Table 5
Comparison of the numbers of *ad-3* mutants among spontaneous and chemical-induced specific-locus mutations: Chemicals that induce *ad-3* mutants by both gene/point mutation (*ad-3*^{R}) and multilocus deletion mutation ([*ad-3*]^{IR})

Expt. no.	Origin [a]	Gene/point mutations		Multilocus deletion mutations			*ad-3*^{R}/[*ad-3*]^{IR} ratio	*p*-value
		ad-3A^{R}	*ad-3B*^{R}	(*ad-3A*)^{IR}	(*ad-3B*)^{IR}	(*ad-3A ad-3B*)^{IR}		
Σ [b]	SP	41	104	6	12	4	6.59	N.A.
12-039	TEM	400	396	16	4	7	29.48	**$\ll 0.001$**
12-064	MMS	186	323	3	14	9	19.58	**< 0.001**
12-125	EDB	60	130	9	6	26	4.63	**0.004**
12-189	PMMT	58	127	3	1	6	18.50	**0.008**
12-194	PDMT	28	24	1	2	9	4.33	**$\ll 0.001$**
12-199	DEP	68	120	4	2	8	13.43	**0.009**
12-217	DEO	40	53	9	25	27	1.52	**$\ll 0.001$**
12-650	2AP	66	138	16	11	1	7.29	0.159
12-780	ETO	147	356	4	3	9	31.43	**$\ll 0.001$**

[a] See abbreviations in Section 2: Materials and methods.
[b] Summation (Σ) of spontaneous *ad-3* mutants in experiments 12-001 through 12-094.

also a p-value of < 0.0001. This heterogeneity test demonstrates significant quantitative differences among chemical-induced *ad-3B*^{R} mutants.

In Table 6, the percentages found for complementing (*ad-3B*$^{R\text{-}C}$) mutants with either non-polarized or polarized complementation patterns, and the percentages of non-complementing (*ad-3B*$^{R\text{-}NC}$) mutants are listed for *ad-3B*^{R} mutants of spontaneous origin and those induced by each of the 2822 chemical mutagens. These data have been tabulated with the percentages of non-complementing *ad-3B*^{R} mutants with non-polarized complementation patterns in ascending order. To determine the basis for the heterogeneity among these 22 samples of chemical-induced *ad-3B*^{R} mutants, each sample was compared with the spontaneous *ad-3B*^{R} mutation sample. The results of the χ^2-tests on the pairwise comparisons with the spontaneous *ad-3B*^{R} mutants gave the p-values listed in the last column. Statistically significant differences in these numbers are indicated in bold face type and 11 out of 22 comparisons demonstrate *qualitative differences* between the types of genetic damage at the molecular level resulting in the spontaneous and chemical-induced samples of *ad-3B*^{R} mutants.

This tabulation demonstrates that 15 of these

Table 6
Comparison of the numbers and percentages of spontaneous and chemical-induced non-complementing and complementing *ad-3B*^{R} mutants

Expt. no.	Origin [a]	Genotype [b,c]						*ad-3B*$^{R\text{-}C}$/*ad-3B*$^{R\text{-}NC}$ ratio	p-values
		$\sum$*ad-3B*^{R}		$\sum$*ad-3B*$^{R\text{-}C}$		$\sum$*ad-3B*$^{R\text{-}NC}$			
		No.	%	No.	%	No.	%		
$\sum$ [d]	Spontaneous	104	100.0	56	53.8	48	46.2	1.17	N.A.
12-758	HC	329	100.0	328	99.7	1	0.3	328.00	**$\ll$ 0.001**
12-733	AHA	153	100.0	139	90.8	14	9.2	9.93	**$\ll$ 0.001**
12-027	MNNG	755	100.0	618	81.9	137	18.1	4.51	**$\ll$ 0.001**
12-264	SQ18506	149	100.0	117	78.5	32	21.5	3.66	**$\ll$ 0.001**
12-683	PROCARB	131	100.0	99	75.6	32	24.4	3.09	**$\ll$ 0.001**
12-004	NA	320	100.0	239	74.7	81	25.3	2.95	**$\ll$ 0.001**
12-650	2AP	138	100.0	99	71.7	39	28.3	2.54	**0.006**
12-265	FANFT	154	100.0	107	69.5	47	30.5	2.28	**0.015**
12-197	4HAQO	137	100.0	93	67.9	44	32.1	2.11	**0.037**
12-189	PMMT	127	100.0	83	65.4	44	34.6	1.89	0.101
12-163	ENU	142	100.0	91	64.1	51	35.9	1.78	0.137
12-196	4NQO	124	100.0	78	62.9	46	37.1	1.70	0.212
12-039	TEM	396	100.0	247	62.4	149	37.6	1.66	0.141
12-217	DEO	53	100.0	30	56.6	23	43.4	1.30	0.874
12-780	ETO	356	100.0	194	54.5	162	45.5	1.20	0.996
12-064	MMS	323	100.0	170	52.6	153	47.4	1.11	0.918
12-199	DEP	120	100.0	62	51.7	58	48.3	1.07	0.848
12-267	AF-2	172	100.0	87	50.6	85	49.4	1.02	0.688
12-125	EDB	130	100.0	63	48.5	67	51.5	0.94	0.492
12-180	EI	115	100.0	47	40.9	68	59.1	0.69	0.074
12-058	ICR-170	715	100.0	183	25.6	534	74.7	0.34	**$\ll$ 0.001**
12-194	PDMT	24	100.0	5	20.8	19	79.2	0.26	**0.007**

