mated 1978 figures on the numbers of households were used to arrive at state totals, consistent with the survey totals. Any errors so introduced are probably not serious in comparison to the uncertainties involved in many of the surveys. The error introduced by the use of 30-year average heating demands should tend to average out across the nation; the 1978–1979 winter was colder than normal in some areas and warmer in others, for example.

7. There are 238 Air Quality Control Regions designated by the Environmental Protection Agency. The regions are usually groups of counties and vary considerably in size and population.
8. The uncertainty in this total estimate of 35 million cords can be derived as follows: for 18 states, usage figures are available from surveys or from local estimates; these total 14,364,000 cords, a figure taken as accurate for the purpose of statistical model development. The uncertainty thus derives from the estimates for the other 30 states. There are several levels of uncertainty involved. First, Eq. 1 contains uncertainty as to how well it represents the 64 New England counties from which it was derived. However, this uncertainty is not relevant to the problem of applying this particular equation (or any other equation) to other states; the uncertainty derives from how well the equation fits the state data. Figure 2 displays this uncertainty at the state level. The standard error involved in the regression of predicted wood usage against observed wood usage may be derived in two different ways, either from use of cords per household in each state as the measure or else from just the total state estimate in cords. The goodness of fit was about the same in each case, with about 65 percent of the variance explained and a standard error of about 37 percent. Since the average predicted state usage for the 48 states (about 736,000 cords) is comparable to the average prediction for the 18 states (787,000 cords), the 18-state sample is regarded as representative of the whole country, including the error of prediction for each state (about 306,000 cords). Thus, assuming that all states have the same probability distribution of prediction error, the error for the sum of the 30 nonsampled states is given by $306{,}000\sqrt{30}$ or 1.68×10^6 cords. The 95 percent confidence limits for the U.S. total would be from 31.1 to 38.2 million cords. This would be equivalent to about 0.70 to 0.86 quad, or from 9.0 to 11.0 percent of U.S. space heating input. The error involved in basing fuel usage on degree days referenced to 65°F may be more important, since it is doubtful that homeowners in climates where only occasional space heating is required would make the investment required for wood heating systems.
9. D. G. DeAngelis, D. S. Ruffin, J. A. Peters, and R. B. Reznik ["Source assessment: Residential combustion of wood" (EPA-600/2-80-042b, Environmental Protection Agency, Washington, D.C., March 1980)] estimated that about 16.6×10^6 metric tons of wood were burned in the residential sector in 1976; this corresponds to about 0.3 quad of energy.
10. J. O. Davies III and P. Cohen, "Wood as a viable home heating alternative" (Gallup Organization, Princeton, N.J., June–July 1979).
11. Figure 2 shows the best fit regression line for all 18 states; it has a slope of 1.06 ± 0.4. The 1:1 correspondence line falls within these 95 percent confidence limits. If the three most outlying states (Oregon, Montana, and Minnesota) are dropped, the slope is 1.11 ± 0.10. Thus, the relation between observed firewood usage and the usage predicted by Eq. 1 appears to be satisfactory.
12. Survey data from Missoula County, Montana (J. McNairy, "Energy use in Missoula," report prepared for Missoula Valley Energy Conservation Board, June 1981) agree quite well with Eq. 1: 0.8×10^{12} Btu and 0.87×10^{12} Btu, respectively. The statewide survey may not be representative.
13. T. D. Warner and J. A. Shelley, *J. For.* **80** 284 (May 1982).
14. R. A. Pellerin and E. D. Markwardt, "Wood energy survey 1979" (Cooperative Extension Agricultural Engineering Department, Cornell University, Ithaca, N.Y., 1979).
15. Data used in Fig. 3 are based on a typical average number of persons per household (3.1) and 6500 heating degree days per year. At about 3.1 persons per household, this population density level corresponds to about 2.7 houses per acre. Since Eq. 1 yields 0.209 cord per household at this density and the average household depending entirely on wood uses about 5 or 6 cords annually, this peak value corresponds to about 4 percent of urban households heating exclusively with wood. Casual use of wood by others would reduce this figure. The uncertainties in the parameters of Eq. 1 result in a large uncertainty in Fig. 3, which should be viewed as an example.
16. Small cities often have population densities higher than 2000 persons per square mile. But many entire counties also have densities in the range of maximum air quality impact; for example, Denver (5100); Suffolk, Mass., which includes Boston (12,907); and suburban New York and New Jersey counties (4000 to 7000).
17. Particulate emissions from wood burning are 20 to 60 times higher per input (Btu) than emissions from liquid or gaseous fuels [F. W. Lipfert, in *Residential Solid Fuels, Environmental Impacts and Solution*, J. A. Cooper and D. Malik, Eds. (Oregon Graduate Center, 1981)]. The substitution of wood fuel for 10 percent of the heat input may result in the doubling or tripling of the total emissions of particulate matter.
18. We thank our colleagues at Brookhaven for helpful discussions and assistance with computations, J. Martino for manuscript preparation, and an anonymous reviewer for statistical suggestions. Supported by the Department of Energy contract DE-AC02-76CH00016.

