Mutagenicity of Toxic Weed Seeds in the Ames Test: Jimson Weed (*Datura stramonium*), Velvetleaf (*Abutilon theophrasti*), Morning Glory (*Ipomoea* spp.), and Sicklepod (*Cassia obtusifolia*)

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Commercial grain, such as soybean and wheat, may be contaminated with nongrain impurities such as toxic weed seeds that coexist with harvested crops. The present study investigated the genotoxic potential of seeds from four nongrain sources: jimson weed (*Datura stramonium*), velvetleaf (*Abutilon theophrasti*), sicklepod (*Cassia obtusifolia*), and morning glory (*Ipomoea* spp.). Mutagenic responses of methanolic extracts of these seeds were determined by using four bacterial strains (TA98, TA100, TA102, and TA2637) with and without microsomal activation. Relative potencies were compared by using the following parameters derived from dose–response curves: (a) mutagenic potency or revertants per milligram equivalent seeds (*R*/MES), which is defined as the maximal mutagenic response produced by 1 mg of seeds, and (b) mutagenic potential, which relates minimal effective dose (MED) with the estimated number of seeds required to produce a significant mutagenic response. Although the seed extracts elicited responses in all four bacterial strains, TA102 was the most sensitive. The following numbers of toxic weed seeds per 15 g of grain are estimated to constitute a minimal effective dose with TA102 and microsomal activation: morning glory, 1; sicklepod, 6; velvetleaf, 50; jimson weed, 566. These results show that morning glory and sicklepod seeds contain high levels of mutagens. Possible sources of mutagens and possible reasons for the observed variation of relative mutagenic potency are discussed. These observations provide a rational basis for relating seed composition to genotoxic effects and for assessing the possible safety of low levels of weed seeds in the diets of food-producing animals and in human diets.

INTRODUCTION

Commercial grain shipments may contain nongrain contaminants, including the seeds from plants that coexisted with the harvested crops. Some of these seeds may contain moderately or highly toxic components and may not be readily separable during the normal cleaning process. Such seeds have been documented to present serious problems, including illness and death of livestock and concern over food safety for humans (Dugan et al., 1989).

Although it is generally recognized that certain problem weed seeds contain toxic principles, species variability in identity and concentration of toxins is unknown. The extent of human and animal exposure to the toxic principles is also unknown, as are the genotoxic effects of toxic weed seeds in humans and animals. The current genotoxicity database is insufficient for possible risk assessment and the setting of tolerance standards for these food contaminants.

Two basic kinds of information need to be integrated to establish maximum tolerance levels for toxic seeds in grain: (1) qualitative and quantitative compositional data on the variation of the toxic principles found in each seed species and (2) potency data in animals for the known and unknown toxic principles, alone and in combinations as found in whole seeds.

The main objective of this study was to screen extracts of seeds from four toxic weeds for potential mutagenicity by using the Ames test (point mutation in bacteria). The overall goal is to assess the possible significance of genotoxicity for the purpose of setting tolerance levels of the weed seeds in edible grain such as wheat and soybeans. This study complements previously described analytical, compositional, chemical, nutritional, and toxicological studies of some of these same seeds (Crawford and Friedman, 1990; Crawford et al., 1990; Dugan et al., 1989; Friedman and Levin, 1989; Friedman and Dao, 1990; Friedman et al., 1989).

MATERIALS AND METHODS

Materials. Seed samples of sicklepod (*Cassia obtusifulia*), jimson weed (*Datura stramonium*), morning glory (*Ipomoea purpurea*), and velvetleaf (*Abutilon theophrasti*) were obtained from the Federal Grain Inspection Service (Kansas City, MO) or the Valley Seed Co. (Fresno, CA). Seed samples were picked through to remove debris and then ground in a Wiley mill, as previously described (Friedman and Levin, 1989; Dugan et al., 1989). Aflatoxin B_1, danthron, emodin, rutin, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). Extraction solvents employed were hexane and chloroform (glass distilled; EM Sciences, Cherry Hill, NJ), methanol (HPLC grade; Fischer Scientific, Fair Lawn, NJ), and Milli-Q deionized water.

Seed Extraction Methodology. The extraction scheme used was modified from one described by van der Hoeven et al. (1983) for vegetable extraction. Our extraction procedure started with two hexane extractions instead of petroleum ether extraction to remove seed oils which can interfere with the scoring of Ames test plates. The deoiled seed samples were subsequently extracted with chloroform, methanol, and Milli-Q water. All extractions were performed in a Soxhlet apparatus starting with 400 mL of solvent and 5–15 g of seed sample weighed in a Whatman cellulose thimble (33 × 94 mm). Solvent boiling was adjusted with a rheostat so that the numbers of fills of the thimble were as follows: hexane, 20 fills in 3 h; chloroform, 20 fills in 3 h; methanol, 40 fills in 6 h; Milli-Q water, 20 fills in 6 h.

The extracts were evaporated in a rotary evaporator from 400 to 100 mL or less. They were divided into two samples of equal volume and evaporated to dryness in preweighed 50-mL boiling flasks. Evaporation temperatures were as follows: 45 °C, hexane; 45 °C, chloroform; 45 °C, methanol; and 65 °C Milli-Q water. The first sample was weighed, reconstituted in DMSO at 45 °C

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for 1 h, and left unhydrolyzed. The second sample was weighed and then hydrolyzed in a boiling water bath for one 1 h with 2.5 mL of 2 N HCl and 5.0 mL of ethanol. Acid/ethanol hydrolysis has been frequently used for the hydrolysis of flavonoid glycosides (Brown and Dietrich, 1979). We observed a 93% conversion of pure rutin to quercetin (data not shown).

