

**ATTACHMENT I--FINAL RISK ASSESSMENT OF  
*Aspergillus oryzae***

(February 1997)

**I. INTRODUCTION**

*Aspergillus oryzae* is an asexual, ascomycetous fungus used for hundreds of years in the production of soy sauce, miso and sake without recorded incidents. There are conflicting opinions about whether *A. oryzae* can be isolated in nature. Although the details of the genetic relationship between *A. oryzae* and *A. flavus* remain unclear, the two species are so closely related that all strains of *A. oryzae* are regarded by some as natural variants of *A. flavus* modified through years of selection for fermenting of foods. *A. oryzae* is regarded as not being pathogenic for plants or animals, though there are a handful of reports of isolation of *A. oryzae* from patients. There are also several reports of products of *A. oryzae* fermentations, e.g.  $\alpha$ -amylase, that seem to be associated with allergic responses in certain occupations with high exposure to those materials. *A. oryzae* can produce a variety of mycotoxins when fermentation is extended beyond the usual time needed for production of these foods. While wild *A. flavus* isolates readily produce aflatoxins and other mycotoxins, *A. oryzae* has not been shown to be capable of aflatoxin production.

**History of Commercial Use and Products Subject to TSCA  
Jurisdiction**

*Aspergillus oryzae* has apparently been an essential part of oriental food production for centuries and is now used in the production of many different oriental foods such as soy sauce, sake and miso. Potential uses under TSCA include fermentations of numerous enzymes, e.g., amylase, protease, B-galactosidase, lipase, and cellulase, and organic compounds such as glutamic acid. While these products have a variety of potential commercial uses, some of them are mostly frequently used in food processing.

The experience of safe commercial use of *A. oryzae* is extraordinarily well established. As a "koji" mold it has been used safely in the food industry for several hundred years. *A. oryzae* is also used to produce livestock probiotic feed supplements. Even the commercialization of byproducts of the fermentation was established nearly a century ago. The "koji" mold enzymes were among the first to be isolated and commercialized. In 1894, Dr. J. Takamine isolated and sold

Takadiastase from a commercial firm he started in Clifton, New Jersey (Bennett, 1985a).

EPA has reviewed, under TSCA, two genetically modified strains of *A. oryzae* used for the production of enzymes (Premanufacture Notice (PMN) numbers P89-134 and P94-1475).

## II. IDENTIFICATION AND TAXONOMY

### A. Overview

The candidate species is a member of the genus *Aspergillus* and belongs to the group of fungi that are generally considered to reproduce asexually (Fungi Imperfecti or Deuteromycetes), although perfect forms (forms that reproduce sexually) of some aspergilli have been found. The form genus *Aspergillus* represents a taxonomic grouping of a very large number of asexual fungi which are characterized by the production of spores on large black or brown conidia in phialides arranged on a characteristic spherical conidiophore termed the vesicle. This definition leads to inclusion of a complex assortment of organisms within the taxon. To simplify the taxonomy of such a large number of organisms, the genus *Aspergillus* has been divided into sections or groups based on color, size and roughness of the spore, conidiophore and vesicle as well as the arrangement of phialides and the presence of sclerotia. The separation of individual species into groups is somewhat tenuous and based on distinguishing measured characters with overlapping means. This resulted in the 132 species arranged in 18 groups by Raper and Fennell (1965) due to overlapping morphological or physiological characteristics. However, it is important to remember that taxonomy is "dealing with living variable organisms and that species and group concepts must be reasonably elastic" (Raper & Fennell, 1965).

As is the case of many fungi, the taxonomy of *Aspergillus* is primarily based on morphological features, rather than physiological, biochemical features and genetic characteristics often used to classify bacteria. Nomenclature problems of the genus *Aspergillus* arise from their pleomorphic life cycle. The newer findings show that this group of fungi has both a perfect (teleomorphic) and an imperfect (anamorphic) state.

