ATTACHMENT I--FINAL RISK ASSESSMENT FOR SACCHAROMYCES UVARUM

(February 1997)

I. INTRODUCTION

Saccharomyces uvarum is one species of a genus which has an extensive history of use in the fermentation and food processing industry. S. uvarum, also called S. bayanus, is referred to as a "wine yeast" due to their broad use in the production of wines. Taxonomically, S. uvarum is closely related to Saccharomyces cerevisiae. S. cerevesiae is the used extensively in the baking of bread and the production of beer. S. cerevisiae is the subject of extensive genetic research and genomic mapping. It is expected that research on S. cerevisiae will lead to greater understanding and better characterization of the entire genus including S. uvarum. Despite S. uvarum's broad use and exposure, there is no reported incidence of adverse effects to either humans or the environment.

History of Commercial Use and Products Subject to TSCA Jurisdiction

Saccharomyces uvarum has a history of safe use. It is widely used in the making of beer and wine and in alcohol production and is found most frequently in grape must and wine. The commercial use of the genus Saccharomyces arose from the fermentation of small grains and fruit for the production of alcoholic beverages. Schwann coined the term "Zuckerpilz" or "sugar fungus" to describe the small bodies in beer. The genus name, Saccharomyces, was derived from this term. At the present time Saccharomyces is not used for the production of so-called specialty chemicals (e.g., antibiotics, culturable enzymes, etc.); however, these yeasts are used to produce alcohol for beverages and industrial purposes. Alcoholic beverages are regulated under statutes other than TSCA, although the use of alcohol for industrial purposes may fall under TSCA jurisdiction (Stewart and Russell, 1985). There are also a limited number of reports in the literature about the production of specialty proteins (El-Refai et al., 1985).

Saccharomyces is the organism of choice for the production of alcohols due to its high level of metabolic activity and its tolerance of high alcohol concentrations. The selection of species is based upon the stock (medium) used in the fermentation system and the requirement for alcoholic tolerance. With the rising costs of conventional energy sources there is a shifting

to "alternative" fuels which include the generation of alcohol from various sources (Stewart and Russell, 1985).

II. IDENTIFICATION AND CLASSIFICATION

A. Taxonomy and Characterization

Saccharomyces uvarum is, under most conditions, a poorly sporogenous yeast most commonly used in the production of alcoholic beverages and vitamin assays. This organism falls into the category of yeasts referred to as the "wine yeasts" due to the broad utility of these fungi in the production of wines. In addition to S. uvarum, the other fungi that comprise the wine fungi are Saccharomyces cerevisiae, the yeast used in the production of beer, Saccharomyces chevalieri, Saccharomyces bayanus, and Saccharomyces italicus (Rosini et al., 1982). The wine yeasts are characterized by both an ability to ferment sugars at a high rate and a high tolerance to alcohol. S. uvarum is a well-characterized species based on morphological and biochemical characteristics.

Morphologically, S. uvarum has been described as spheroidal, ovoid, ellipsoidal, cylindrical or elongate in shape. There are also some filamentous forms. The nonfilamentous forms can occur singularly, in pairs, or clusters. Strains of this species have been determined by the size of the individual cells.

Beyond the use of morphological criteria for taxonomy, scientists have historically applied the use of fermentation of selective sugars as criteria for speciation. However, the relative facility with which Saccharomyces spp. changes its fermentation patterns limits the utility of sugar metabolism as a criterion for speciation.

DNA homology studies have been employed as tools to delineate species within the genus but with conflicting results. Rosini et al. (1982) tested over 1,000 strains of yeast (classified as S. cerevisiae, S. uvarum, S. italicus, S. bayanus, and S. chevalieri) for DNA homology employing both reassociation and DNA composition. They found a high degree of relatedness between S. uvarum and S. bayanus. However, the DNA homology criteria was at odds with the conventional systematic criteria. The authors noted that despite the high degree of homology between S. uvarum and S. bayanus, the fermentation potential of the two strains varied substantially.

Despite the discrepancy between the phenotypic traits and genotype, there appears to be a weight of data that supports the inclusion of S. bayanus with S. uvarum. Martini and Kurtzman (1985) have presented what appears to be the definitive classification on Saccharomyces spp. They have noted the

shortcomings of the current classification system and characters employed and recommended the use of DNA homology studies to develop the new scheme. The authors have proposed a reclassification of S. uvarum into S. bayanus. However, none of these organisms or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

B. Related Species of Concern

None of the above strains or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

III. HAZARD ASSESSMENT

A. Human Health Hazards

1. Colonization and Pathogenicity

S. uvarum has been used for a variety of purposes throughout history, predominantly for the processing of food. This history of use is noted by Stewart and Russell (1985) who remarked "No other group of microorganisms has been more intimately associated with the progress and well-being of the human race than Saccharomyces cerevisiae and its closely related species". Saccharomyces uvarum has been used extensively in the production of beer and other foodstuffs, and for the production of ethanol. This legacy provides a history of long use. Within this record, there are no reported incidences of adverse effects to humans from S. uvarum. This experience is consistent not only with S. uvarum, but also with those species that are proposed to comprise the new genus S. bayanus.