The hydrolysate was reconstituted in 25 mL of ethanol and evaporated three times: first at 70 °C, then in the water bath warming from 70 to 100 °C, and finally at 100 °C. In a duplicate series, unhydrolyzed samples from the methanol extract were subjected to boiling, ethanol reconstitution, and ethanol evaporation for the purpose of verifying that heat alone was not responsible for mutagen formation.

Unhydrolyzed and hydrolyzed samples were reconstituted in a desired volume of DMSO at 45 °C for 1 h. Particulate DMSO-insoluble material was removed by filtration through a single layer of Schleicher and Schuell 595 filter paper. DMSO-insoluble oils were removed by separation in a pipet. The reconstituted samples were checked for UV absorbance in the 260–400-nm range.

Mutagenicity Assays with Seed Extracts. The Ames *Salmonella*/microsome assay was performed by using *Salmonella typhimurium* tester strains TA98, TA100, TA102, and TA2637 kindly provided by Dr. Bruce Ames (University of California, Berkeley, CA). The methodology employed was previously used in related studies (MacGregor and Friedman, 1977; Friedman and Smith, 1984; Friedman et al., 1980, 1982, 1990a,b; MacGregor et al., 1980, 1989) and is described in detail by Maron and Ames (1983). The DMSO-soluble seed extract (0.1 mL) was added to culture tubes containing 2.0 mL of top agar and 0.1 mL of tester strain. If microsomal activation was required, 0.5 mL of "high" S9 mix was added to the culture tubes after addition of bacteria. The cultures were then plated. The S9 liver homogenate was prepared from the livers of 200 male Wistar rats induced with Aroclor 1254 (Simonsen Laboratories, Gilroy, CA). The tester strains and S9 used in this study were all taken from the same in-house lot to compare revertant per plate responses within strains. The extracts were checked for sterility by plating 0.1-mL high dose samples with 2.0 mL of top agar.

Negative (DMSO) controls were run with each experiment. The range of revertant colonies per plate (R/P) for three to five experiments used to generate the figures and tables for each strain were as follows: TA98 with S9, 43 ± 5; TA98 without S9, 36 ± 3; TA100 with S9, 159 ± 17; TA100 without S9, 156 ± 18; TA102 with S9, 491 ± 49; TA102 without S9, 378 ± 23; TA2637 with S9, 50 ± 4; and TA2637 without S9, 26 ± 3.

The ranges of R/P responses for the positive controls with S9 were as follows: aflatoxin B_1, 0.3 μg/plate, TA98, 459 ± 36; aflatoxin B_1, 0.3 μg/plate, TA100, 951 ± 57; danthron, 45 μg/plate, TA102, 1413 ± 171; and emodin, 30 μg/plate, TA2637, 322 ± 25. Sparse pinpoint colonies of "lawn" growth and the presence of DMSO-insoluble material were taken as indicators of toxic or excessive doses, respectively.

Duplicate plates from tester strains dosed with seed extracts were scored for revertant colonies by hand-counting with a dissecting microscope for TA98 and TA2637 and by a combination of hand-counting and Artek Model 980 automated scoring for TA100 and TA102. Each individual TA100 and TA102 experiment was scored by hand-counting four to six plates from the lowest value to higher values for TA100 (highest manual count, 1072) and TA102 (highest manual count, 962). The remaining plates were scored by the Artek Model 980 counter with settings of 0.2 mm for colony size, 5.3 for area, and 6.0 for sensitivity. Regression analysis with the "Ames fit" model (see below) was used to find manual versus Artek slopes in seven individual experiments with TA100 and TA102. Slope values for TA100 ranged from 1.21 to 1.50. Slope values for TA102 ranged from 2.21 to 3.44. Slope values were accepted for P zero slope of $P < 0.05$. Artek values times accepted slope value plus intercept was used to estimate revertants per plate.

Since exogenous histidine has been shown to influence R/P counts (Maron and Ames, 1983; Friedman et al., 1990a), histidine concentration in the hydrolyzed methanol extracts was measured by using an amino acid analyzer. The highest value was 0.0025 mg/mL hydrolyzed sicklepod methanol extract.

Table I. Effects of Hydrolysis on Mutagenic Potency of Methanol Extracts from Seeds of Morning Glory, Sicklepod, Velvetleaf, and Jimson Weed Seeds Using TA102 with S9[a]

seed	yield, mg	% recovery after hydrolysis of ethanol treatment	UV absorbance 0.001× dilution of 38 MES stock solution[a]	mutagenic potency (TA102 + S9; R/MES)[b]
morning glory				
unhydrolyzed	1107	(–)	0.68[c]	16
heated	952	108	0.60[c]	19
hydrolyzed	1095	82	0.43[d]	1225, 1283[e]
hydrolyzed	1086	67	0.25[d]	534, 536[e]
sicklepod				
unhydrolyzed	437	(–)	0.38[f]	20
heated	365	85	1.45[f]	49
hydrolyzed	533	47[g]	0.41[h]	415
hydrolyzed	413	85[i]	0.72[h]	625, 378[e]
velvetleaf				
unhydrolyzed	984	(–)	0.31[j,k]	9
heated	883	82	0.31[j,k]	2
hydrolyzed[e]	941	78	1.19[j,k]	432
hydrolyzed[e]	915	80	1.80[j,k]	1160
jimson weed				
unhydrolyzed	563	(–)	0.45[j]	ns
hydrolyzed	439	86	0.77	8

[a] MES = milligram equivalent seeds. [b] R/MES = revertants per milligram equivalent seeds. [c] Peak = 332.5 nm. [d] Peak = 286.5 nm. [e] Duplicate values. [f] Peak = 280.5 nm. [g] Started with 15 g of seeds, filtered. [h] Peak = 282 nm. [i] Started with 6 g of seed, not filtered. [j] Peak = 260 nm. [k] Stock solution diluted 0.01× peak = 320 nm.

Addition of this amount of pure histidine did not influence colony counts in any of the strains tested (data not shown).