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Activation of 2-Aminofluorene by Cultured Plant Cells

Abstract. *Cultured tobacco plant cells activated 2-aminofluorene to an agent mutagenic to* Salmonella typhimurium *strain TA98. The plant activation of 2-aminofluorene is heat-inactivated and may not involve solely cytochrome P-450. The kinetics of activation demonstrated both time- and concentration-dependent responses.*

Environmental mutagens constitute a threat to public health (*1*). A relatively new topic in genetic toxicology is the activation of chemicals into mutagens by green plants. A promutagen is a chemical that is not mutagenic in itself but can be biologically transformed into a mutagen. Plant activation is the process by which a promutagen is activated into a mutagen by the biological action of a plant system. Plant activation is analogous to the mammalian microsome activation systems that are used routinely in most short-term microbial mutagen assays. In order for an agent to be defined as a plant promutagen, it is necessary that the plant activation process be distinct from the genetic end point used to assay for mutagenicity (*2*).

We provide here evidence for the metabolic activation of 2-aminofluorene (2-AF) to a mutagen by plant cells in culture. Aromatic amines such as 2-AF and 2-acetylaminofluorene are well-characterized mammalian promutagens and procarcinogens (*3*). Their carcinogenicity and in vivo reactivity are dependent upon metabolic activation. The first step in the mammalian metabolic activation of these agents is *N*-hydroxylation (*3, 4*). In the presence of mammalian hepatic microsomes, the *N*-hydroxylation of aromatic amines is dependent upon the cytochrome P-450 enzyme system that functions as a terminal monooxygenase (*4*). Plant cells contain exceedingly little cytochrome P-450 (*5*).

Our discovery of plant activation of 2-AF is based on a new technique in which cultured tobacco cells *(Nicotiana tabacum)* were coincubated with the bacterial genetic indicator organism *Salmonella typhimurium* strain TA98 (*6*). The *N. tabacum* and *S. typhimurium* cells were coincubated in a culture medium with 2-AF, after which the plant cells were removed by centrifugation. Induction of reverse mutation at the *his* locus in TA98 was determined by plating on a minimal medium.

A liquid suspension culture of *N. tabacum* cell line TX1 was grown aseptically at 28°C in Murashige and Skoog medium containing 0.4 mg of 2,4-dichlorophenoxyacetic acid per milliliter (*7*); 50-ml aliquots of plant cells were harvested from logarithmic-phase cell suspensions by centrifugation at 50*g*. The cell pellet was resuspended in 10 ml of fresh cell culture medium and used immediately.

A single colony isolate of TA98 was inoculated into nutrient broth and incubated at 37°C overnight. The bacterial cell pellet was resuspended in a standard S-9 buffer (*8*). For incubation studies requiring mammalian microsomal activation, an Aroclor-induced rat hepatic fraction (S-9) was used to prepare a standard activation mix (*8*). The bacterial cells were maintained according to established practice (*8*).

