should therefore be possible.

DMNA exhibits a covalent binding index for rat liver DNA of 6000 (Meier-Bratschi et al.., 1983); i.e., it is by a factor of 400 more potent than gyromitrin with respect to liver DNA methylation. The DMNA content in the average human diet amounts to approximately 1 μg/day (Spiegelhalder et al., 1980). Thus, a daily intake of 400 μg of gyromitrin would produce the same degree of methylation of liver DNA as an intake of 1 μg of DMNA. Hereby, it must be assumed that the dose-binding relationship is linear down to environmental doses of methylating agent. Good data are available to show that it is indeed the case for the generation of 7-methylguanine from doses of DMNA as little as 10 μg/kg (Diaz Gomez et al., 1977). The relatively low methylating potency of gyromitrin does not allow the detection of DNA methylations at such low dose levels. In a preliminary experiment with the first small batch of [^{3}H]gyromitrin, two rats were treated with 0.15 and 0.24 mg/kg. We could not detect radiolabel in the 7-methylguanine fraction of any DNA sample. The corresponding limit of detection, CBI < 33 and CBI < 19, respectively, for liver DNA was near the true value measured in the main experiment using a 50-fold higher dose.

In the dose range studied, there is therefore no indication for a higher methylating potency at lower doses, so that the liver DNA damage produced by DMNA and gyromitrin can tentatively be compared. Since the use of false morels can be easily avoided in contrast to the intake of DMNA in food, a much lower risk seems to be acceptable for gyromitrin. Although no rules exist for defining such risks, we believe that the acceptable DNA damage from gyromitrin should be at least 100 times lower than that from the ubiquitous and therefore unavoidable DMNA. The acceptable daily gyromitrin intake would then maximally amount to 400/100 = 4 μg. Pyysalo and Niskanen (1977) calculated a permitted intake of 35 μg of gyromitrin man^{-1} day^{-1} derived from the no-effect level of gyromitrin in chicken (0.05 mg kg^{-1} day^{-1}) divided by a security factor of 100. It is reasonable that our approach, based on potentially irreversible effects of gyromitrin, produces a lower limit for an acceptable daily intake.

The above risk estimate has to be based upon the assumption that our data in the rat can be extrapolated to humans. An additional uncertainty has to be faced when the acceptable levels of gyromitrin intake are to be expressed in terms of the whole mushroom. Reported contents range between 10 and 3000 mg/kg of dried mushroom (Pyysalo and Niskanen, 1977; Schmidlin-Mészaros, 1974; Stijve, 1978) so that 4 μg of gyromitrin can be present in as little as 0.4 g of mushroom or less. On the other hand, the chemical instability of gyromitrin during cooking must be taken into account. A conclusive risk estimate therefore will require an analysis of the amount of gyromitrin and other methylating agents on the fork.

ACKNOWLEDGMENT

We are indebted to P. Schmid for performing the GC/MS analyses.

Registry No. [*N*-methyl-^{3}H]Gyromitrin, 85650-34-6; formylhydrazine, 624-84-0; gyromitrin, 16568-02-8; 7-methylguanine, 578-76-7; acetaldehyde, 75-07-0; acetaldehyde *N*-formylhydrazone, 85650-35-7; [^{3}H]methyl iodide, 72165-55-0.