RESULTS AND DISCUSSION

Mutagenic Potencies. The Ames fit microcomputer method of Moore and Felton (1983) was used to evaluate significant mutagenicity from dose–response curves. Dose was defined in terms of milligram equivalent seeds (MES) and the response observed was revertants per plate (R/P). The slopes calculated from the Ames fit and used for statistical analysis were R/P per MES or "mutagenic potency".

Milligram equivalent seeds (MES) relates amounts of starting seed material to milligram dosage of extract added to Ames test plate such that MES = $W \times 1000 \times 0.1$ mL/$2V$, where MES is milligrams equivalent seeds; W is the weight of starting material (usually 5–15 g); 1000 is milligrams per gram; 0.1 mL is the volume of extract added to the Ames plate; 2 is the factor which accounts for dividing the sample into two parts for unhydrolyzed and hydrolyzed treatment; and V is the reconstituted volume after evaporation of extract (usually 2–20 mL).

Table I shows the effects of hydrolysis on mutagenic potencies of four seed extracts, major UV absorbance peaks (used as a concentration check in duplicate extracts), and the calculated mutagenic potencies. Table II shows examples of calculated Ames fit data for quercetin and extracts of two seeds.

The Ames fit method calculates two probabilities (P). First, the P for linearity of the dose–response curve defines a linear portion of the curve for $P > 0.05$. When P linearity is calculated to be <0.05, the computer program warns that the "model does not fit". This usually happens when a toxic dose decreases the revertants per plate (R/P) response at the peak of the dose–response curve (e.g., Table II, toxic dose of quercetin, 50 μg/plate) or when colony counts are too high for accurate manual vs Artek Model 980 correlations (e.g., Table II, sicklepod, 19 MES/plate). The second probability value signifies a positive, linear,

Table II. Ames Fit Data with Quercetin and Hydrolyzed Methanolic Extracts from Jimson Weed and Sicklepod in TA102 with Microsomes[a]

sample	dose,[b] μg/plate	revertants per plate[c]	no. of doses	P (linearity)	P (zero slope)	slope (R/μg)
quercetin	0	467, 412				
	0.4	491, 451				
	0.8	504, 465	3	0.99	0.25	56.3
	1.6	557, 504	4	1	0.0515	55.0
	3.1 MED	612, 551	5	0.98	0.0079*	44.8
	6.3 hs	852, 710	6	0.98	0.0006*	52.5 hs
	12.5	1041, 952	7	0.89	0.0001*	45.1
	25.0	1378, 1160	8	0.34	0*	33.9
	50.0 t	1319, 1182	9	0.0019 mnf	0	17.7
jimson weed	0	648, 612				
	0.3[d]	793, 631				
	0.6[d]	631, 725	3	0.67	0.81	80.0
	1.2[d]	665, 634	4	0.61	1	−2.1
	2.4[d]	604, 670	5	0.70	0.91	−13.1
	4.8[d]	839, 771	6	0.33	0.0544	25.8
	9.5[d]	816, 692	7	0.35	0.0572	12.6
	19.0**[d]	793, 809	8	0.31	0.0137*	7.9**
	38.0[d]	962, 757	9	0.54	0.0062*	5.3
sicklepod	0	476, 557				
	0.15[d]	529, 488				
	0.3**[d]	661, 747	3	0.10	0.0332*	625**
	0.6[d]	832, 744	4	0.19	0.0055*	506
	1.2[d]	977, c	5	0.22	0.0022*	416
	2.4[d]	1193, 1106	6	0.0994	0.0004*	255
	4.8[d]	2239, 2019	7	0.128	0*	320
	9.5[d]	2611, 3439	8	0.70	0*	269
	19.0[d]	3395, 2684	9	0.0134 mnf	0	147

[a] Abbreviations: R = revertants; MED = minimum effective dose—see text; MES = milligram equivalent seeds; hs = highest slope value observed; t = toxic dose; mnf = model does not fit linearity parameter $P < 0.05$; ** = minimal effective dose and highest slope value (mutagenic potency—see text) are identical; c = contaminated plate; * = accepted slope value for P zero slope < 0.05 with a linear fit. [b] MES per plate for jimson weed. [c] Contaminated plate. [d] MES/plate.

mutagenic response. We chose P = zero slope < 0.05 as the cutoff point for comparison of seed extract mutagenic potencies. The slopes chosen for tabulation were the highest slope value and dose or mutagenic potency and the minimal effective dose (MED) or lowest dose observed for a significant mutagenic response. Quercetin's highest accepted slope value in TA102 with S9 was found to be 52.5 revertants/μg at 6.3 μg/plate, and its MED was 3.1 μg/plate with a slope of 44.8 revertants/μg (Table II). Jimson weed and sicklepod data demonstrate conditions where the mutagenic potency or the number of revertants per milligram equivalent seed (R/MES) can be equal to MED and where one seed extract is much more potent than another (i.e., Table II, sicklepod has an R/MES value of 625 versus jimson weed with a corresponding value of 7.9 in TA102 with S9).

The dose range used to compare the methanol seed extracts for mutagenic response was 19–0.019 milligram equivalent seeds (doses reduced by 0.5× intervals). Doses of 38–150 milligram equivalent seeds for this group of seed extracts produced revertant per plate values that were not accepted by the Ames fit model either because colony counts were too high for accurate manual vs Artek correlations or the doses were toxic to the bacteria.

The minimal effective dose was also used to estimate the number of seeds required for a significant mutagenic response. Thus, if there are 151 seeds per gram of jimson weed and the percent required for MED is 25% (percent required for MED in TA102 with S9 equals R/MES divided by starting dose of 75 R/MES times 100) then, 0.25 × 2265 (number of seeds in 15 g of starting material) = 566 seeds required for MED. This calculated mutagenic potential allows for visual field inspection of possible mutagenic seed contamination.