[a] For abbreviations see Section 2: Materials and methods.
[b] Summation of *ad-3B*^{R} mutants in the following 13 genotypic classes: (1) *ad-3B*^{R}, (2) *ad-3B*^{R} + *RL*^{CL}, (3) *ad-3A*^{R} + *ad-3B*^{R}, (4) *ad-3A*^{R} + *ad-3B*^{R} + *RL*^{CL}, (5) *ad-3A*^{R} + *ad-3B*^{R} + *nic-2*^{R}, (6) *ad-3A*^{R} + *ad-3B*^{R} + *nic-2*^{R} + *RL*^{CL}, (7) *ad-3B*^{R} + *nic-2*^{R,} (8) *ad-3B*^{R} + *nic-2*^{R} + *RL*^{CL}, (9) *ad-3B*^{R} + *RL*, (10) *ad-3A*^{R} + *ad-3B*^{R} + *RL*, (11) *ad-3A*^{R} + *ad-3B*^{R} + *nic-2*^{R} + *RL*, (12) *ad-3B*^{R} + *nic-2*^{R} + *RL, and* (13) *(ad-3A)*^{IR} + *ad-3B*^{R}.
[c] Complementing *ad-3B*^{R} mutants = *ad-3B*$^{R\text{-}C}$ (includes both non-polarized, complementing *ad-3B*^{R} mutants = *ad-3B*$^{R\text{-}NP}$; and polarized, complementing *ad-3B*^{R} mutants = *ad-3B*$^{R\text{-}P}$); non-complementing *ad-3B*^{R} mutants = *ad-3B*$^{R\text{-}NC}$.
[d] Summation ($\sum$) of all data on *ad-3B*^{R} mutants in experiments 12-001 through 12-094

Table 7
p-values in all possible pairwise comparisons of the numbers of *ad-3* mutants among 13 chemicals that induced specific-locus mutations predominantly, or exclusively, by gene/point mutation (*ad-3*^{R})

Expt. no.	Origin	*ad-3B*^{R}/*ad-3A*^{R} ratio	*p*-value												
			12-733	12-758	12-197	12-683	12-163	12-267	12-180	12-196	12-058	12-264	12-265	12-004	12-027
12-733	AHA	1.31	–	0.277	0.220	0.242	0.070	**0.042**	0.071	**0.034**	**0.001**	**0.042**	**< 0.001**	**≪ 0.001**	**≪ 0.001**
12-758	HC	1.56	0.277	–	0.737	0.777	0.318	0.223	0.296	0.164	**0.014**	0.223	**0.006**	**≪ 0.001**	**≪ 0.001**
12-197	4HAQO	1.67	0.220	0.737	–	0.951	0.644	0.543	0.587	0.406	0.206	0.543	0.048	**≪ 0.001**	**≪ 0.001**
12-683	PROCARB	1.67	0.242	0.777	0.951	–	0.622	0.522	0.568	0.391	0.197	**0.522**	**0.046**	**≪ 0.001**	**≪ 0.001**
12-163	ENU	1.86	0.070	0.318	0.644	0.622	–	0.983	0.990	0.769	0.597	0.983	0.155	**≪ 0.001**	**≪ 0.001**
12-267	AF-2	1.91	**0.042**	0.223	0.543	0.522	0.983	–	0.929	0.840	0.672	0.927	0.169	**≪ 0.001**	≪ 0.001
12-180	EI	1.92	0.071	0.296	0.587	0.568	0.990	0.929	–	0.879	0.751	0.929	0.224	**0.001**	**≪ 0.001**
12-196	4NQO	2.03	**0.034**	0.164	0.406	0.391	0.769	0.840	0.879	–	0.983	0.840	0.337	**0.003**	**≪ 0.001**
12-058	ICR-170	2.07	**0.001**	**0.014**	0.206	0.197	0.597	0.672	0.751	0.983	–	0.672	0.206	**≪ 0.001**	**≪ 0.001**
12-264	SQ18506	2.25	**0.042**	0.223	0.543	0.522	0.983	0.927	0.929	0.840	0.672	–	0.169	**< 0.001**	**≪ 0.001**
12-265	FANFT	2.53	**< 0.001**	**0.006**	0.048	**0.046**	0.155	0.169	0.224	0.337	0.206	0.169	–	0.070	**0.006**
12-004	NA	3.68	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**0.001**	**0.003**	**≪ 0.001**	**< 0.001**	0.070	–	0.459
12-027	MNNG	4.23	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.00 1**	**0.006**	0.459	–