LITERATURE CITED

Boyd, M. R. In "Organ-Directed Toxicity"; Brown, S. S.; Davies, D. S., Eds.; Pergamon Press: Oxford, 1981; p 267.
Braun, R.; Greeff, U.; Netter, K. J. *Xenobiotica* **1980**, *10*, 557.
Diaz Gomez, M. I.; Swann, P. F.; Magee, P. N. *Biochem. J.* **1977**, *164*, 497.
Franke, S.; Freimuth, U.; List, P. H. *Arch. Toxikol.* **1967**, *22*, 293.
Guisti, G. V.; Carnevale, A. *Arch. Toxicol.* **1974**, *33*, 49.
Hawks, A.; Magee, P. N. *Br. J. Cancer* **1974**, *30*, 440.
Lawley, P. D. *IARC Sci. Publ.* **1976**, *No. 12*, 181.
List, P. H.; Luft, P. *Tetrahedron Lett.* **1967**, *20*, 1893.
List, P. H.; Luft, P. *Arch. Pharm. (Weinheim, Ger.)* **1968**, *301*, 294.
List, P. H.; Luft, P. *Arch. Pharm. (Weinheim, Ger.)* **1969**, *302*, 143.
Lutz, W. K. *Mutat. Res.* **1979**, *65*, 289.
Markov, G. G.; Ivanov, I. G. *Anal. Biochem.* **1974**, *59*, 555.
Meier-Bratschi, A.; Lutz, W. K.; Schlatter, C. *Food Chem. Toxicol.* **1983**, in press.
O'Connor, P. J. *J. Cancer Res. Clin. Oncol.* **1981**, *99*, 167.
Pyysalo, H.; Niskanen, A. *J. Agric. Food Chem.* **1977**, *25*, 644.
Schmidlin-Mészaros, J. *Mitt. Geb. Lebensmittelunters. Hyg.* **1974**, *65*, 453.
Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. *Oncology* **1980**, *37*, 211.
Stijve, T. *Mitt. Geb. Lebensmittelunters. Hyg.* **1978**, *69*, 492.
Toth, B. *Int. J. Cancer* **1972**, *9*, 109.
Toth, B.; Nagel, D. *J. Natl. Cancer Inst. (U.S.)* **1978**, *60*, 201.
Toth, B.; Shimizu, H. *Cancer Res.* **1973**, *33*, 2744.
Toth, B.; Smith, J. W.; Patil, K. D. *JNCI, J. Natl. Cancer Inst.* **1981**, *67*, 881.
Viviani, A.; Lutz, W. K. *Cancer Res.* **1978**, *38*, 4640.
von Wright, A.; Pyysalo, H.; Niskanen, A. *Toxicol. Lett.* **1978**, *2*, 261.

Annelis Meier-Bratschi
Brian M. Carden
Jürg Lüthy
Werner K. Lutz*
Christian Schlatter

Institute of Toxicology
Swiss Federal Institute of Technology and University of Zurich
CH-8603 Schwerzenbach, Switzerland

Received for review December 20, 1982. Accepted March 31, 1983. This work was financially supported by the Swiss Federal Office of Public Health. B.M.C. was supported by D.G.R.S.T., France.

Effect of Bioregulators on the Accumulation of Rubber in Guayule

Previous work on the use of bioregulators to increase the yield of rubber from guayule was expanded to include additional bioregulators, concentrations as low as 125 ppm, and longer treatment periods. A ^{13}C NMR analysis showed substantial increases in rubber as compared to that for the control. A 91% increase in total rubber content was obtained with 2-(3,4-dimethylphenoxy)triethylamine. No statistically significant differences in the percent rubber values were obtained.

Research interest in guayule (*Parthenium argentatum* A. Gray) continues to grow as the worldwide demand for natural rubber increases (D'Ianni et al., 1978). Previously we reported on the increase in rubber content of guayule

Table I. Effects of Several Bioregulators on Rubber Content of Guayule

treatment	rubber content[a]	%[b]
control	15.5 ± 3.9	5.3 ± 0.7
250 ppm of 2-(3,4-dichlorophenoxy)-triethylamine	23.6 ± 6.5	5.0 ± 1.4
125 ppm of 2-(3,4-dichlorophenoxy)-triethylamine	23.5 ± 6.2	6.7 ± 0.5
500 ppm of 2-(2,4-dichlorophenoxy)-triethylamine	24.3 ± 4.2	6.0 ± 1.5
1000 ppm of *N*-methylbenzylhexyl-amine	26.4 ± 7.4	6.6 ± 0.2
500 ppm of 2-(3,4-dimethylphenoxy)-triethylamine	29.6 ± 8.4	6.6 ± 0.7

[a] Grams of rubber per plant ± the standard deviation for four to six plants. [b] Percent of rubber ± the standard deviation for four to six plants.

plants treated with one bioregulator (the application of 5000 ppm of 2-(3,4-dichlorophenoxy)triethylamine caused the bioinduction of additional rubber) for a time period of only 10 months (Yokoyama et al., 1977). In the present report we extend this work to several other bioregulators, a treatment period of 27 months, and lower concentrations of bioregulator.

MATERIALS AND METHODS

The bioregulators reported here include 2-(3,4-dichlorophenoxy)triethylamine (125 and 250 ppm), 2-(2,4-dichlorophenoxy)triethylamine (500 ppm), *N*-methylbenzylhexylamine (1000 ppm), and 2-(3,4-dimethylphenoxy)triethylamine (500 ppm). The *N*-methylbenzylhexylamine was synthesized by the method of Poling et al. (1982). The rest were synthesized according to the procedures of Schuetz and Baldwin (1958). In addition to bioregulator, the spray formulation included 0.3% Ortho X-77 spreader (Chevron Chemical Co., San Francisco, CA) and 500 ppm of (diethylamino)ethanol.