The history of use of S. uvarum extends through several scenarios of exposure. Industrial application, particularly in the earlier periods of use, involved substantial human exposure both in the production facility and the research environment. Presently, the consumption of yeast (generic application) is a common source of vitamins. While the condition of "data linking the organism to disease" can be taken as an exculpatory evidence for pathogenicity, a history of significant exposure with incidence of disease in a nondebilitated condition contributes to a history of safe use.

2. Pathogenicity of closely related species for Humans

Closely related species also have a history of extensive use without significant incidence of disease. The most intensely studied of those species closely related to S. uvarum (bayanus)

is S. cerevisiae. S. cerevisiae is not considered a pathogenic microorganism, but has rarely been reported as a cause of opportunistic infections. Eng et al. (1984) described five cases of such infections and reviewed the literature on eight other S. cervisiae infections (also briefly reviewed by Walsh and Pizzo, 1988). All of the patients had underlying disease. Some of them had also received antibiotic therapy, thereby suppressing normal bacterial flora and allowing mycotic organisms to become established.

A low concern for the pathogenicity of S. cerevisiae is also illustrated by a series of surveys conducted at hospitals over the last several years. S. cerevisiae accounted for less than 1% of all yeast infections isolated at a cancer hospital and in most of the cases the organism was isolated from the respiratory system (Kiehn et al., 1980). At Yale-New Haven Hospital over the past five years, there have been 50 isolates of S. cerevisiae recovered from patients; however, most of the isolates were considered contaminants (Dynamac, 1991).

3. Toxin Production

There were no reports found in the literature that indicate that S. uvarum produces toxins to humans or animals.

4. Measure of the Degree of Virulence

Although information is not available on the potential virulence of S. uvarum, there is information available on the closely related S. cerevisiae. A number of individual virulence factors have been identified as being associated with the ability of yeasts to cause disease. The principal virulence factors associated with yeasts appear to be phospholipase A and lysophospholipase. It is believed that these enzymes enhance the ability of the yeast to adhere to the cell-wall surface and result in colonization as a first step in the infectious process. Nonpathogenic yeast had considerably lower phospholipase activities. Of a wide range of fungi assayed for phospholipase production, S. cerevisiae was found to have the lowest level of activity (Barrett-Bee et al., 1985). Therefore, based on the phospholipase virulence factor S. cerevisiae is considered a nonpathogenic yeast.

A second factor associated with virulence in yeast is the ability of a fungus to impair the host's immune capabilities. The cell walls of most fungi have the capacity to impede the immune response of the host. In a study that determined the overall pathogenicity of a number of yeasts used in industrial processes animals exposed to both high levels of S. cerevisiae, and cortisone demonstrated a greater ability of the fungus to colonize compared with those animals treated with only the yeast. However, the animals suffered no ill-effects from the

introduction of S. cerevisiae, (Holzschu et al., 1979). Therefore, this study suggests that even with the addition of high levels of an immuosuppresive agent, S. cerevisiae appears to be nonpathogenic.

5. Conclusions

There is no information in the literature associating S. uvarum with pathogenicity in humans. Although the absence of data on the potential pathogenicity of an organism usually cannot serve as confirmation of lack of toxicity, the legacy of use and continuous exposure, both through ambient exposure and industrial contact, contribute to an extensive history of safe use. There is much more information available on the related species S. cerevisiae due to its more widespread use. Even with this organism, reported incidences of infection are extremely rare and invariably in individuals with existing debilitating conditions. The body of evidence clearly indicates that the S. uvarum component of S. bayanis would not be expected to produce disease states with industrial application as long as no traits that would enhance the infectivity, ability to colonize, and virulence or toxin producing capability of organism are introduced.

S. uvarum has been used extensively for a great many years for making beer and wine and for alcohol production under conditions which at times must have resulted in considerable human exposure with no recorded instances of colonization or infection. Nor has it been reported to cause disease in animals.

Since S. uvarum has no reported history of pathogenicity during its long and extensive use, and its closely related species have but very low reported instances of infection, full exemption status is recommended.