chemicals have induced *ad-3B*^{R} mutants with lower percentages of non-complementing mutants than *ad-3B*^{R} mutants of spontaneous origin. In addition, another 7 chemicals have induced *ad-3B*^{R} mutants with higher percentages of non-polarized patterns than *ad-3B*^{R} mutations of spontaneous origin and that another 9 chemicals have lower percentages of non-polarized pattern percentages of non-complementing mutants than *ad-3B*^{R} mutants of spontaneous origin. These comparisons demonstrate that the percentages of HC, AHA, MNNG, SQ18506, PROCARB, NA, 2AP, FANFT, 4HAQO, ICR-170, and percentages of non-polarized patterns in *ad-3B*^{R} mutation PDMT-induced *ad-3B*^{R} mutants showing allelic complementation are significantly different ($p < 0.05$) from those of *ad-3B*^{R} mutants of spontaneous origin. These data permit us to conclude that the array of genetic damage at the molecular level in using the criterion 50.0% (11/22) of the percentages of *ad-3B*^{R} mutations showing allelic complementation that chemical-induced *ad-3B*^{R} mutants is *qualitatively different* from *ad-3B*^{R} mutants occurring spontaneously.

These comparisons provided the third demonstration of quantitative differences between the spontaneous and induced *ad-3* mutational spectra that result from *qualitative differences* between the types of genetic damage resulting in the spontaneous and chemical-induced specific-locus mutations in the *ad-3* region.

3.7. Heterogeneity tests and χ^2-tests on the 13 chemicals that induce ad-3 mutants resulting exclusively, or almost exclusively, from gene / point mutation

The final issue to be investigated is whether chemical-induced samples of *ad-3* mutants are different from each other. This involves performing χ^2-tests on all possible pairwise combinations of the numbers of *ad-3* mutants in each of these 22 chemical-induced samples.

A heterogeneity test on the 13 chemical-induced samples of *ad-3* mutants resulting exclusively, or almost exclusively, from gene/point mutation, alone, gave a χ^2-value of 215.5 with 48 degrees of freedom with $p = < 0.0001$. These statistical analyses demonstrated highly significant heterogeneity in the overall data base.

To permit the use of the χ^2-method of analysis, this exercise was performed in 3 separate matrices since many of these 13 chemical-induced samples of *ad-3* mutants resulting exclusively, or almost exclusively, from gene/point mutation (Table 4) have no [*ad-3*]^{IR} mutants in 1 or more of the 3 summary genotypic classes. To perform χ^2-tests on all possible pairwise combinations of this group of 13 chemical-induced *ad-3* mutants, the numbers in each of the 3 genotypic classes of [*ad-3*]^{IR} mutations were eliminated.

To determine the basis for this heterogeneity and

Table 8
p-values in comparison of the distribution of the numbers of *ad-3* mutants among chemicals that induce *ad-3* mutants by both gene/point mutation (*ad-3*^{R})and multilocus deletion mutation ([*ad-3*]^{IR})

Expt. no.	*ad-3B*^{R}/ *ad-3A*^{R} ratio	Origin [a]	*p*-value								
			12-194	12-039	12-217	12-064	12-199	12-650	12-125	12-189	12-780
12-194	0.84	PDMT	–	**≪ 0.001**	**0.011**	**≪ 0.001**	**0.005**	**≪ 0.001**	**0.043**	**≪ 0.001**	**≪ 0.001**
12-039	0.99	TEM	**≪ 0.001**	–	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-217	1.38	DEO	**0.011**	**≪ 0.001**	–	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-064	1.74	MMS	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	–	0.082	**≪ 0.001**	**≪ 0.001**	**0.030**	**0.010**
12-199	1.78	DEP	**0.005**	**≪ 0.001**	**≪ 0.001**	0.082	–	**0.001**	**0.014**	0.519	0.073
12-650	2.08	2AP	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.001**	–	**≪ 0.001**	**0.001**	**≪ 0.001**
12-125	2.17	EDB	**0.043**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.014**	**≪ 0.001**	–	**0.006**	**≪ 0.001**
12-189	2.28	PMMT	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.030**	0.519	**0.001**	**0.006**	–	0.572
12-780	2.42	ETO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.010**	0.073	**≪ 0.001**	**≪ 0.001**	0.572	–

[a] See abbreviations in Section 2: Materials and methods.

to determine whether there are significant differences between the numbers of chemical-induced *ad-3* mutants in the two genotypic subclasses, namely *ad-3A*^{R} and *ad-3B*^{R}, each of the 13 chemical-induced *ad-3* mutational spectra was compared with all others in all possible pairwise combinations. The results of these χ^2-tests are given in Table 7 and are tabulated as a function of the *ad-3B*^{R}/*ad-3A*^{R} ratio. Statistically significant differences in these numbers are indicated in bold face type. These data demonstrate that 38.5% (30/78) of all possible pairwise combinations) are significantly different from each other with 66.7% (20/30) having *p*-values of 0.001 or lower.

3.8. Heterogeneity tests and χ^2-tests on the 9 chemicals that induce ad-3 mutants by gene / point mutation and multilocus deletion mutation

The next series of χ^2-tests were performed on all possible pairwise combinations of the 9 chemicals (Table 5) that induce *ad-3* mutants both by gene/point mutation and multilocus deletion mutation.