Table III. Mutagenic Activity of the Hydrolyzed Methanol Extracts of Jimson Weed, Morning Glory, Sicklepod, and Velvetleaf Seeds in the Ames *Salmonella*/Microsome Test

seed	strain, S9, − or + (TA)	mutagenic potency, R/MES[a]	mutagenic potential[b] (derived from MED[c] and MES[d])	% required for MED[c]	estimated no. of seeds/15 g required for MED[c,e]
morning glory	98, −	18	1.2	1.6	7
	98, +	36	1.2	1.6	7
	100, −	72	1.2	1.6	7
	100, +	112	1.2	1.6	7
	102, −	316	0.6	0.8	4
	102, +	1225	0.15	0.2	1
	2637, −	11	2.4	3.2	14
	2637, +	53	1.2	1.6	7
sicklepod	98, −	855	0.04	0.1	1
	98, +	358	0.08	0.2	2
	100, −	11	4.8	12.6	91
	100, +	68	1.2	3.2	23
	102, −	174	1.2	3.2	23
	102, +	625	0.3	0.8	6
	2637, −	621	0.15	0.4	3
	2637, +	200	0.04	0.1	1
velvetleaf	98, −	9	2.4	6.3	100
	98, +	3	4.8	12.6	200
	100, −	22	4.8	12.6	200
	100, +	19	4.8	12.6	200
	102, −	158	1.2	3.2	50
	102, +	432	1.2	3.2	50
	2637, −	5	2.4	6.3	100
	2637, +	4	9.5	25.0	400
jimson weed	98, −	3	9.5	12.6	285
	98, +	7	4.8	6.3	143
	100, −	0	ns[f]	ns	ns
	100, +	2	38.0	50.0	1132
	102, −	0	ns	ns	ns
	102, +	8	19.0	25.0	566
	2637, −	0	ns	ns	ns
	2637, +	0	ns	ns	ns

[a] R/MES = revertants per milligram equivalent seeds. [b] For definition, see text under Mutagenic Potencies. [c] MED = minimal effective dose. [d] MES = milligram equivalent seeds. [e] Based on number of seeds per gram: 151 for jimson weed, 29 for morning glory, 48 for sicklepod, and 106 for velvetleaf. [f] ns = not significant.

Factors Influencing Mutagenicity. Maron and Ames (1983) recommend the following bacterial strains for the detecting of several classes of mutagens: TA98 for frameshift mutagens; TA100 for base pair substitutions at guanine–cytosine (G–C) base pairs; TA102 for frameshift mutations at adenine–thymidine (A–T) base pairs; and TA2637 for frameshift mutagens at a site with several cytosine residues (similar in specificity to TA97). We used TA2637 instead of TA97 because we were unable to observe reliable spontaneous reversion with TA97 strain. Previous work with naturally occurring flavonoids, flavones, and anthraquinones has shown that TA98 is most sensitive for detecting mutagenic flavonoids (MacGregor 1984), TA100 is most sensitive for detecting mutagenic flavones (Elliger et al., 1983), and TA2637 is most sensitive for detecting mutagenic anthraquinones (Tikkanen et al., 1983).

Our experimental system for mutagenicity studies of weed seed extracts generated a series of chemically undefined products. Previous studies and our present results (Tables I–III and Figures 1–4) collectively define major factors that can affect mixtures and the interpretation of mutagenicity data in the Ames test. These factors include the following: (1) mutagen glycosylation [e.g., hydrolysis of nonmutagenic rutin produces a mutagen: quercetin (Figure 5a; Brown, 1980)]; (2) mutagen toxicity to *S. typhimurium* (e.g., emodin is toxic at doses above approximately 30 μg/plate; Tikkanen et al., 1983); (3)

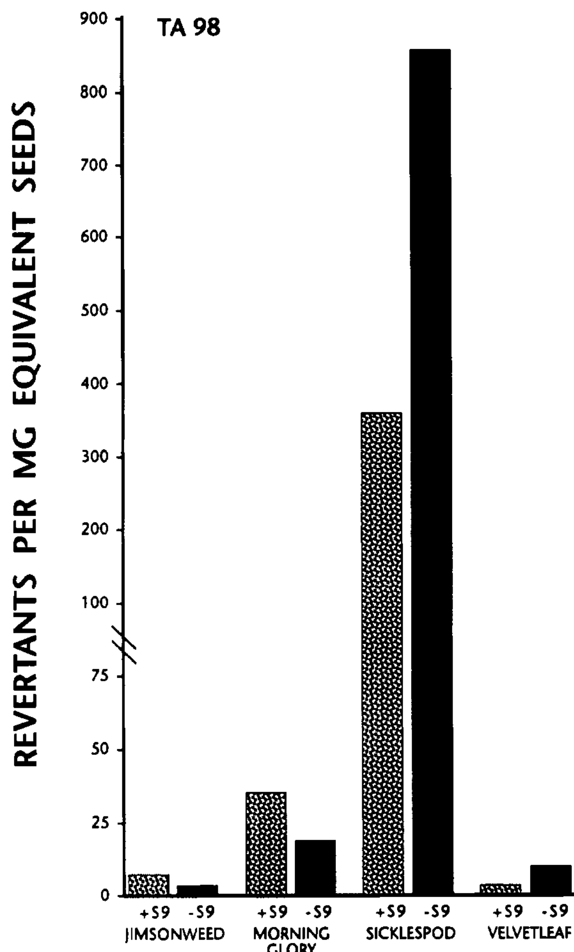

Figure 1. Mutagenic potencies of methanolic extracts of four toxic weed seeds in revertants per milligram equivalent seed (*R*/MES) in strain TA98, with and without microsomal activation.

