ATTACHMENT I--FINAL RISK ASSESSMENT OF SACCHAROMYCES CEREVISIAE

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I. INTRODUCTION

Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages. With a prolonged history of industrial applications, this yeast has been either the subject of or model for various studies in the principles of microbiology. Jacob Henle based his theories of disease transmission on studies of strains of Brewer's Yeast. Currently, S. cerevisiae is the subject of a major international effort to characterize a eucaryotic genome (Anderson, 1992).

History of Commercial Use and Products Subject to TSCA Jurisdiction

Saccharomyces cerevisiae, in addition to its use in food processing, is widely used for the production of macromolecular cellular components such as lipids, proteins including enzymes, and vitamins (Bigelis, 1985; Stewart and Russell, 1985).

The Food and Drug Administration rates Brewer's Yeast extract as Generally Recognized as Safe (FDA, 1986). Furthermore, the National Institutes of Health in its Guidelines for Research Involving Recombinant DNA Molecules (DHHS, 1986) considers S. cerevisiae a safe organism. Most experiments involving S. cerevisiae have been exempted from the NIH Guidelines based on an analysis of safety (see Appendix C-II of the NIH Guidelines). While alcoholic beverages, vitamins, and bread leavening are covered under the Federal Food, Drug and Cosmetic Act, the production of enzymes and other macromolecules may be subject to TSCA regulation. The abundance of information on S. cerevisiae, derived from its role in industry, has positioned it as a primary model for genetic studies and, by extension, as a strong candidate for genetic manipulation for TSCA applications (Dynamac, 1990).

II. IDENTIFICATION AND CLASSIFICATION

A. Taxonomy and Characterization

Saccharomyces cerevisiae is a yeast. The organism can exist either as a single-celled organism or as pseudomycelia. The cells reproduce by multilateral budding. It produces from one to four ellipsoidal, smooth-walled ascospores. S. cerevisiae can be differentiated from other yeasts based on growth characteristics and physiological traits: principally the ability to ferment individual sugars. Clinical identification of yeast is conducted using commercially available diagnostic kits which classify the organism through analysis of the ability of the yeast to utilize distinct carbohydrates as sole sources of carbon (Buesching et al., 1979; Rosini et al., 1982). More recently, developments in systematics have led to the design of sophisticated techniques for classification, including gas-liquid chromatography of lysed whole cells (Brondz and Olsen, 1979).

As a result of the application of newer techniques arising from innovative approaches, the taxonomy of Saccharomyces is subject to greater scrutiny. The initial classification was based principally on morphological characteristics with specific physiological and biochemical traits used to differentiate between isolates with similar morphological traits. Using these criteria, there are as many as 18 species listed in the literature. In addition, what had been classified as one large heterogeneous species, S. cerevisiae, may, in the future, be divided into four distinct species based on DNA homology studies. The four species are S. cerevisiae, S. bayanus (also known as S. uvarum), S. pasteurianus (also known as S. carlsbergensis), and S. paradoxus. All four represent industrially important species. 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.

Any assessment of Saccharomyces must take into consideration the malleability of the current classification. For this assessment of S. cerevisiae the reviews of the organism are based on the classification proposed by Van der Walt (1971).

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. cerevisiae is a commonly used industrial microorganism and is ubiquitous in nature, being present on fruits and

vegetables. Industrial workers and the general public come into contact with S. cerevisiae on a daily basis through both inhalation and ingestion (see section IV). Saccharomyces spp. are frequently recovered from the stools and throats of normally healthy individuals. This indicates that humans are in constant contact with these yeasts.

There are individuals who may ingest large quantities of S. cerevisiae every day, for example, people who take the yeast as part of a "health food" regimen. Therefore, studies were conducted to ascertain whether the ingestion of large numbers of these yeasts might result in either colonization, or colonization and secondary spread to other organs of the body. It was found that the installation of very large numbers of S. cerevisiae into the colons of animals would result in both colonization and passage of the yeasts to draining lymph nodes. It required up to 10^{10} S. cerevisiae in a single oral treatment to rats to achieve a detectable passage from the intestine to the lymph nodes (Wolochow et al., 1961). The concentrations of S. cerevisiae required were well beyond those that would be encountered through normal human daily exposure.

S. cerevisiae is not considered a pathogenic microorganism, but has been reported rarely as a cause of opportunistic infections. Eng et al. (1984) described five cases of such infections and reviewed the literature on eight other S. cerevisiae infections (also briefly reviewed by Walsh and Pizzo, 1988). All of the patients in the cases 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).

2. Toxin Production

There have been no reports of isolates of S. cerevisiae that produce toxins against either humans or animals. However, S. cerevisiae has been shown to produce toxins against other yeasts. These toxins, termed "killer toxins", are proteins or glycoproteins produced by a range of yeasts. The yeasts have been genetically modified to alter activity and are used in industrial settings as a means of controlling contamination of fermentation systems by other yeasts (Sid et al., 1988).

