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Project Summary

Detection of Carcinogenicity Based on Mutagenicity in *Arabidopsis*

George Redei and Gregoria N. Acedo

Thirty-seven synthetic chemicals plus two mycotoxins were tested for mutagenicity in an *Arabidopsis* embryo system. The results of this test, prokaryotic repair tests, bacterial mutation assays, eukaryotic cell systems and *in vivo* tests were compared to the carcinogenicity classifications of the chemicals.

Thirty-two of the 37 chemicals tested were correctly identified as either mutagenic or nonmutagenic in the *Arabidopsis* assay. To compare these results with those of the other assays, we defined three criteria. "Sensitivity" indicated the percentage of tested carcinogens that were mutagenic in a system. Of 20 carcinogens tested in the *Arabidopsis* assay, 19 were mutagenic (sensitivity 95%). For the other assays, sensitivities ranged from 16% to 88%. "Specificity" indicated the percentage of noncarcinogens that were nonmutagenic in a system. Three of twelve noncarcinogens were nonmutagenic to *Arabidopsis* (specificity 25%). For the other assays, specificities ranged from 20% to 100%. The noncarcinogens pyrene, 4-acetylaminofluorene, 1-naphthylamine, isopropyl-N-(chlorophenyl) carbamate, azoxybenzene, and ascorbic acid were mutagenic in the *Arabidopsis* assay and other assays. "Accuracy" indicated the percentage of correctly identified chemicals based on the predicted classification. For the *Arabidopsis* assay, 22 of 32 chemicals were correctly identified (accuracy 69%). Overall accuracies for the other assays ranged from 57% to 62%.

The *Arabidopsis* assay was the most sensitive and accurate short-term test in our comparison. Therefore, *Arabidopsis* can be used to supplement a battery of short-term tests for identifying carcinogens.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In an international program sponsored by the British Medical Research Council, the U.S. National Institute of Environmental Health Sciences, the U.S Environmental Protection Agency (EPA), and the National Cancer Institute of Japan, workers from 63 laboratories evaluated the mutagenic effects of 42 chemicals using different short-term biological assays, such as prokaryotic repair tests, bacterial mutation assays, *in vitro* eukaryotic cell assays, and *in vivo* tests with *Drosophila* and mice (the results of these studies were published in *Evaluation of Short-Term Tests for Carcinogens*, F.D. deSerres and J Ashby, eds., 1981, Elsevier/North Holland, New York). From the results of previous animal assays, the chemicals were classified as proven carcinogens, harmless analogues of carcinogens, and noncarcinogens This program was intended to help researchers select the most effective series of short-term tests. Because no single system consistently identified all the carcinogens and noncarcinogens, we retested 37 chemicals using an *Arabidopsis* assay and compared the results to those of the other assays.

Arabidopsis thaliana (L.) Heynh. seeds were soaked in water for 24 hr and then treated for 15 hr with chemicals dissolved in either medium with nutrients and minerals or dimethylsulfoxide (DMSO). As a control, some seeds were concurrently exposed only to the solvents. Approximately 250 seeds per treatment were planted on Promix medium, moistened, placed in covered glass vessels, and incubated at 24° C under a light intensity of 800 foot-candles. After germination, 100 to 200 plants per culture vessel were examined for mutations (fruits with pale or white embryos). The mutation frequency for exposed seeds minus control frequency was recorded as "mutation %."

To compare the assays, we defined three criteria. "Sensitivity" indicated the percentage of tested carcinogens that were mutagenic to a system. "Specificity" indicated the percentage of noncarcinogens that were nonmutagenic to a system. "Accuracy" indicated the percentage of correctly identified chemicals based on the predicted classification.

The *Arabidopsis* assay has many advantages: (i) it is a eukaryotic test, (ii) forward mutations are scored at more than 10,000 loci, which ensures a representative sample of the genome, (iii) the results of the embryo assays can be supplemented by progeny tests, (iv) the assay does not rely on any supplement for activation; the plant seems to convert most promutagens into ultimate mutagens, (v) the test is less expensive than most higher eukaryotic assays, and (vi) according to literature survey, 88% of the tested carcinogens are mutagenic to *Arabidopsis*.