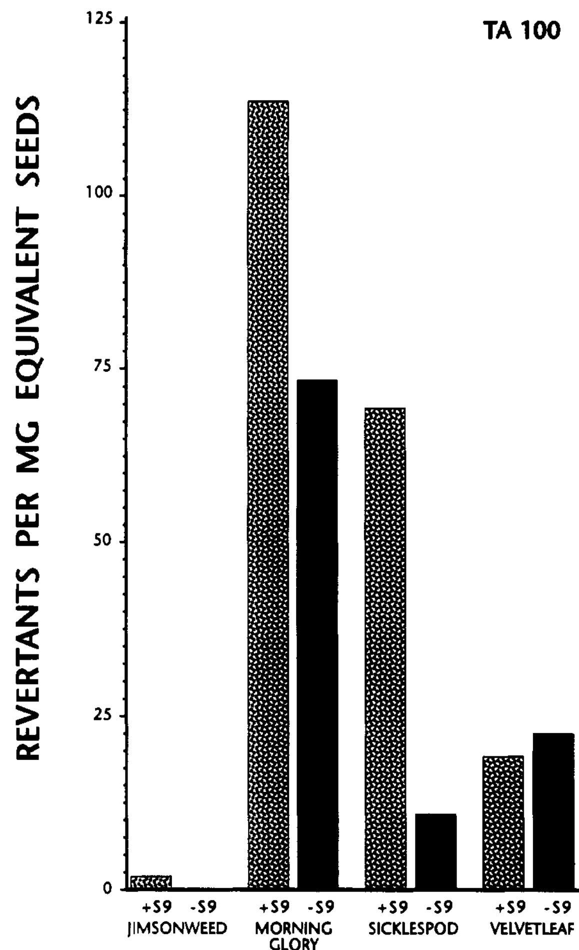

Figure 2. Mutagenic potencies of methanolic extracts of four toxic weed seeds in revertants per milligram equivalent seed (*R*/MES) in strain TA100, with and without microsomal activation.

structural requirements in a related series (e.g., hydroxyanthraquinones; Westendorf et al., 1990); (4) the presence of antimutagens or S9 inhibitors (see discussion on sicklepod); (5) the presence of more than one mutagen (see discussion on sicklepod) and (6) the possible contamination of seeds with exogenous mutagens, e.g., mycotoxin (Friedman et al., 1982), herbicides sprayed on seeds, and mutagens trapped in dirt or dust. Minor factors that were checked include the (1) presence of histidine, (2) genotoxic artifacts generated by extraction, and (3) mutagens generated by heat (Friedman et al., 1990b).

This combination of conditions and factors creates a large number of experiments. Rangefinder studies were used to determine which conditions significantly influence mutagenic potency and minimal effective dose.

First, methanol extraction accounted for nearly all mutagenic potency in all four seed sources. For example, with TA102 plus S9, hydrolyzed methanol extracts as compared to hexane, chloroform, or water extracts produced 98% of total activity for sicklepod, morning glory, and velvetleaf and 61% for jimson weed (data not shown). Strain TA102 was selected for this comparison because it gave positive results with all four seeds and its mutagenic response was the least variable (Figures 1–4). Second, using TA102 plus S9, we found that hydrolysis increased mutagenic potency in the methanol extracts of morning glory (58×), sicklepod (48×), velvetleaf (14×), and jimson weed (approximately 4×—estimated because unhydrolyzed activity was not significant) (Tables I and III). Third, the increases were found not to be due to heat or evaporation; i.e., heating unhydrolyzed methanol extracts with evaporation temperatures did not produce similar increases in mutagenic potency. Finally, the presence of S9 in the Ames test reaction mixture either increased or decreased mutagenic potency.

We expected variability in mutagenic potencies from different seed lots due to potent variation in mutagen concentration or composition. We therefore carried out our experiments with a single lot of each seed source. Extraction and mutagenic data for each seed source and their interpretation are elaborated below.

Jimson Weed (*D. stramonium*) Seeds. Of the four seed extracts tested for mutagenicity, jimson weed was found to have the least amount of mutagenic potency and potential. Activities from the methanol extracts ranged from not significant (i.e., TA2637 with and without S9, Table III) to a comparatively weak response to TA98 with S9 of 7 revertants/milligram equivalent seeds (Table III). This activity was in contrast to, for example, sicklepod, which, in TA98 with S9, generated 358 *R*/MES. Table III also shows estimates of the number of seeds required per 15 g of starting material for a minimal effective dose (MED). In TA98 with S9, methanol extracts of jimson weed required an estimated 143 seeds for MED, whereas sicklepod only required an estimated 2 seeds. A visual scan of histograms in Figures 1–4 also demonstrates that jimson weed is comparatively inactive compared to other seeds. This inactivity also suggests that genotoxic artifacts are not formed during the extraction procedure.

Jimson weed seeds containing the toxic tropane alkaloids atropine and scopolamine occasionally contaminate commercial grain (Friedman and Levin, 1989). A recent 90-