The coincubation experiments were conducted such that either the time of coincubation with 2-AF or the concentration of 2-AF was the variable. The coincubation and the incubation tests were conducted while shaking at temperatures of 25°C and 37°C, respectively. The solvent controls consisted of 50 μl of dimethyl sulfoxide (DMSO) added to each different control suspension. The experimental test suspensions contained 2-AF in 50 μl of DMSO. A heat-inactivated control consisted of either plant cells or S-9 mix, killed or denatured by incubation at 70°C for 15 minutes prior to their use in the suspension tests. Samples (1 ml) were withdrawn at the designated times, diluted in 9 ml of 0.5 percent NaCl at 4°C, and mixed vigorously. The

plant cells were pelleted by gentle centrifugation for 1 minute. Eight milliliters of the supernatant fluid were removed and centrifuged at 3000*g* for 10 minutes. The bacterial cell pellet was resuspended in 800 μl of standard S-9 buffer, and 100 μl of the suspension were plated onto minimal medium (*8*). The titer of viable bacterial cells was determined by plating onto nutrient agar 100 μl of a 1.33×10^5-fold dilution of each suspension.

The results of the time-dependent coincubation experiments are presented in Table 1. Activation was not observed for 2-AF alone, heat-killed plant cells alone, or 2-AF coincubated with heat-killed plant cells. Viable plant cells coincubated with TA98 but without 2-AF did not induce mutations. When plant cells were coincubated with TA98 for 0 to 120 minutes in the presence of 250 μ*M* 2-AF, the mean number of *his* revertants per plate increased from 1.3 to 432.5, corresponding to from 0.2 to 188.0 revertants per 10^7 recovered bacteria. Thus the activation of 2-AF by plant cells in culture demonstrated time-dependent kinetics. For the incubation tests involving mammalian microsomal activation, 2-AF alone, S-9 mix alone, heat-inactivated S-9 mix alone, or 2-AF incubated with heat-inactivated S-9 did not activate 2-AF. A positive control of incubating 2-AF plus S-9 mix with TA98 induced a time-dependent increase in revertants.

In the concentration-dependent experiments (Table 1) the time of coincubation was constant at 60 minutes while the 2-AF concentrations were varied. The protocol was the same as described above except that 3 ml of the coincubation suspension was distributed into each of five 50-ml Delong culture flasks. After the suspension had incubated for 5 minutes, 30 μl of a known concentration of 2-AF was added to each flask. Samples (2.5 ml) were withdrawn after 60 minutes of coincubation and added to 10 ml of 0.5 percent NaCl at 4°C. The plant cells were separated from the bacteria, which were recovered subsequently by centrifugation. The bacterial pellet was resuspended in 2 ml of standard S-9 buffer. The reversion frequencies of TA98 and the cell titer were determined as outlined above.

Table 1. The activation of 2-AF by plant tissue culture cells or mammalian S-9 and the resulting mutagenicity in *Salmonella typhimurium* strain TA98 after time- or concentration-dependent exposure; HI, heat-inactivated; S.E., standard error of the mean. For each treatment, four or six minimal medium plates were prepared and scored for revertant colonies.

2-AF concentration (μ*M*)	Incubation time (min)	S-9	Plant cells	Mean revertants per plate ± S.E.	Revertants per 10^7 recovered bacteria
			Time-dependent experiments		
250	0	−	HI	1.8 ± 0.4	0.3
250	120	−	HI	5.3 ± 1.5	0.4
0	0	−	+	3.0 ± 1.1	0.7
0	30	−	+	1.7 ± 0.7	0.2
0	60	−	+	2.0 ± 0.4	0.3
0	120	−	+	3.0 ± 0.4	0.3
250	0	−	+	1.3 ± 0.5	0.2
250	30	−	+	72.8 ± 3.7	18.7
250	60	−	+	296.7 ± 5.9	70.6
250	120	−	+	432.5 ± 10.0	188.0
250	0	HI	−	10.3 ± 1.2	1.2
250	105	HI	−	16.7 ± 2.1	2.1
0	0	+	−	6.3 ± 1.1	1.2
0	10	+	−	8.7 ± 0.8	1.3
0	25	+	−	7.3 ± 1.1	1.4
0	40	+	−	2.7 ± 1.3	0.6
0	105	+	−	6.7 ± 0.6	1.4
250	0	+	−	20.2 ± 1.2	7.2
250	10	+	−	314.8 ± 9.2	90.0
250	25	+	−	1091.0 ± 49.6	363.7
250	40	+	−	1328.7 ± 82.0	428.7
250	105	+	−	1364.7 ± 79.3	1137.5
			Concentration-dependent experiments		
0	60	−	+	9.8 ± 0.6	0.7
10	60	−	+	99.0 ± 3.5	6.4
50	60	−	+	297.5 ± 17.5	28.9
100	60	−	+	368.3 ± 16.3	38.4
200	60	−	+	607.8 ± 38.2	50.6
0	60	−	−	13.0 ± 2.5	1.2
10	60	−	−	11.5 ± 2.5	0.9
50	60	−	−	11.0 ± 2.6	1.0
200	60	−	−	24.8 ± 2.8	2.4
0	60	+	−	14.8 ± 3.5	0.4
10	60	+	−	99.3 ± 4.5	8.6
50	60	+	−	684.0 ± 13.5	90.8
100	60	+	−	1338.3 ± 27.2	151.0
200	60	+	−	1973.0 ± 12.0	8750.0