The morphological approach to taxonomy has led to the existence of several synonyms for the genus *Aspergillus*. They are: *Alliospora* Pim; *Aspergillonsis* Spegazzini; *Cladaspergillus* Ritg; *Cladosparum* Yuill and Yuill; *Euaspergilus* Ludwig; *Gutturomyces* Rivolta; *Raperia* Subramaniam and Grove; *Sceptromyces* Corda; *Spermatoloncha* Spegazzini; *Sphaeromyces* Montagne; *Sterigmatocystis* Cramer; and *Stilbothamnium* Hennings (Bennett, 1985b).

Aspergilli are ubiquitous in nature. They are geographically widely distributed and have been observed in a broad range of habitats, because they can colonize a wide variety of substrates.

### **B. The *Aspergillus flavus* Group**

*Aspergillus oryzae* is a member of the *A. flavus* group of *Aspergillus* species. The *A. flavus* group, which also now includes *A. sojae*, *A. nomius* and *A. parasiticus* (see below) is defined by the production of spore chains in radiating heads which range in color from yellow-green to olive brown. The conidiophores are roughened and colorless. The spores themselves have conspicuous ridges and echinulations (spines). Sclerotia are occasionally produced (Raper & Fennell, 1965). *A. oryzae/flavus* species have never been connected to a sexual or teleomorphic stage. However, the teleomorphic stages of other *Aspergillus* species have been demonstrated by the formation of cleistothecia. These species belong to the genera *Emericella*, *Neosartorya* and *Eurotium*, all belonging to the ascomycetous family *Eurotiaceae* (Fennel, 1973). Either the sexual stages of the *A. flavus* group have not been recognized as such, being identified as completely different species based on morphology, or this group of fungi are "degenerate", having lost the ability to form sexual spores and mycelia.

*A. oryzae* is considered by some experts to be a domesticated variant of *A. flavus* (Kurtzman et al. 1986). Through long-time use, *A. oryzae* strains seem to have been selected to exhibit reduced sporulation, have more aerial mycelia and exhibit no environmental survival structures like sclerotia or the presence of aflatoxins that might function to inhibit grazing by insects. These morphological features that differentiate *A. oryzae* from *A. flavus* may represent adaptations to the artificial culture conditions of the koji fermentation. Misidentification of new isolates not obtained from well established cultures is always a possibility, since the key morphological differences between the two species seem related to culture adaptation. However, the source of *A. oryzae* strains for industrial fermentations today is likely to be standard culture collections. Environmental isolates of aspergilli would likely be identified as *A. flavus* rather than the laboratory-adapted *A. oryzae*.

### **C. Related Species of Concern**

The taxonomy of *Aspergillus* has public health implications due to the production of potent mycotoxins by members of this genus. Most notable is the association of aflatoxins with members of the *A. flavus* group (Bennett, 1985b; Semeniuk et al., 1971). *A. oryzae* is a member of that group and in spite of the above mentioned morphological distinctions, *A. oryzae* appears to be very closely related to *A. flavus*. Numerous studies have been

done to distinguish the koji molds from their toxicogenic relatives. The results are unambiguous in their confirmation of the conspecificity of *A. oryzae* and *A. flavus*. (see Section IV. below).

In a similar way, *A. sojae* is considered to be a domesticated form of *A. parasiticus* and shares a 92% DNA homology with its wild progenitor. *A. sojae* also has a history of safe use in the food industry. *A. parasiticus* in nature is an active colonizer of cereal grains and seeds with concurrent mycotoxin production. While these species can be distinguished from *A. flavus/oryzae* using morphological criteria, all four species intergrade. The hazard concerns for these species, thus, are equivalent to those associated with *A. flavus/oryzae*.

*A. nomius* is a newly classified species of toxigenic strains originally described in the *A. flavus* group, but not having the same level of DNA homology as shown among the four varieties mentioned above (Kurtzman et al., 1987). *A. nomius* produces aflatoxin and includes strains isolated from diseased bees. *A. oryzae* is distinguishable both morphologically and genetically from *A. nomius*.