A heterogeneity test, with $\alpha = 0.05$, on these 9 chemicals gave a χ^2-value of 499.7 with 32 degrees of freedom and a *p*-value of < 0.0001. These heterogeneity tests demonstrate significant quantitative differences among the numbers of *ad-3* mutants in these 5 major genotypic classes, namely gene/point mutation and multilocus deletion mutation.

To determine the basis for this heterogeneity and to determine whether there are significant differences between the numbers of chemical-induced *ad-3* mutants in these 5 major genotypic classes, each of the 9 chemical-induced *ad-3* mutational spectra were compared with each other in all possible pairwise combinations. The results of these χ^2-tests are given in Table 8. Statistically significant differences in these numbers are indicated in bold face type. They demonstrate that 88.9% (32/36 of all possible pairwise combinations) are significantly different from each other and 78.1% (25/32) exhibit *p*-values of 0.001 or lower.

3.9. Chi-square tests on the 13 chemical-induced ad-3 mutants resulting exclusively, or almost exclusively, from gene / point mutation and the 9 chemicals that induce ad-3 mutants by gene / point mutation and multilocus deletion mutation

The final comparison (Table 9) involved χ^2-tests on all possible pairwise combinations of the 13 chemicals that induced *ad-3* mutants exclusively, or almost exclusively, from gene/point mutation and

Table 9
p-values in all possible pairwise comparisons of the distribution of the numbers of *ad-3* mutants among 13 chemicals that induced specific-locus mutations predominantly, or exclusively, by gene/point mutation (*ad-3*^{R}) as compared with 9 chemicals the induced specific-locus mutations by both gene/point mutation (*ad-3*^{R}) and multilocus deletion mutation ([ad-3]^{IR})

Expt. no.	Origin [a]	*p*-value								
		12-194	12-039	12-217	12-064	12-199	12-650	12-125	12-189	12-780
12-733	AHA	**≪ 0.001**	**0.011**	**≪ 0.001**	**0.002**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-758	HC	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-197	4HAQO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.026**	**0.003**	**≪ 0.001**	**≪ 0.001**	**0.002**	**0.020**
12-683	PROCARB	**≪ 0.001**	**0.001**	**≪ 0.001**	**0.031**	**0.004**	**≪ 0.001**	**≪ 0.001**	**0.003**	**0.021**
12-163	ENU	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	0.056	**0.011**	**≪ 0.001**	**≪ 0.001**	**0.007**	0.126
12-180	EI	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	0.260	0.344	**≪ 0.001**	**≪ 0.001**	0.382	0.580
12-267	AF-2	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.041**	**0.006**	**≪ 0.001**	**≪ 0.001**	**0.010**	0.118
12-196	4NQO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.039**	**0.008**	**≪ 0.001**	**≪ 0.001**	**0.019**	0.150
12-058	ICR-170	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-264	SQ18506	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.041**	**0.006**	**≪ 0.001**	**≪ 0.001**	**0.010**	**0.118**
12-265	FANFT	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.005**	**0.005**	**≪ 0.001**	**≪ 0.001**	0.059	0.266
12-004	NA	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.001**
12-027	MNNG	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**

[a] See abbreviations in Section 2: Materials and methods.

Table 10
p-values in all possible pairwise comparisons of the distribution of the numbers of non-complementing and complementing *ad-3B*^{R} mutants among 22 chemical-induced samples

Expt. no.	Origin [a]	*p*-value									
		12-758	12-733	12-027	12-264	12-683	12-004	12-650	12-265	12-197	12-189
12-758	HC	–	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-733	AHA	**≪ 0.001**	–	**0.009**	**0.005**	**0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-027	MNNG	**≪ 0.001**	**0.009**	–	0.402	0.117	**0.010**	**0.009**	**0.001**	**< 0.001**	≪ 0.001
12-264	SQ18506	**≪ 0.001**	**0.005**	0.402	–	0.657	0.430	0.233	0.097	0.057	**0.021**
12-683	PROCARB	**≪ 0.001**	**0.001**	0.117	0.657	–	0.938	0.566	0.311	0.311	0.096
12-004	NA	**≪ 0.001**	**≪ 0.001**	**0.010**	0.430	0.938	–	0.587	0.278	0.167	0.062
12-650	2AP	**≪ 0.001**	**≪ 0.001**	**0.009**	0.233	0.566	0.587	–	0.769	0.572	0.324
12-265	FANFT	**≪ 0.001**	**≪ 0.001**	**0.001**	0.097	0.311	0.278	0.769	–	0.868	0.544
12-197	4HAQO	**< 0.001**	**≪ 0.001**	**< 0.001**	0.057	0.208	0.167	0.572	0.868	–	0.761
12-189	PMMT	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.021**	0.096	0.062	0.324	0.544	0.761	–
12-163	ENU	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.009**	0.054	**0.027**	0.214	0.389	0.587	0.929
12-039	TEM	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**0.008**	**< 0.001**	0.061	0.143	0.292	0.617
12 – 196	4NQO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.040**	**0.040**	**0.019**	0.164	0.304	0.475	0.785
12-217	DEO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.018**	**0.018**	**0.011**	0.068	0.123	0.197	0.348
12-780	ETO	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**0.002**	**0.009**	**0.043**
12-064	MMS	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.019**
12-199	DEP	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.001**	**0.004**	**0.012**	**0.040**
12-267	AF-2	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**< 0.001**	**0.003**	**0.015**
12-125	EDB	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**	**< 0.001**	**0.002**	**0.009**
12-180	EI	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< 0.001**
12-058	ICR-170	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
12-194	PDMT	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**