3. Measure of the Degree of Virulence

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

4. Ability to Transfer Virulence Factor Genes

S. cerevisiae does not carry virulence factors to humans or animals. However, the species does carry linear, double-stranded plasmids which can be transmitted to other Saccharomyces. These plasmids carry genes that encode the "killer toxins" discussed above can be transferred from one Saccharomyces to another. Therefore, gene constructs involving the incorporation of traits using these linear plasmids should be considered to be nonstable.

5. Summary

In conclusion, S. cerevisiae is a organism which has an extensive history of safe use. Despite considerable use of the organism in research and the presence of S. cerevisiae in food, there are limited reports in the literature of its pathogenicity to humans or animals, and only in those cases where the human had a debilitating condition. Factors associated with the virulence of yeasts (i.e., phospholipases) indicate that this organism is nonpathogenic. The organism has not been shown to produce toxins to humans.

B. Environmental Hazards

S. cerevisiae is ubiquitous in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. S. cerevisiae is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables (Phaff et al., 1966). The only adverse effect to the environment noted in the literature is the presence of the "killer toxins" which is active against other strains of Saccharomyces.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

S. cerevisiae 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. cerevisiae. 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. cerevisiae is a normal inhabitant of soils and is widespread in nature. S. cerevisiae is able to take up a wide variety of sugars and amino acids. These traits enhance the organism's ability for long term survival. S. cerevisiae can be isolated from fruits and grains and other materials with a high concentration of carbohydrates (LaVeck, 1991).

2. Releases

Estimates of the number of S. cerevisiae 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 cerevisiae 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 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. cerevisiae 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. cerevisiae 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. cerevisiae 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. cerevisiae in surface water for the uncontrolled/untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. S. cerevisiae 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. cerevisiae, it would be expected to survive well in soil. These releases could result in human and environmental exposure (Versar, 1992). It is

currently estimated that over one million tons of naturally-occurring yeast are produced annually during brewing and distilling practices (LaVeck, 1991).

6. Summary

Although direct monitoring data are unavailable, worst case estimates do not suggest high levels of exposure of S. cerevisiae 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. cerevisiae with a very limited 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. cerevisiae is currently classified as a class 1 containment organism under the NIH Guidelines based largely on the extensive history of safe use.

Factors associated with the development of disease states in fungi have been reviewed. Data suggests that only with the ingestion of high levels of S. cerevisiae or with the use of immunosuppressants can S. cerevisiae colonize in the body. Even under those conditions, there were no noted adverse effects. In the few cases which S. cerevisiae was found in association with a disease state, the host was a debilitated individual, generally with an impaired immune system. In other cases the organism was recovered from an immunologically privileged site (i.e., respiratory tract). Many scientists believe that under appropriate conditions any microorganism could serve as an opportunistic pathogen. The cases noted in the above Human Health Assessment, where S. cerevisiae was found in association with a disease state, appear to be classic examples of opportunistic pathogenicity (see III.A.3).

The organism is not a plant or animal pathogen. Despite the fact that S. cerevisiae is ubiquitous in nature, it has not been found to be associated with disease conditions in plants or animals. The only adverse environmental condition that was noted is the production of "killer toxins" by some strains of the yeast. These toxins have a target range that is limited to susceptible yeasts. The toxins, proteins and glycoproteins, are not expected to have a broad environmental effect based largely on the anticipated short persistence of the toxins in soil or

water and by the limited target range. S. cerevisiae "killer toxin" has been used industrially to provide a level of protection against contamination by other yeasts in the fermentation beer.

The current taxonomy of Saccharomyces is under revision based on the development of alternative criteria. However, this should not have a major effect on the risk associated with closely related species. Saccharomyces, as a genus, present low risk to human health or the environment. Criteria used to differentiate between species are based on their ability to utilize specific carbohydrates without relevance to pathogenicity. Nonetheless, this risk assessment applies to those organisms that fall under the classical definition of S. cerevisiae as described by van der Walt (1971).

S. cerevisiae is a ubiquitous organism which, despite its broad exposure, has very limited reported incidence of adverse effects. The extensive history of use, the diversity of products currently produced by the organism, and the attention given this organism as a model for genetic studies collectively makes this organism a prime candidate for full exemption. The increased knowledge derived from the ongoing research should further enhance this organisms' biotechnological uses.

B. Recommendation

Saccharomyces cerevisiae 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.

LaVeck, G. 1991. Exposure assessments of microorganisms considered for 5(h)(4) exemptions under the proposed biotech

rule. 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; Notice. 51 FR 16958-16985.

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 the 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.