### III. HAZARD ASSESSMENT

#### A. Human Health Hazards

##### 1. Toxin Production by *A. oryzae*

The close relationship between *A. oryzae* and *A. flavus* and the production of highly toxic mycotoxins by the latter has resulted in careful examination of the toxigenic potential of *A. oryzae*. However, *A. oryzae*, as a koji mold, has toxigenic potential in its own right. Those aspergilli used for manufacture of Japanese fermented foods have long been called koji molds. Prominent among the 25 koji molds listed is *A. oryzae* (Manabe et al., 1984). This fungus is used for sake, an alcoholic beverage, miso a soy bean paste, shoyu, soy sauce, amasake, a sweet beverage, and shouchu, a distilled liquor.

*A. flavus* commonly colonizes damaged cereal grains, soybeans and peanuts, actively producing mycotoxins (Stoloff, 1982). Certain strains of *A. oryzae* have themselves been shown to produce the mycotoxins aspergillic, kojic, cyclopiazonic and B-nitropropionic acids and maltoryzine (Ciegler & Vesonder, 1987).

Even with the food industry strains, a caveat of safety is that the fungal incubation not exceed the normal three day period. *A. oryzae* has been shown to produce toxic compounds under incubations longer than the typical koji fermentation

(Semeniuk et al, 1971; Yokotsuka & Sasaki, 1986). The following are toxins produced by some strains of *A. oryzae*.

a. Kojic acid

Kojic acid (discovered by Saito, 1907) is produced by koji, a solid culture of the koji mold. It is a commonly produced metabolite that possesses antibacterial and antifungal activity. Few oral studies exist for this byproduct. Giroir reported toxic effects on chickens at four to eight mg/kg feed. Older studies (Friedemann, 1934, Werch et al. 1957, Morton et al., 1945) using intravenous or intraperitoneal challenges show moderate toxicity for kojic acid. Later work had similar results (Ueno and Ueno, 1978). Kojic acid also is reported to have moderate cardiotoxic and cardiostimulant activity (Manabe et al., 1984., Bajpai et al. 1982). Nineteen of 47 *A. oryzae* strains tested produced kojic acid (Manabe et al., 1984). Even though it is apparent that the koji molds, including *A. oryzae* can produce the toxin kojic acid, this toxin may not be present in the fermented foods. The incubation period for sake, shoyu and miso is about two days and no kojic acid is found at that time (Manabe et al., 1984). However, these authors concluded that they were unable to prove kojic acid was not present in any fermented food in Japan, because conditions of production and materials were different for each industry, and were often uncontrolled. Semeniuk et al. (1971) warned that even with food industry strains, fungal incubation must not exceed three days. Thus, as the culture adjusts to changing conditions, *A. oryzae* may produce toxic compounds when incubation time exceeds typical koji fermentation time.

b. Maltoryzine

Maltoryzine, another toxic metabolite isolated and characterized by Iisuka and Iida (1962), was produced by *A. oryzae* var. *microsporus*. This metabolite was determined to be the cause of poisoning among dairy cows. While highly toxic (LD<sub>50</sub> 3 mg/kg; Iizuka, 1974; Ciegler and Vesonder, 1987), the substance may only be found in one or a very few strains of *A. oryzae*. The single isolate, IAM 2950, produced enough of the toxin when grown on malt rootlets to poison some milk cows, prompting the determination of its LD<sub>50</sub>. The production of these toxins is related to the composition of the growth substrate and usually occurs in stationary phase cultures. Commercial strains of *A. oryzae* and *A. sojae* apparently do not produce maltoryzine.

c. Cyclopiazonic acid

Pitt and Cruickshank (1990), note that many isolates of *Aspergillus oryzae* are found to produce cyclopiazonic acid. Orth (1977), reporting on food industry strains of *A. oryzae*, indicated that eight of 16 strains produced cyclopiazonic acid.