Results

Thirty-two of the 37 chemicals tested with the *Arabidopsis* assay could be correctly identified as mutagens or nonmutagens. Of these 32 chemicals, 28 were mutagenic and four were nonmutagenic. Nineteen of 20 carcinogens were mutagenic in the *Arabidopsis* assay (sensitivity 95%). The sensitivity ranges for the other tests were: prokaryotic repair tests, 16% to 73%; bacterial mutation assays, 27% to 76%; eukaryotic cell systems, 8% to 88%; and *in vivo* tests, 17% to 52%.

Table 1 compares the sensitivities of all assays combined, bacterial mutation assays, and the *Arabidopsis* assay. Of the 25 carcinogens, 14 (Group I) were correctly identified as mutagens by all assays combined (sensitivity 60% or better) and bacterial mutation assays (sensitivity 70% or better). The *Arabidopsis* assay identified 10 of the 14 Group I compounds as mutagens (sensitivity 71%). The identification of Group II carcinogens was more difficult. The bacterial mutation and combined assay sensitivities were below 49% for these carcinogens. However, *Arabidopsis* identified 9 of 11 Group II carcinogens as mutagens (sensitivity 82%).

Table 1 *A comparison of the sensitivities of all assays combined, the bacterial mutation tests, and the* Arabidopsis *assay for 25 carcinogens. Sensitivity is defined as the percentage of carcinogens that are mutagenic in a system. The symbols ●, -, ?, and NT indicate mutagenicity, nonmutagenicity, inconclusive results, and not tested, respectively*

	Sensitivity		
Carcinogens	***All assays***	***Bacterial mutation***	Arabidopsis
Group I			
β-Propiolactone	*93*	*100*	●
4-Nitroquinoline-N-oxide	*89*	*100*	*NT*
2-Acetylaminofluorene	*86*	*100*	●
Benzo(a)pyrene	*83*	*100*	●
Epichlorohydrin	*82*	*95*	●
Methylazoxymethanolacetate	*81*	*73*	●
Methylene bis(2-chloroaniline)	*77*	*84*	●
2-Naphtylamine	*75*	*95*	?
Cyclophosphamide	*74*	*74*	●
Hydrazine sulphate	*71*	*80*	●
Dimethylanthracene	*70*	*88*	?
Benzidine	*68*	*85*	●
Dimethylcarbamoyl chloride	*67*	*76*	?
Nitrosomorpholine	*60*	*70*	●
Group II			
Auramine	*49*	*48*	●
Dimethylaminobenzene	*48*	*45*	●
O-Toluidine	*44*	*33*	●
Hexamethylphosphoramide	*38*	*11*	?
Safrole	*38*	*20*	●
Urethane	*38*	*26*	●
Ethylenethiourea	*32*	*20*	●
Ethionine	*28*	*14*	●
Diethylstilbestrol	*24*	*14*	●
Chloroform	*20*	*11*	●
Aminotriazole	*20*	*10*	-

Only three of the 12 presumably noncarcinogenic chemicals were nonmutagenic in the *Arabidopsis* assay (specificity 25%). Specificity ranges for the other assays were: prokaryotic repair tests, 35% to 75%; bacterial mutation assays, 59% to 82%; eukaryotic systems, 23% to 85%; and *in vivo* tests, 20% to 100%. Although classified as noncarcinogens, pyrene, 1-naphthylamine, 4-acetylaminofluorene, isopropyl-N-(chlorophenyl) carbamate, azoxybenzene, and ascorbic acid were mutagenic in the *Arabidopsis* assay and other assays.

Table 2 compares the pooled accuracies of four assay systems with the accuracies of the *Arabidopsis* assay for 42 chemicals. The *Arabidopsis* assay correctly identified 69% of the chemicals and was the most accurate assay. Overall accuracies for the other systems were: prokaryotic repair tests, 58%; bacterial mutation assays, 62%; eukaryotic tests, 60%; and *in vivo* tests, 57%.