[a] See abbreviations in Section 2: Materials and methods.

the 9 chemicals that induced *ad-3* mutants both by gene/point mutation and multilocus deletion mutation.

A heterogeneity test, with $\alpha = 0.05$, on all of the 22 chemicals gave a χ^2-value of 1611.2 with 84 degrees of freedom and a *p*-value of < 0.0001. This heterogeneity test demonstrates, as expected, highly significant quantitative differences among the numbers of *ad-3* mutants in these 5 major genotypic classes, namely gene/point mutation and multilocus deletion mutation.

The results of χ^2-tests on all possible pairwise combinations of these 22 samples of chemical-induced *ad-3* mutants are given in Table 10. Statistically significant differences in these numbers are indicated in bold face type. These data demonstrate that 90.6% (106/117) of all possible pairwise combinations) were significantly different from each other. The majority (63.2% (67/102) of the pairwise combinations) had *p*-values of 0.001 or lower.

4. Discussion

The *p*-values for comparisons of *ad-3* mutants occurring spontaneously and those induced by 22 different chemical treatments have been determined for each the following: (1) the numbers of mutants contributing to the ratios of gene/point mutations and multilocus deletion mutations (*ad-3*^{R}/[*ad-3*]^{IR} ratio); and (2) the numbers of mutants contributing to the ratio of complementing to non-complementing *ad-3B*^{R} mutants showing allelic complementation (*ad-3B*$^{R\text{-}C}$/*ad-3B*$^{R\text{-}NC}$ ratio).

The total data base developed in the present χ^2-tests is summarized in Figs. 1–3. The larger the number of '+' responses and the darker the shading, the greater the difference in each comparison.

In Fig. 1, the combined data base is given for all possible pairwise combinations of the 13 chemicals that induced exclusively, or almost exclusively, gene/point mutations as well as comparisons of

12-163	12-039	12-196	12-217	12-780	12-064	12-199	12-267	12-125	12-180	12-058	12-194
≪ 0.001	**≪ 0.001**	≪ 0.001	≪ 0.001	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
≪ 0.001	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
≪ 0.001	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.009	**< 0.001**	**0.040**	**0.018**	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.054	**0.008**	**0.040**	**0.018**	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.027	**< 0.001**	**0.019**	**0.011**	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.214	0.061	0.164	0.068	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.389	0.143	0.304	0.123	**0.002**	**< 0.001**	**0.004**	**< 0.001**	**< 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.587	0.292	0.475	0.197	**0.009**	**0.004**	**0.012**	**0.003**	**0.002**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.929	0.617	0.785	0.348	**0.043**	**0.019**	**0.040**	**0.015**	**0.009**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
–	0.794	0.943	0.428	0.064	**0.029**	0.057	**0.022**	**0.013**	**≪ 0.001**	**≪ 0.001**	**< 0.001**
0.794	–	1.000	0.509	**0.034**	**0.011**	0.047	**0.011**	**0.007**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**
0.943	1.000	–	0.536	0.128	0.064	0.100	**0.047**	**0.029**	**< 0.001**	**≪ 0.001**	**< 0.001**
0.428	0.509	0.536	–	0.889	0.698	0.664	0.542	0.403	0.083	**≪ 0.001**	**0.008**
0.064	**0.034**	0.128	0.889	–	0.683	0.666	0.452	0.282	**0.015**	**≪ 0.001**	**0.003**
0.029	**0.011**	**0.064**	0.698	0.683	–	0.941	0.734	0.484	**0.040**	**≪ 0.001**	**0.005**
0.057	0.047	0.100	0.664	0.666	0.941	–	0.949	0.704	0.126	≪ 0.001	0.011
0.022	**0.011**	**0.047**	0.542	0.452	0.734	0.949	–	0.804	0.135	**≪ 0.001**	**0.012**
0.013	**0.007**	**0.029**	0.403	0.282	0.484	0.704	**0.804**	–	**0.288**	**≪ 0.001**	**0.023**
< 0.001	**≪ 0.001**	**< 0.001**	0.083	**0.015**	**0.040**	0.126	0.135	0.288	–	**0.001**	0.107
≪ 0.001	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**< < 0.001**	**< < 0.001**	**≪ 0.001**	**≪ 0.001**	**≪ 0.001**	**0.001**	–	0.779
< 0.001	**≪ 0.001**	**< 0.001**	**0.008**	**0.003**	**0.005**	**0.011**	**0.012**	**0.023**	0.107	0.779	–

these 13 chemical-induced mutations with those of spontaneous origin. The quantitative differences in the numbers of *ad-3A*^{R} and *ad-3B*^{R} mutants demonstrates that: (1) 100% (13/13) of the chemically induced *ad-3* mutant samples are *quantitatively different* from the spontaneous sample; and (2) 85.9% (67/78) of the combinations of the 13 chemically induced samples are quantitatively different from each other. We interpret these data as indicating that these results are due to the induction of by each of these 13 chemicals of a *qualitatively different* spectrum of genetic damage than occurs spontaneously and a *qualitatively different* spectrum from each other.