This acid is a natural contaminant of foods and feeds and is produced by several molds including those used in fermented food production. These included *A. flavus*, *A. versicolor*, *A. tamarii*, several *Penicillium* species, including *P. camemberti*, and *A. oryzae*. This mycotoxin has been shown to occur naturally in corn, cheese, peanuts and in Kodo millet that was implicated in natural human intoxication in India (CAST Task Force Report No. 116, 1989a). Benkhemmar et al. (1985) showed that when cyclopiazonic acid producing (CPA+) strains are mated with CPA- strains, the CPA+ phenotype is dominant in the heterokaryon. Oral administration produced effects at levels ranging from 0.25 to >50 mg/kg with dogs among the most sensitive species and rats among the least (Purchase, 1971; Nuehring et al., 1985). LO(A)ELs for sensitive species were at or under 1mg/kg. Nishie et al. (1985) noted that Rao and Husain (1985) identified cyclopiazonic acid as the cause of debilitating illnesses in cattle and man in India.

#### d. b-nitropropionic acid

*A. oryzae* can produce b-nitropropionic acid, along with other food-borne molds (Gilbert et al., 1977). Its mode of action is apparently irreversible succinate dehydrogenase inhibition which can cause a variety of symptoms often neurological in nature. These symptoms have been studied in mice (Gould and Gustine, 1982; Umezawa, 1967) and rats (Hamilton and Gould, 1987) where intravenous or subcutaneous LD<sub>50</sub>s of 20-50 mg/kg were determined. Reports of livestock poisoning via ingestion in feed (James et al., 1980; James, 1983) showed that ingestion of b-nitropropionic acid could produce significant toxic effects up to and including death. When *A. oryzae* (ATCC 12892) was studied for its ability to produce b-nitropropionic acid on various high protein and carbohydrate-rich foods, it flourished and produced this toxin in cooked sweet potato, potato and ripe banana (Penel and Kosikowski, 1990). Ames type assays for mutagenicity (Dunkel, 1985) showed positive responses with and without activation for two *Salmonella* strains, but not for three others. This assay uses multiple indicator strains in order to ensure that each potential mutation mode is detectable; the failure in three strains merely implies that the mutation modes to which each is sensitive are not the ones associated with the test substance.

#### 2. Taxonomic and Genetic Relationship to Other Aspergilli

The closest taxon to *A. oryzae* is *A. flavus* which Kurtzman et al. (1986) regard as conspecific. Many strains of *A. flavus* produce aflatoxins which are acutely toxic to mammals (oral LD<sub>50</sub>s ranging from 1 to 15 mg/kg depending on test species (Ceigler, 1975). Aflatoxins are animal carcinogens (Barnes and Butler, 1964; Dickens and Jones, 1964; Sinhuber, 1968) and also probable

human carcinogens (Council for Agricultural Science and Technology, 1989). Developmental effects have also been found (Elis and DiPaolo, 1967, Le Breton et al., 1964).

While the koji molds like *A. oryzae* are distinguishable from, they are nevertheless very closely related to, *A. flavus*. Distinguishing between *A. oryzae* and *A. flavus* by physical traits is elusive. The toxigenic subspecies/variety *A. flavus* has numerous spore chains that remain yellow-green; sterigmata that are always biseriate; spiny (echinulate) individual spores; roughened conidiophores up to 600µm in length and sclerotia often present. The variety called *A. oryzae* specifically has fewer spore chains, fading to brown with age; longer average conidiophores (about two to three mm); smoother individual spores; sterigmata usually in 1 series and sclerotia rarely produced (Raper & Fennel, 1965).

### 3. Lack of Aflatoxin Production in *A. oryzae*

Despite this strong similarity between the two species, production of aflatoxins has not been demonstrated by *A. oryzae*. Many studies affirm that the currently available strains confirmed to be *A. oryzae* are not capable of producing aflatoxins (Wei and Jong, 1986; Yokotsuka and Sasaki, 1986). In one test, no strains of *A. oryzae* or *A. sojae* (another koji mold) produced detectable levels of aflatoxins, while 33% and 85% of the strains of *A. flavus* and *A. parasiticus*, respectively, were toxigenic. As mentioned above, Kurtzman, et al. (1986) regard *A. oryzae* and *A. sojae* as domesticated varieties of their respective subspecies. Only one study (El-Hag and Morse, 1976) describes aflatoxin production by a strain reported to be *Aspergillus oryzae* (NRRL strain 1988). This observation is notable as an exception to the rule of no aflatoxin production by *A. oryzae*.