Two-month-old greenhouse seedlings (N565 II variety) were planted by the California Department of Food and Agriculture on leased private farmland in the Bakersfield, CA, area in July 1979. Fertilization was accomplished by nutrient addition to the drip irrigation water. Plants were first treated with bioregulator 5 months after planting and at approximately 4-month intervals thereafter for a total of five treatments. Plants were treated by hand-sprayer application of the spray formulation until the entire plant was wetted and dripping (ca. 100 mL/plant). Control plants were not sprayed at all. Plants were harvested March 1982, air-dried, defoliated by hand, ground in a Model C comminuting machine (W. J. Fitzpatrick Co., Chicago, IL), and frozen for further analysis.

After drying overnight in an oven at 50 °C and grinding in a Wiley mill (20-mesh screen), we analyzed 0.3-g samples by a modification of the ^{13}C NMR method of Hayman et al. (1982). The B peak only in rubber's ^{13}C NMR spectra was integrated as described by Visintainer et al. (1981).

RESULTS AND DISCUSSION

From Table I it can be seen that there is a nearly 2-fold increase in total rubber for the 2-(3,4-dimethylphenoxy)-triethylamine treatment as compared to that for the control sample. The increase in rubber content for the other treatments was also substantial but less than that above. No substantial differences in the percentage figures were observed.

These results are reported as total rubber per plant in addition to the percentage figure more commonly reported in the literature [for example, see Tipton and Gregg (1980)]. There are two advantages to the total rubber content figures used here. First, the bias against larger more quickly growing plants is eliminated. That is, when the percentage method of reporting results is used, greater amounts of "induced rubber" in one of the two samples will be masked if the plant is also growing faster. Second, the commerical viability of guayule is dependent on the yield of rubber per acre of guayule, a figure more closely related to the units used here rather than to a percentage.

The figures in Table I are the means and standard deviation obtained from four to five replicate samples. Statistical analyses of the data (*F* test and *t* test) indicate significantly different results at the 90% or greater confidence level for the total rubber figures only.

Results from preliminary experimentation indicated lower concentrations of bioregulator applied at the earlier stages of development would be beneficial. This indeed seems the case as the concentration of bioregulator was lowered from 5000 ppm in earlier work to as low as 125 ppm in this report. The results from several other bioregulators and concentrations were not included because of insufficient sample size.

Work is continuing on the effectual use of bioregulators of guayule through a larger scale study.

ACKNOWLEDGMENT

We thank I. A. Siddiqui of the California Department of Food and Agriculture for his kind help.

Registry No. 2-(3,4-Dichlorophenoxy)triethylamine, 65202-07-5; 2-(2,4-dichlorophenoxy)triethylamine, 1974-14-7; *N*-methylbenzylhexylamine, 81404-52-6; 2-(3,4-dimethylphenoxy)-triethylamine, 63955-87-3.

LITERATURE CITED

D'Ianni, J. D.; Rogers, T. H.; Finelli, A. F.; Sinclair, E. A. In "Guayule, Reencuentro en el Desierto"; Guzman, G. W., Ed.; Centro de Investigacion en Quimica Applicada: Saltillo, Mexico, 1978.

Hayman, E. P.; Yokoyama, H.; Schuster, R. *J. Agric. Food Chem.* **1982**, *30*, 399.

Poling, S. M.; Hsu, W. J.; Yokoyama, H. *Phytochemistry* **1982**, *21*, 601.

Schuetz, R. D.; Baldwin, R. A. *J. Am. Chem. Soc.* **1958**, *80*, 162.

Tipton, J. L.; Gregg, E. C. *HortScience* **1980**, *15*, 87.

Visintainer, J.; Beebe, D. H.; Myers, J. W.; Hirst, R. C. *Anal. Chem.* **1981**, *53*, 1570.

Yokoyama, H.; Hayman, E. P.; Hsu, W. J.; Poling, S. M.; Bauman, A. J. *Science (Washington, D.C.)* **1977**, *197*, 1976.

Ernest Hayman*
Henry Yokoyama
Seth Gold

Fruit and Vegetable Chemistry Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Pasadena, California 91106

Received for review December 20, 1982. Accepted May 23, 1983. This investigation was supported in part from funds from the National Science Foundation under Grant EFR 78-09567. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.