In Fig. 2, the combined data base is given for all possible pairwise combinations of the numbers of *ad-3* mutants induced by the 9 chemicals that induced both gene/point mutations and multilocus deletion mutations as well as comparisons of these 9 chemical-induced mutations with those of spontaneous origin. The quantitative differences in the numbers of *ad*-3 mutants in these two major genotypic classes demonstrates that: (1) 100% (9/9) of the chemically induced *ad-3* mutant samples is *quantitatively different* from the spontaneous sample; and (2) that 100% (36/36) of the combinations of the 9 chemically induced samples are quantitatively different from each other. We interpret these data as indicating that these results are due to the induction of by each of these 9 chemicals of a *qualitatively different* spectrum of genetic damage than occurs spontaneously and a *qualitatively different* spectrum from each other.

In Fig. 3, the combined data base is given for all possible pairwise combinations of the 13 chemicals that induced exclusively, or almost exclusively, gene/point mutations and the 9 chemicals that induced both gene/point mutations and multilocus deletion mutations. The data used in these χ^2-tests included all of the numbers in the two major genotypic classes induced by all 22 chemicals. We have interpreted the data in this summary as indicating

that the *quantitative differences* in the numbers of *ad-3* mutants in these two major genotypic classes demonstrates that 95.7% (112/117) result from the induction of a *qualitatively different* spectrum of genetic damage.

Consideration of the overall data base on these comparisons between spontaneous and chemical induced *ad-3* mutants in the data summaries in Figs. 1–3 demonstrates that: (1) all 22 chemicals induced a spectrum of specific-locus mutations that was *qualitatively different* from that occurring spontaneously; and (2) 93.1% (215/231) of all possible pairwise combinations ($22 \times 21/2 = 231$) of these 22 chemicals show statistically significant *qualitative differences* from each other.

The data on these 22 chemical mutagens have confirmed and extended our observations in an earlier paper (de Serres and Webber, 1996) that demonstrated *qualitative differences* between the *ad-3* mutants induced by 7 different radiation treatments and those occurring spontaneously as well as the fact that the majority (90.5% (19/21)) of radiation-induced specific-locus mutations are *qualitatively different* from each other.

5. Implications for genetic risk assessment

The data to determine the numbers of *ad-3* mutants of spontaneous origin in each of the 22 samples of chemical-induced *ad-3* mutants presented in Table 1 have demonstrated striking *quantitative differences* between the spontaneous frequency of *ad-3* mutants and the frequencies induced by all 22 chemicals. In addition, comparisons of the samples of chemical-induced *ad-3* mutants with those occurring spontaneously demonstrated striking *qualitative differences* between the spontaneous mutational spectrum and