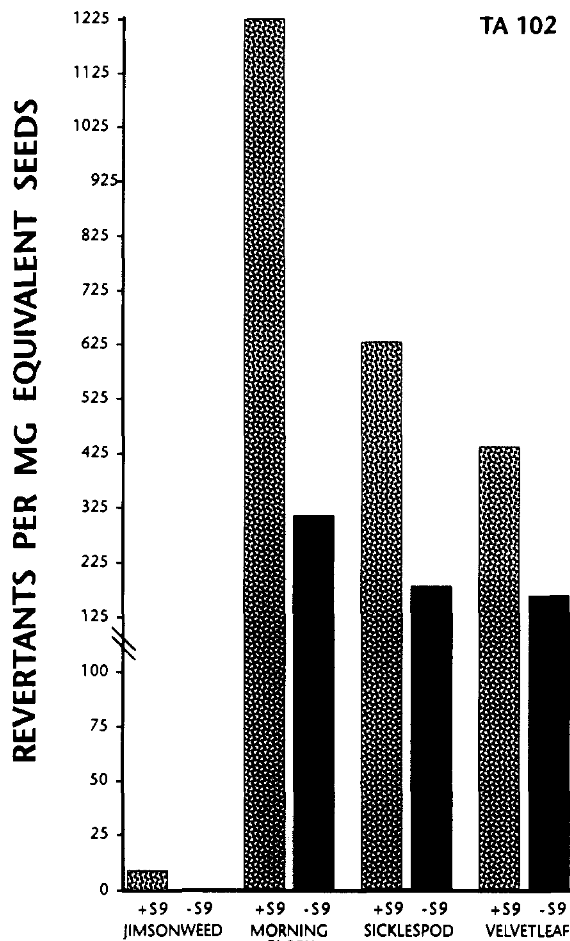

Figure 3. Mutagenic potencies of methanolic extracts of four toxic weed seeds in revertants per milligram equivalent seed (R/MES) in strain TA102, with and without microsomal activation.

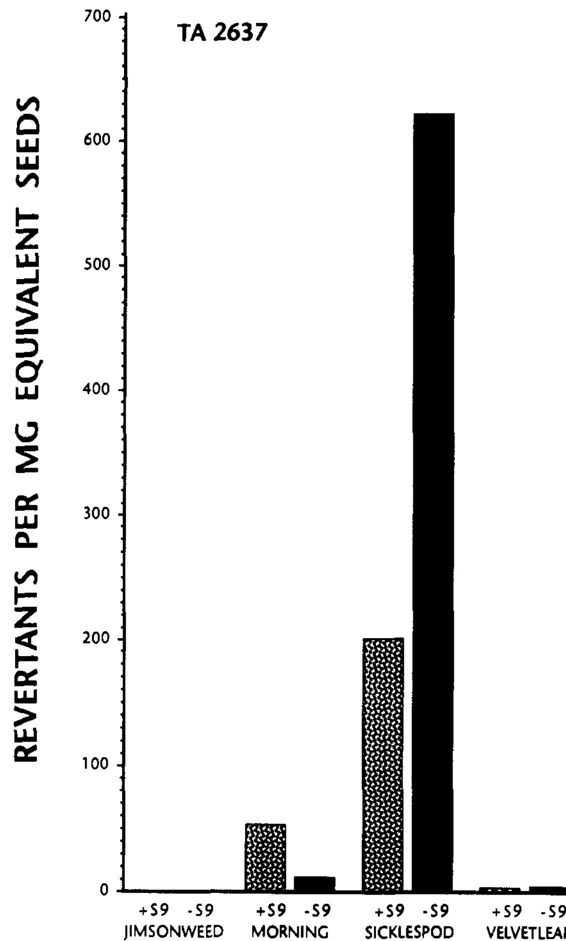

Figure 4. Mutagenic potencies of methanolic extracts of four toxic weed seeds in revertants per milligram equivalent seed (R/MES) in strain TA2637, with and without microsomal activation.

day feeding study in rats showed that jimson weed seeds produce adverse physiological effects, i.e., weight loss and changes in plasma enzymes, but no observable clastogenic (chromosome-damaging) activity in the bone marrow micronucleus test (Dugan et al., 1989). Negative micronucleus test results have also been observed with scopolamine given intraperitoneally to mice (data not shown). Atropine and scopolamine were also negative in the Ames test in TA98 (Glatt et al., 1983; McCann et al., 1975; unpublished results). Atropine failed to induce β-galactosidase gene expression in the umu gene test system using *S. typhimurium* strain TA1535/pSK 1002 (Nakamura et al., 1987). Jin-fu et al. (1988), however, demonstrated a small but significant increase in chromosome damage by scopolamine in human lymphocyte cell cultures. This effect could arise from alkylation of DNA or other sensitive site by the epoxide group of scopolamine, as illustrated in Figure 5b. Our results suggest that jimson weed seeds are toxic due to the presence of tropane alkaloids, whose stereochemistry is still being elucidated (Schmidt and Honigberg, 1989), but have negligible genotoxicity in bacteria and in vivo in bone marrow cells.

Velvetleaf (*A. theophrasti*) Seeds. The methanol extracts of velvetleaf seeds were most active in strain TA102, producing 423 R/MES with a minimal effective dose (MED) of 1.2 MES and a requirement of 50 seeds/15 g of starting material for MED (Table III). These activities required hydrolysis (e.g., a 40× increase vs unhydrolyzed extracts, Table I) but produced variable responses with S9. For example, S9 increased mutagenic potency 2× that in TA102, did not influence activity in TA100 or TA2637, and decreased activity 3× in TA98 (Figures 1–4). The magnitude of differences in these responses indicates that S9 had little influence on the active component(s) in velvetleaf seeds.

Hydrolysis of the methanol extract produced DMSO-insoluble material which was separated by filtration. The reason for the precipitation is not known. Unhydrolyzed and hydrolyzed extracts produced different UV absorbance spectra, indicating that chemical changes occurred during hydrolysis (Table I).

Plant phenolics with alleopathic and mutagenic potential are reported to occur in the coats of velvetleaf seeds (Paszkowski and Kremer, 1988). The components (in relative order of concentration) were delphinidin = quercetin > (+)-catechin = myricetin > (–)-epicatechin and cyanidin. The condensed tannin, delphinidin, is negative in the Ames assay but positive in vitro for micronuclei clastogenicity in V79 cells (Ferguson et al., 1985). Cyanidin, (+)-catechin, and (–)-epicathechin are negative in the Ames test. However, quercetin and myricetin are strong mutagens in TA98 (MacGregor, 1984; Friedman and Smith, 1984).