The results in Table 1 indicate that the plant cells activated 2-AF into a mutagen. The kinetics of mutation induction demonstrated a concentration-dependent response. The mean number of revertants per plate ranged from 9.8 at 0 μ*M* 2-AF to 607.8 at 200 μ*M* 2-AF. The positive controls of S-9 mix and the same concentration range of 2-AF showed a concentration-dependent response.

We disrupted *N. tabacum* cells with a pressure bomb to investigate the relationship of plant cytochrome P-450 and the activation of 2-AF. A 9000*g* supernatant fluid was analyzed for cytochrome P-450 with a spectrophotometer (Perkin-Elmer 552A) according to the methods of Omura and Sato (*9*). We detected 8.7 pmole of cytochrome P-450 per milligram of cellular protein. The mammalian S-9 that was used in the incubation experiments contained 1.3 nmole of cytochrome P-450 per milligram of protein. The concentrations of protein were equal in the coincubation and S-9 incubation experiments. The amount of cytochrome P-450 in the S-9 positive control was approximately 150 times greater than the amount in the coincubation experiments. The difference in activation was about three times greater for S-9 incubation than for plant coincubation in the concentration range of 10 to 100 μ*M* 2-AF. Thus the difference in the ratio of plant activation to S-9 activation of 2-AF is not concordant with the ratio of plant cytochrome P-450 to S-9 cytochrome P-450. These data suggest that the activation of 2-AF by plants may differ in some fundamental manner from mammalian microsomal activation.

The plant activation of 2-AF is enzymatic and exhibits time- and concentration-dependent kinetics. These kinetics encompass (i) the transport of 2-AF into the plant cell, (ii) its activation by the plant cell, (iii) the migration of a proximal mutagen or ultimate mutagen to a bacterium, and (iv) the induction of mutation in *S. typhimurium*.

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Quaternary Pollen Record from Laguna de Tagua Tagua, Chile

Abstract. *Pollen of southern beech and podocarp at Laguna de Tagua Tagua during the late Pleistocene indicates that cooler and more humid intervals were a feature of Ice Age climate at this subtropical latitude in Chile. The influence of the southern westerlies may have been greater at this time, and the effect of the Pacific anticyclone was apparently weakened. The climate today, wet in winter and dry in summer, supports broad sclerophyll vegetation that developed during the Holocene with the arrival of paleo-Indians and the extinction of mastodon and horse.*

The basin of Laguna de Tagua Tagua (34°30′S, 71°10′W), some 50 km^2 in area, is located 120 km southwest of Santiago, Chile, at an elevation of 200 m on the eastern edge of the coastal cordillera (Fig. 1). The basin is formed by Andean laharic deposits overlain by lacustrine sediments (*1*). Laguna de Tagua Tagua (*2*) was visited early in the 19th century by the naturalist Gay (*3*) who described the "*grand et superbe lac*" with its remarkable floating islands. Later, it was visited by Darwin (*4*) who also wrote of the islands: "As the wind blows, they pass from one side of the lake to the other, and often carry cattle and horses as passengers." In the mid-19th century, a ditch was dug to drain the laguna, at which time the bones of extinct Pleistocene mammals, notably mastodon and horse, were exhumed (*5*). At a depth of 2.3 to 2.4 m, artifacts of human industry were found in association with the animal remains. Charcoal from this level was dated at 11,380 ± 320 radiocarbon years before the present; the age of sediments 1.0 m deep is 6130 ± 115 years (*6, 7*).