Conclusion

A higher percentage of carcinogens and presumed noncarcinogens were mutagenic to *Arabidopsis* than to the prokaryotic repair systems, bacterial mutation assays, eukaryotic tests, and *in vivo* tests. This high sensitivity can be attributed to the large number of target loci in the *Arabidopsis* genome. Also, the *Arabidopsis* system correctly identified carcinogens and noncarcinogens more consistently than the other assays. Therefore, the *Arabidopsis* assay could be a valuabe addition to a battery of short-term tests for identifying carcinogens.

Table 2 *A comparison of the pooled accuracies of four assay systems and the accuracy of the Arabidopsis assay for 42 chemicals. Accuracy is defined as the percentage of correctly identified chemicals based on the predicted classification. The symbols ●, -, NT, and ? indicate carcinogenicity, noncarcinogenicity, not tested, and inconclusive results, respectively.*

		Accuracy (%)					
Chemicals		*Repair tests*	*Bacterial mutation*	*Eukaryotic systems*	*In vivo tests*	*Arabidopsis assays*	*Total*
Nitroquinoline-N-oxide	●	100	100	91	20	NT	89
Methylnitroquinoline N-oxide	-	0	0	5	60	NT	8
Benzidine	●	57	85	68	40	100	68
Tetramethylbenzidine	-	71	95	88	88	?	88
Dimethylaminobenzene	●	0	45	64	40	100	48
Dimethylaminobenzene sulphonic acid-Na	-	71	45	65	100	NT	62
β-Propiolactone	●	100	100	92	33	100	93
γ-Butyrolactone	-	67	90	71	100	?	81
Benzo(a)pyrene	●	71	100	71	83	100	83
Pyrene	-	75	57	53	100	0	62
Ethionine	●	29	14	35	40	100	28
Methionine	-	67	90	76	100	100	81
Chloroform	●	17	11	31	0	100	20
Trichloroethane	-	83	94	67	100	0	81
2-Acetylaminofluorene	●	71	100	73	100	100	86
4-Acetylaminofluorene	-	33	0	50	100	0	27
2-Naphthylamine	●	71	95	75	29	?	75
1-Naphthylamine	-	50	30	36	78	0	41
Nitrosomorpholine	●	43	70	56	50	100	60
Diphenylnitrosomine	-	57	70	65	80	0	66
Urethane	●	38	26	41	100	100	38
Isopropyl-N-(3-chlorophenyl) carbamate	-	50	100	38	100	0	67
Methylazoxymethanol-acetate	●	86	73	83	100	100	81
Azoxybenzene	-	17	37	46	67	0	38
Dinitrosopentamethylene tetramine	-	17	90	56	75	?	67
Hydrazine sulphate	●	83	80	21	0	100	71
Hexamethylphosphoramide	●	17	11	48	88	?	38
Safrole	●	86	20	39	20	100	38
Diethylstilbestrol	●	29	14	26	33	100	34
Cyclophosphamide	●	67	74	73	100	100	74
Epichlorohydrine	●	80	95	20	20	100	82
Auramine	●	57	48	50	0	100	49
Methylene bis(2-chloro-aniline)	●	100	84	54	66	100	77
Toluidine	●	43	33	64	0	100	44
Ethylenethiourea	●	67	20	38	0	100	32
Aminotriazole	●	17	10	46	0	0	20
Dimethylcarbamoyl chloride	●	71	76	64	40	?	67
Dimethylformamide	-	100	82	76	100	0	82
Dimethylanthracene	●	57	88	70	0	?	70
Anthracene	-	63	89	92	100	100	85
Sucrose	-	100	100	75	100	100	92
Ascorbate	-	67	84	58	100	0	72
Accuracy of the systems		58 3	62 1	59 5	57 3	68 8	60 8

George Redei and Gregoria N. Acedo are with the University of Missouri, Columbia, MO 65211.
***Shahbeg S. Sandhu** is the EPA Project Officer (see below).*
The complete report, entitled "Detection of Carcinogenicity Based on Mutagenicity in Arabidopsis, *" (Order No. PB 83-225 078, Cost: $10.00, subject to change) will be available only from:*

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Health Effects Research Laboratory
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