B. Environmental Hazards

S. uvarum has been isolated from such natural sites as honey, phyllosphere, on the surfaces and inside rotten fruit, and in fruit juice. Despite the ubiquity of S. uvarum in nature there are no reports in the literature indicating that the organism is pathogenic to animals, plants or other microorganisms. Furthermore, there are no reports of this organism producing toxins against animals or plants.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

S. uvarum is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Recombinant

DNA Molecules (U.S. Department of Health and Human Services, 1986).

No data were available for assessing the release and survival specifically for fermentation facilities using S. uvarum. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, i.e., near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

S. uvarum is a normal inhabitant of soils and the surfaces of plants. S. uvarum is capable of utilizing a diverse group of substrates as carbon and nitrogen sources. The nutritional characteristics, along with the ability to produce ascospores, enhances the ability of the organism to survive in nature (Versar, 1992).

2. Releases

Estimates of the number of S. uvarum organisms released during production are tabulated in Table 1 (Reilly, 1991). The uncontrolled/untreated scenario assumes no control features for the fermentor off-gases, and no inactivation of the fermentation broth for the liquid and solid waste releases. The containment criteria required for the full exemption scenario assume the use of features or equipment that minimize the number of viable cells in the fermentor off-gases. They also assume inactivation procedures resulting in a validated 6-log reduction of the number of viable microorganisms in the liquid and solid wastes relative to the maximum cell density of the fermentation broth.

TABLE 1. Estimated Number of Viable Saccharomyces uvarum Organisms Released During Production

Release Media	Uncontrolled/ Untreated (cfu/day)	Full Exemption (cfu/day)	Release (days/year)
Air Vents	$2x10^{8}$ - 1x1011	$<2x10^{8}$ - 1x1011	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{12}$	$7x10^{6}$	90
Soil/Landfill	$7x10^{14}$	$7x10^{8}$	90

Source: Reilly, 1991

These are "worst-case" estimates which assume that the the maximum cell density in the fermentation broth for fungi is 10^{7} cfu/ml, with a fermentor size of 70,000 liters, and the separation efficiency for the rotary drum filter is 99 percent.

3. Air

Specific data which indicate the survivability of S. uvarum in the atmosphere after release are currently unavailable. Survival of vegetative cells during aerosolization is typically limited due to stresses such as shear forces, desiccation, temperature, and UV light exposure. As with naturally-occurring strains, human exposure may occur via inhalation as the organisms are dispersed in the atmosphere attached to dust particles, or lofted through mechanical or air disturbance.

Air releases from fermentor off-gas could potentially result in nonoccupational inhalation exposures due to point source releases. To estimate exposures from this source, the sector averaging form of the Gaussian algorithm described in Turner (1970) was used. For purposes of this assessment, a release height of 3 meters and downward contact at a distance of 100 meters were assumed. Assuming that there is no removal of organisms by controls/equipment for off-gases, potential human inhalation dose rates are estimated to range from 3.0×10^3 to 1.5×10^6 cfu/year for the uncontrolled/untreated scenario and less than that for systems with full exemptions. It should be noted that these estimates represent hypothetical exposures under reasonable worst case conditions (Versar, 1992).

4. Water

The concentrations of S. uvarum in surface water were estimated using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/l). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers (IFD) database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers facilities that have a NPDES permit to discharge to surface water). Tenth percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year period. The use of this methodology to estimate concentrations of S. uvarum in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process. Estimated concentrations of S. uvarum in surface water for the uncontrolled/untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. Saccharomyces uvarum Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	7Q10	Mean	7Q10
Uncontrolled/Untreated				
10th Percentile	156	5.60	4.5×10^{4}	1.25×10^{6}
50th Percentile	768	68.13	9.11×10^{3}	1.03×10^{5}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{-2}	1.25×100^{0}
50th Percentile	768	68.13	9.11×10^{-3}	1.03×10^{-1}

*MLD = million liters per day
Source: Versar, 1992

5. Soil

Since soil is a natural habitat for S. uvarum, it would be expected to survive well in soil. These releases could result in human and environmental exposure (Versar, 1992). However, it is anticipated that industrial strains which have adapted to well-defined media would be less competitive than the original wild-type strain (Sayre, 1992).

6. Summary

Although direct monitoring data are unavailable, worst case estimates do not suggest high levels of exposure of S. uvarum to either workers or the public resulting from normal fermentation operations.

V. INTEGRATED RISK ASSESSMENT

A. Discussion

There is an extensive history of use of and exposure to S. uvarum with no record of adverse effects to the environment or human health. Yeast has been used for centuries as a leavening for bread and fermenter of beer without records of virulence.

S. uvarum is not a plant or animal pathogen. Although it has been used extensively in the fermentation and food processing industry, S. uvarum has not been found to be associated with disease conditions in plants or animals.