Of the components reported to occur in velvetleaf seed methanol extracts, quercetin appears to be the most likely candidate for an active mutagenic compound. If this is true, our data imply that TA102 with an MED of 1.2 MES should appear more sensitive for detecting quercetin than TA98, with an MED of 4.8 MES (Table III). We tested this hypothesis by determining the following MED values in micrograms per plate for pure quercetin with S9: TA98, 1.6; TA100, 3.1; TA102, 3.1; and TA2637, 3.1. Thus, even

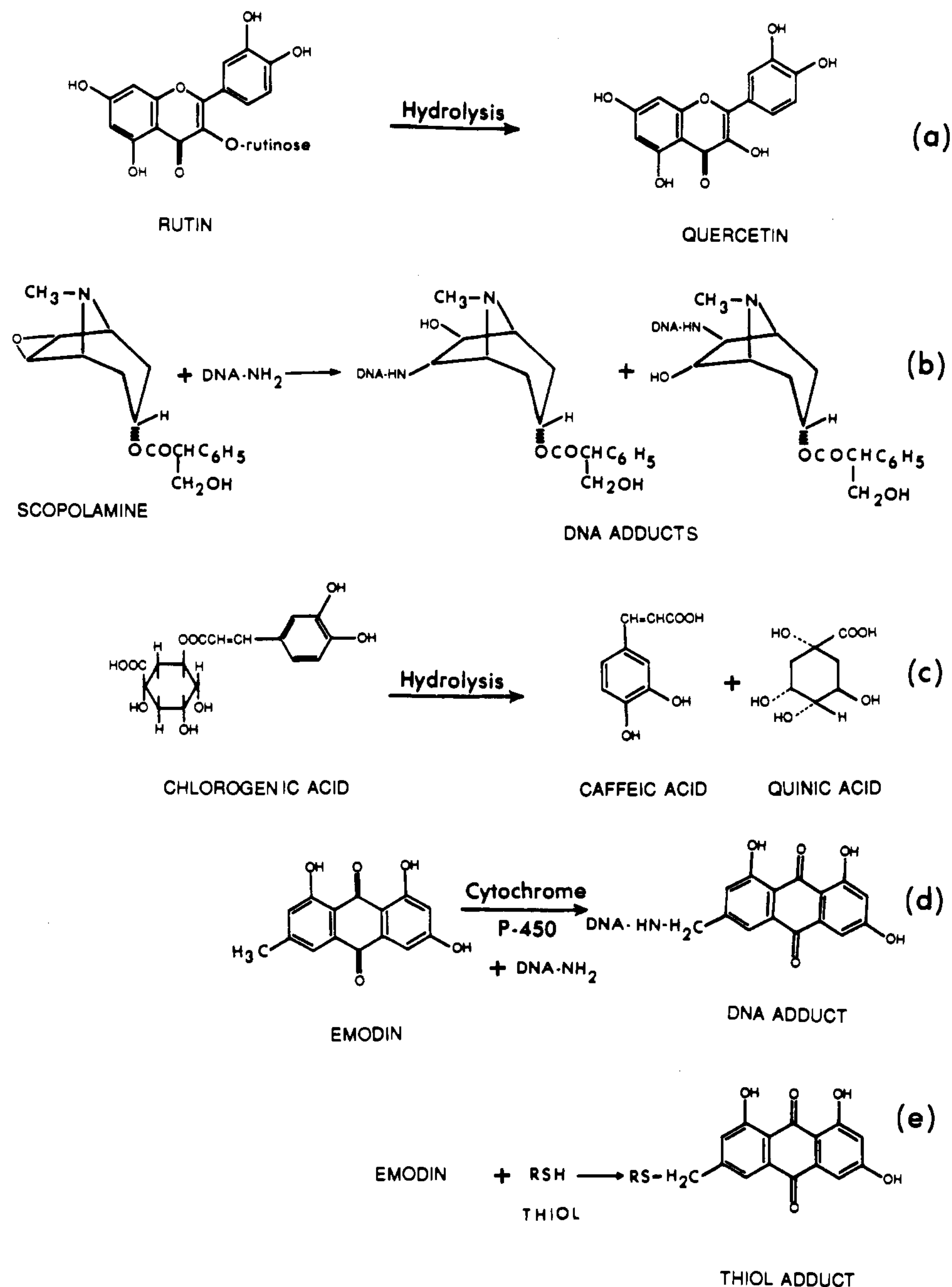

Figure 5. Possible mechanisms of genotoxicity of some compounds in toxic weed seeds. (a) Hydrolysis of the nonmutagenic glycoside rutin to the mutagenic aglycon quercetin. (b) Postulated alkylation of an amino group of DNA by an epoxide group of scopolamine to form a DNA adduct. The epoxide group can be opened at either side of the ring to form two stereoisomers. (c) Hydrolysis of nonmutagenic but genotoxic chlorogenic acid to caffeic and quinic acids. (d) Postulated oxidation of the methyl group of emodin by cytochrome P-450 to a reactive, resonance-stabilized carbonium ion intermediate which then combines with an amino group of DNA to form a DNA adduct. (e) Postulated transformation of biologically active emodin to an inactive thiol adduct.

though TA102 was the most sensitive strain for velvetleaf seeds (Table III), TA98 remains the most sensitive strain for the detection of pure quercetin mutagenicity with a MED of 1.6 μg/plate. However, our data do not rule out quercetin as a contributor (either by itself or by rutin hydrolysis; Figure 5a) to velvetleaf seed mutagenicity. Furthermore, some plant phenolics such as quercetin have been suspected as carcinogens. However, in vivo results are contradictory (MacGregor, 1984). Other phenolic compounds are reported to have antimutagenic properties (Smith and Rosin, 1984; Shinohara et al., 1988).