The site of the laguna, at present largely cultivated, is surrounded by broad sclerophyll vegetation with hilltops over 500 m high occupied by species of deciduous southern beech, *Nothofagus obliqua* and *N. glauca* (*8, 9*). Evergreen beech, *N. dombeyi*, occurs in the Andes north to about 34°40′S at an altitude close to 1500 m where it grows with *N. obliqua*, *N. glauca*, and *N. alpina*. At about 35°50′S, *N. antarctica* reaches the northern end of its range at about 1750 m; *N. pumilio* is its associate north to about 36°40′S. Species of podocarp, important along with beech in the vegetational history at the laguna, are the montane *Podocarpus andinus*, which ranges to around 36°S in the Andes at altitudes of about 1700 m, and the lowland *P. salignus* to 35°30′S (*9, 10*). The regional climate is dry in summer, influenced by the Pacific anticyclone, and wet in winter when cyclonic storms of the southern westerlies move northward (*11*). Precipitation annually averages about 800 mm with 84 percent received during autumn and winter; summer is virtually without rain. Average temperature in summer is about 20°C and in winter 8°C (*12*).

The pollen stratigraphy (Fig. 2) of sediments in the basin was studied (*13*) from a 10.7-m core with an age of more than 45,000 years (QL-1674). The coring location is where an access road from the upland in the northeastern sector crosses the drainage ditch, some 1200 m from the shoreline before the lake was drained (*14*). Pollen of the chenopods and amaranths (Chenopodiaceae-Amaranthaceae), grasses (Gramineae), and composites (Compositae) dominates Holocene sediments (pollen assemblage zone 1). The chronological setting of zone 1 is estimated from the ages of 11,380 and 6130 years (*6, 7*). Percentages of chenopods and amaranths, indicative of warm and dry intervals, reach maximums in zone 1c and secondary peaks in zones 1a and 1e. Grasses increase in zone 1b, after about 6000 years ago along with trees, and in zone 1d. They increase in conjunction with *Gunnera* and Umbellif-

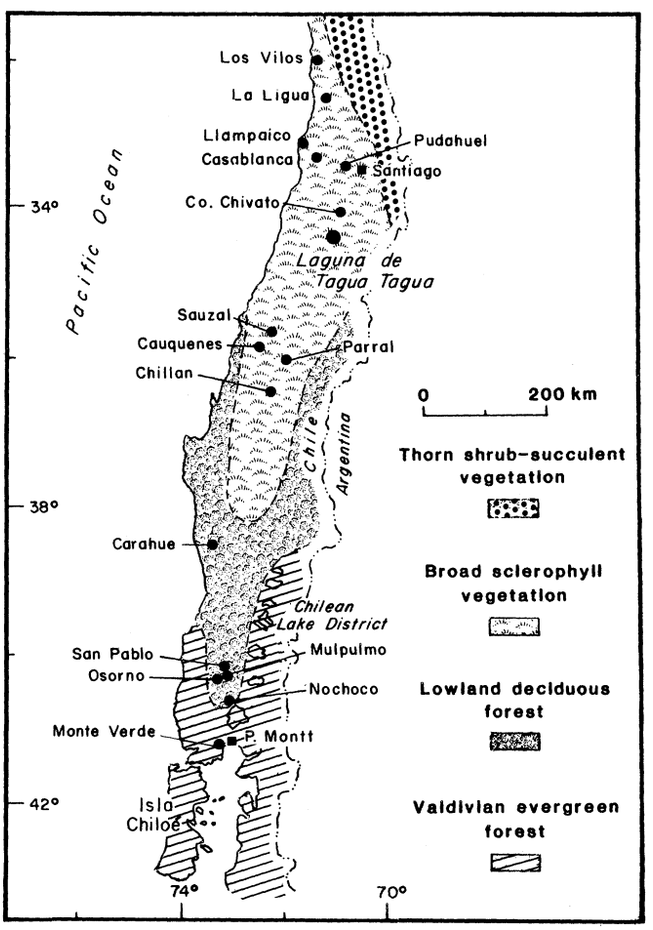

Fig. 1. Location of Laguna de Tagua Tagua in relation to zones of generalized vegetation (*8*) and distribution of late Pleistocene mastodon sites in central and southern Chile (*6, 22–24*).