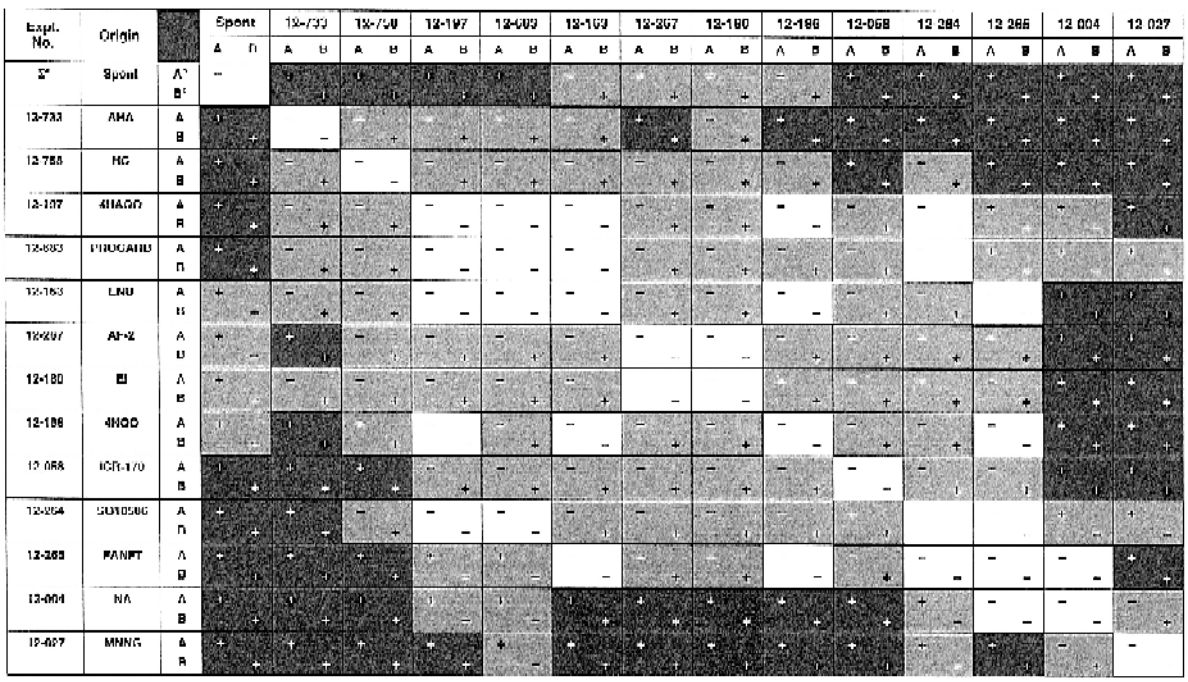

Fig. 1. Summary of the results of χ^2-tests on all possible pairwise combinations of spontaneous and 13 chemical-induced *ad-3* mutants resulting exclusively, or almost exclusively, from gene/point mutation at the *ad-3A* and *ad-3B* loci in heterokaryon 12 of *Neurospora crassa*. [a] Summation (Σ) of spontaneous *ad-3* mutations in experiments 12-001 through 12-094. [b] Results of tests to compare the numbers of gene/point mutations and multilocus deletion mutations. [c] Results of tests to compare the numbers of complementing and non-complementing *ad-3B*^{R} mutants. +, pairwise comparisons demonstrate statistically significant difference between the *ad-3* mutational spectra; −, pairwise comparisons demonstrate no statistically significant difference between *ad-3* mutational spectra.

each of the 22 chemical- and radiation-induced *ad-3* mutational spectra.

The genetic characterization of the specific-locus mutants recovered in somatic cells of the Neurospora specific-locus assay permits a comparison between samples induced by different mutagenic agents that is difficult or impossible in somatic cell assays of other experimental eukaryotic organisms. The Neurospora assay mimics the specific-locus assay for two closely linked genes, namely *dilute* (d) and *short-ear* (se), in the mouse (Russell, 1951). This approach has made it possible to compare mutational spectra at two different, but closely linked, loci in the *ad-3* region as well as at other loci in the immediately adjacent genetic regions (de Serres and Brockman, 1995; de Serres and Malling, 1995).

The appropriate utilization of data from in vitro assays such as the *ad-3* specific-locus assay in Neurospora, however, is still an unresolved challenge in genetic risk assessment of human exposure to mutagenic environmental agents (Bloom and Poskitt, 1988; de Serres, 1994). However, it is clear that in vitro assays can provide unique and critical information as demonstrated in the present paper comparing *ad-3* mutants occurring spontaneously and those induced by chemical mutagens. In a companion report, *ad-3* mutants occurring spontaneously have been demonstrated (de Serres and Webber, 1996) to be *qualitatively different* from those induced by a non-ionizing radiation (UV), and each of 6 ionizing radiations (250 kVp X-rays, 447 MeV protons, 101 MeV carbon ions, 39 MeV helium ions, ^{85}Sr, and ^{32}P). The data on spontaneous and radiation-induced specific-locus mutations in Neurospora are in agreement with similar studies in mammalian in vitro systems (Sankaranarayanan, 1991b).

The importance of the analyses in the present and a previous report (de Serres and Webber, 1996) and earlier paper on radiation-induced specific-locus mutation (de Serres and Webber, op. cit.) on radiation-induced specific-locus mutation in somatic cells of Neurospora is that *both chemical and radiation-in-*

Expt No.	Origin		Spont	PDMT 12-194	TEM 12-039	DEO 12-217	MMS 12-064	DEP 12-199	2AP 12-650	EDB 12-125	PMMT 12-189	ETO 12-780
			A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
7[a]	Spont	A	–	+	+	+	+	+	+	+	+	+
		B		+	–	–	–	–	–	–	–	–
12-194	PDMT	A	+		+	+	+	+	+	+	+	+
		B	+		+	+	+	+	+	+	+	+
12-039	TEM	A	+	+		+	+	+	+	+	+	+
		B	–	+	–	–	+	+	–	+	–	–
12-217	DEO	A	+	+	+	–	+	+	+	+	+	+
		B	–	+	–	–	+	–	–	–	–	–
12-064	MMS	A	+	+	+	+		+	+	+	–	+
		B	–	+	+	+		–	+	–	+	–
12-199	DEP	A	+	+	+	+	+		+	+	–	+
		B	–	+	+	–	–	–	+	–	+	–
12-650	2AP	A	+	+	+	+	+	+		+	+	+
		B	–	+	–	–	+	+	–	+	–	+
12-125	EDB	A	+	+	+	+	+	+	+		+	+
		B	–	+	+	–	–	–	+	–	+	–
12-189	PMMT	A	+	+	+	+	–	–	+	+		–
		B	–	+	–	–	+	+	–	+	–	+
12-780	ETO	A	+	+	+	+	+	+	+	+	–	
		B	+	+	–	–	–	–	+	–	+	–