Morning Glory (*Ipomoea* spp.) Seeds. Of the four seed extracts tested for mutagenic potency, we considered morning glory methanol extracts most potent because they were positive in all four strains and required the fewest average number of seeds for mutagenic potency (calculated from Table III). The seeds' highest mutagenic potency was found in TA102 with S9, where an estimated 1 seed/15 g of starting material was required for a MED of 0.15 MES. All strains required hydrolysis (Table I) and S9 for maximal mutagenic potency (Figures 1–4).

Chlorogenic acid, a major component of morning glory seeds, has a UV absorbance maximum of 328 nm in ethanol (Friedman et al., 1989). In our DMSO-soluble extracts, we observed a peak at 332.5 nm in unhydrolyzed extracts which was lost upon hydrolysis, possibly indicating hydrolysis of chlorogenic acid to caffeic and quinic acids (Figure 5c).

In a rangefinder experiment with doses of up to 75 MES given intraperitoneally, we found weight loss but no evidence for micronuclei formation in mice (data not shown). The genotoxicity data of the major component

of morning glory seeds (chlorogenic acid) did not correlate well with our findings. Chlorogenic acid and its hydrolysis products (caffeic and quinic acids) were found to be negative for the Ames test but did produce chromosome aberration in Chinese hamster ovary (CHO) cells (Fung et al., 1988; Stich et al., 1981). This rules out these plant phenolics as the mutagen principles in our study. Dietary flavonoids such as quercetin and chlorogenic acid may have the potential of producing intestinal injury (Canada et al., 1989). It is also noteworthy that chlorogenic acid is rapidly destroyed under the influence of food-processing conditions such as autoclaving and conventional and microwave baking (Friedman and Dao, 1990).

A second group of chemicals, i.e., the ergot alkaloids, that occur in seeds of the Heavenly Blue variety of morning glory and which are much more heat-resistant than chlorogenic acid (Friedman and Dao, 1990) have apparently not been tested for mutagenicity in the Ames test. Thus, we cannot assign a possible genotoxic principle that may be responsible for the high mutagenicity we find for morning glory seed extracts.

Sicklepod (*C. obtusifolia*) Seeds. Sicklepod seed extracts are reported to be toxic to muscle tissues (Lewis and Shibamoto, 1989; Putnam et al., 1988) and to possess mutagenic anthraquinones such as emodin, chrysophanic acid, and physcion (Crawford et al., 1990). In this study, sicklepod methanol extracts were highly mutagenic, with the most sensitive strains being TA98 without S9 (MED = 0.04 MES) and TA2637 without S9 (MED = 0.15 MES). These activities require only an estimated 1-3 seeds/15 g of starting material (Table III). The S9 (microsome) requirements were ambiguous with TA98 and TA2637 activity inhibited by S9 (2.5× in TA98 and 3.1× in TA2637) (Figures 1 and 2). Sicklepod methanol extracts also required hydrolysis for maximal activity (a 15× increase in activity vs unhydrolyzed, Table I). During extraction with 15 g of seeds, a black residue formed on the boiling flask. The filtered sample gave identical UV absorbance maxima as a duplicate sample with starting material but with no filtration. Filtered and unfiltered samples had similar mutagenic potencies, indicating that the mutagenic component did not precipitate out. Finally, UV absorbance profiles of unhydrolyzed and hydrolyzed extracts did change, indicating chemical changes during hydrolysis.

In the Ames test, anthraquinones are positive in strains TA2637 and TA100 (TA100 is less sensitive than TA2637) and negative in TA98 and TA102 (Bachmann et al., 1979; Tikkanen et al., 1983). The extracts were strongly positive in TA98 and TA102, indicating that components other than, or in addition to, anthraquinones may be responsible for mutagenic potency. The decrease in mutagenic potency in TA98 and TA2637 implies inhibition of microsomal fraction by an antimutagen. In studies with rhubarb extracts, Van der Hoeven et al. (1983) were able to assign emodin as the active component in strain TA1537, a nonplasmid parent strain of TA2637 (Maron and Ames, 1983). However, we were not able to give a similar assignment due to the complex nature of sicklepod extract mutagenicity data. Figure 5d illustrates a possible mechanism for the mutagenicity of emodin (Tanaka et al., 1987) and Figure 5e the trapping of the postulated electrophilic intermediate by a nucleophilic thiol, thus preventing DNA adduct formation (Friedman, 1984).

Our data and related studies suggest that exposure to sicklepod seeds carries with it a risk for genotoxicity as well as for myotoxicity. By themselves, mutagenic anthraquinones are not thought to pose a high cancer risk because of their inability to covalently bind *Salmonella* and rat liver DNA (Bosch et al., 1987). However, further compositional analyses of sicklepod seed extracts are needed to provide a chemical basis for risk assessment (Crawford and Friedman, 1990; Crawford et al., 1990).

CONCLUSIONS

The observed wide variability in the genotoxicity of extracts of different toxic weed seeds should, together with additional compositional–pharmacological–toxicological data, be taken into account in setting maximum tolerance levels for the contamination of commercial grain and other foods and feeds with these seeds. The derived quantitative parameters, milligram equivalent seeds (MES) and minimum effective dose (MED), should facilitate comparison of relative genotoxicities of various grain samples. These parameters merit adoption by other investigators so that the relative potency index can be extended to many other naturally occurring and processing-induced food ingredients. Such an index would help relate consumption of foods and feeds containing toxic weed seed to the safety and health of animals and humans. A better understanding of the chemical bases and molecular mechanisms of toxic, mutagenic, and antimutagenic responses is also needed to facilitate predicting genotoxicities and relative risks to animal and human health from compositional data of complex foods, feeds, toxic weeds, and other materials (Deshpande et al., 1984; Bradfield and Bjeldanes, 1991; De Flora et al., 1989; Loprieno et al., 1991; MacGregor, 1984; Friedman, 1980; Weisburger, 1991).

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