There is the likelihood that the taxonomy of S. uvarum will be modified in the future. The problem with taxonomy is not considered a risk issue. There are no closely related yeasts which pose a human or ecological hazard. Worker and non-occupational human exposures are expected to be low given the conditions of this exemption.

B. Recommendation

Saccharomyces uvarum is recommended for the tiered exemption.

VI. REFERENCES

21 CFR 184.1983.

Anderson, A. 1992. Yeast genome project: 300,000 and counting. Science 256:462.

Barrett-Bree, Y. Hayes, R.G. Wilson, and J.F. Ryley. 1985. A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. J. Gen. Microbiol. 131:1217-1221.

Bigelis, R. 1985. Primary metabolism and industrial fermentations. in Gene Manipulations in Fungi. J.W. Bennet and L.L. Lasure (ed.) Academic Press, New York, NY. PP 357-

Brondz, I., and I. Olsen. 1990. Multivariate analyses of cellular carbohydrates and fatty acids of Candida albicans, Torulopsis glabrata, and Saccharomyces cerevisiae. J. Clin. Microbiol. 28:1854-1857.

Buesching, W.J., K. Kurek, and G.D. Roberts. 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. J. Clin. Microbiol. 9565-569.

Bussey, H., T. Vernet, and A.-M. Sdicu. 1988. Mutual antagonism among killer yeasts: competition between K1 and K2 killers and a novel cDNA-based K1-K2 killer strain of Saccharomyces cerevisiae. Can. J. Microbiol. 34:38-44.

Dynamac. 1990. Evaluation of microorganisms for possible exemption under TSCA Section 5. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Dynamac. 1991. Human health assessment for Saccharomyces cerevisiae. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Holzschu, D.L., F.W. Chandler, L. Ajello, and D.G. Ahearn. 1979. Evaluation of industrial yeasts for pathogenicity. Sabouraudia 17:71-78.

Kiehn, T.E., F.F. Edwards, and D. Armstrong. 1980. The prevalence of yeasts in gastrointestinal inoculation in antibiotic treated mice. Sabouraudia 21:27-33.

Kough, J. 1990. Clarification of taxonomic questions on Saccharomyces cerevisiae. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Martini, A. and C. Kurtzman. 1985. Deoxyribonucleic acid relatedness among species of the genus Saccharomyces sensu stricto. Int. J. System. Bacteriol. 35:508-511.

Nobre, G.N., and A.F. Ferreira. 1986. Enhancement of Streptococcus faecalis infection and complement depletion in yeast-treated mice. J. Gen. Microbiol. 132:1277-1281.

Organization for Economic Cooperation and Development. 1986. Recombinant DNA Safety Considerations. Paris, France.

Phaff, H., M.Miller, and E. Mrak. 1966. The life of yeasts. Harvard University Press. Boston, MA.

Reilly, B. 1991. Analysis of environmental releases and occupational exposure in support of the proposed TSCA 5(h)(4) exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Rosini, G., F. Federici, A.E. Vaughn, and A. Martini. 1982. Systematics of the species of the yeast genus Saccharomyces associated with the fermentation industry. Eur. J. Appl. Biotechnol. 15:188-193.

Sayre, P. 1991. Environmental hazard assessment of Saccharomyces cerevisiae for proposed 5(h)4 exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Stewart, G.C. and I. Russell. 1985. The biology of Saccharomyces, pp. 511-536. In A.L. Demain and N.A. Solomon, (eds.), Biology of industrial organisms. Benjamin Cummins Publishers, Menlo Park.

Tao, J., I. Ginsberg, N. Banerjee, W. Held, Y. Koltin, and J.A. Bruenn. 1990. Ustilago maydis KP6 Killer toxin: structure, expression in Saccharomyces cerevisiae, and relationship to other cellular toxins. Mol. Cell. Biol. 10:1373-1381.

Thayer, D.W. 1990. Personnel communication with C. Felkner. (Cited in Dynamac, 1990).

U.S. Department of Health and Human Services. 1986. Guidelines for research involving recombinant DNA molecules, Appendix F. 51 FR 16971.

Van der Walt, J. 1971. Saccharomyces, pp. 597-605. In J.Lodder, (ed.), The yeasts, a taxonomic study. North Holland Publ. Co. Amsterdam.

Versar. 1992. Screening level exposure assessment of Saccharomyces uvarum for 5(h)(4) exemption under the proposed biotech rule. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Wolochow, H., G.J. Hildegrand, and C. Lamanna. 1961. Translocation of microorganisms across the intestinal wall of the rat: effect of microbial size and concentration. J. Infect. Dis. 116:523-528.