Fig. 2. Summary of the results of χ^2-tests on all possible pairwise combinations of spontaneous and 9 chemical-induced samples of *ad-3* mutants resulting from gene/point mutation and multilocus deletion mutation at the *ad-3A* and *ad-3B* loci in heterokaryon 12 of *Neurospora crassa*. [a] Summation (Σ) of spontaneous *ad-3* mutations in experiments 12-001 through 12-094. [b] Results of tests to compare the numbers of gene/point mutations and multilocus deletion mutations. [c] Results of tests to compare the numbers of complementing and non-complementing *ad-3B*^{R} mutants. +, pairwise comparisons demonstrate statistically significant difference between the *ad-3* mutational spectra; −, pairwise comparisons demonstrate no statistically significant difference between *ad-3* mutational spectra.

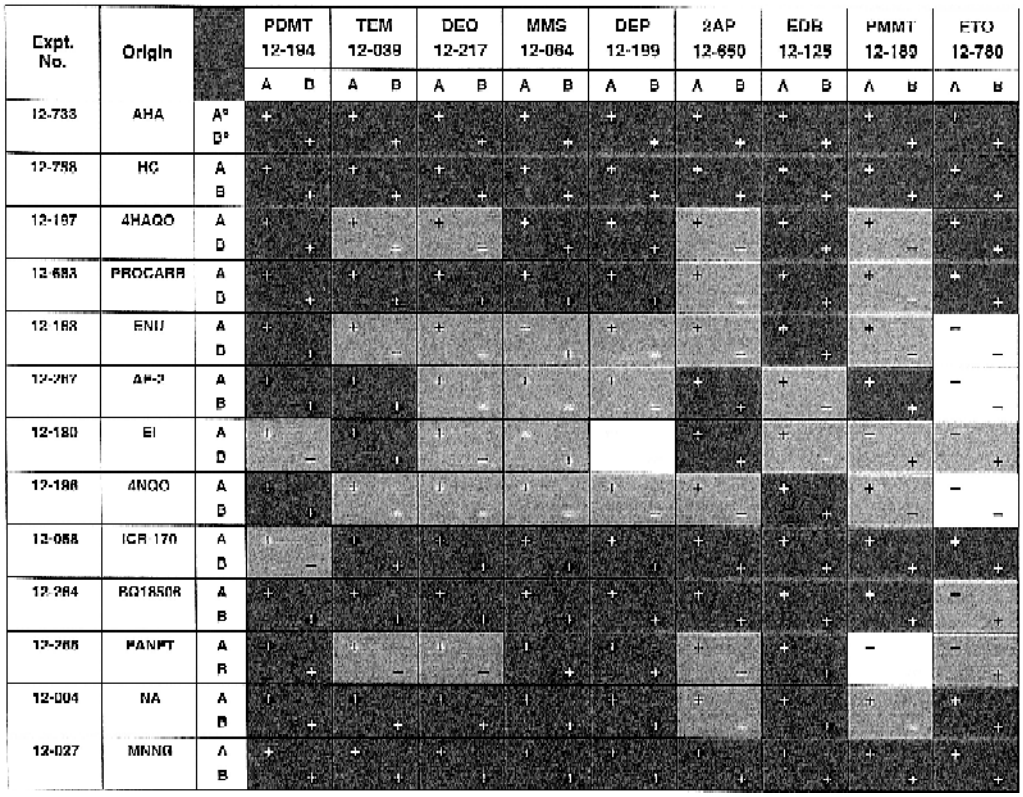

Fig. 3. Summary of the results of χ^2-tests on all possible pairwise combinations among 13 chemical-induced samples of *ad-3* mutants resulting exclusively, or almost exclusively, from gene/point mutation and 9 chemical-induced samples of *ad-3* mutants resulting from gene/point mutation and multilocus deletion mutation at the *ad-3A* and *ad-3B* loci in heterokaryon 12 of *Neurospora crassa*. [a] Results of tests to compare the numbers of gene/point mutations and multilocus deletion mutations. [b] Results of tests to compare the numbers of complementing and non-complementing *ad-3B*^{R} mutants. +, pairwise comparisons demonstrate statistically significant difference between the *ad-3* mutational spectra; −, pairwise comparisons demonstrate no statistically significant difference between *ad-3* mutational spectra.

duced specific-locus mutations are qualitatively different from spontaneous specific-locus mutations. In the simplest of terms, this means that in genetic risk assessment, one cannot assume that a mutagenic agent will give proportional increases in the frequencies of the different genotypic classes of specific-locus mutations that occur spontaneously. In addition, because the spectrum of genetic alterations resulting in induced specific-locus mutations is qualitatively different from the spectrum of genetic alterations in spontaneous specific-locus mutations, then, in humans, the spectrum of induced genetic disease in both somatic and germ cells would be expected to be different from that occurring spontaneously. An additional factor that has emerged from the present and previous study on radiation-induced specific-locus mutation (de Serres and Webber, 1996) is that in somatic cells of Neurospora, *the majority of chemical-induced and radiation-induced specific-locus mutations are different from each other*.

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