It has been noted that *A. flavus* strains upon extended laboratory cultivation lose morphologically distinguishing characteristics, making them appear much like *A. oryzae* (Kurtzman, et al., 1986). Wicklow (1984) details the competitive disadvantages of *A. oryzae* and implies that *A. flavus* is the "wild" form. Kurtzman et al. (1986) ask whether the separation between toxigenic and non-toxigenic *A. flavus* group species occurs through ecological adaptation or chromosomal changes such as translocations or inversions.

The elucidation of metabolic pathways responsible for the production of aflatoxins by *A. flavus* group fungi has progressed rapidly. Recently Payne (Bhatnagar, et al. 1992 and Payne, 1994) reported on the conversion of an aflatoxin non-producing strain of *A. flavus* to aflatoxin B<sub>1</sub> positive using a cosmid library developed from a toxigenic *A. flavus*. While added metabolic precursors could not stimulate toxin production in the mutant, the addition of an appropriate cosmid carrying a <5 kb fragment

of the genome of the toxin producer converted the non-toxigenic strain to significant levels of aflatoxin production. Further work has resulted in isolation of a small segment specifying a regulatory, rather than structural, gene that affects early parts of the pathway. Probes for this regulatory gene, designated *afl R*, have been positive in both *A. oryzae* and *A. sojae*, even though those strains do not produce aflatoxin. In addition, Payne stated that probes for structural genes for aflatoxin production were also positive in some, but not all, *A. oryzae* strains examined.

It appears that evidence is mounting towards multiple reasons for failure to produce aflatoxins in *A. oryzae* cultures. One explanation is a lack of functional regulators, specifically *afl R*, that activate aflatoxin production. Another is that some or all of the structural genes in the aflatoxin pathway may be non-functional. For both types of genes, those sequences could be absent or present in the wrong orientation or split by insertions or modified slightly so as to be non-functional. Except for substantial deletion or absence of the necessary sequences, all of these alternatives are potentially reversible. However, Payne indicated that he doubted that industrial strains of *A. oryzae* were likely to revert to aflatoxin production. He indicated that, even though probes found the presence of appropriate gene sequences, the genes so detected could easily be incomplete enough so as to be completely non-functional.

Thus, complete absence of genetic potential is not the only plausible explanation for the non-expression of characters such as aflatoxin production in *A. oryzae*. In a related study, researchers attempting to improve strains of a mold identified as *A. oryzae* used for food fermentation in Thailand acquired a toxin producing strain by simple UV mutagenesis of a known "safe" strain (Kalayanamitr, et al. 1987). The toxins produced by this strain and other toxigenic *A. oryzae* strains are not aflatoxins but rather other types of mycotoxins. The exact composition of the toxins involved in *A. oryzae* toxicosis in these studies, as in other anecdotal studies, was not determined (Semeniuk, et al., 1971; Wicklow and Dowd, 1989, and Kalayanamitr, et al., 1987). The mechanism for this conversion to toxigenicity was not investigated, but the mutations required could have affected either structural or regulatory genes and produced the new observed toxigenic phenotype.

#### 4. Colonization and Pathogenicity

*Aspergillus oryzae* does not appear to be a human pathogen. Available information documents infections in humans possibly caused by *A. oryzae* in only three instances. The first was a case of meningitis (Gordon, et al., 1976). In the second case, *A. oryzae* invaded the paranasal sinuses, causing fever and right periorbital swelling (Byard, et al., 1986). The third case was a



pulmonary aspergilloma caused by *A. oryzae* (Liao, 1988). Care must be exercised in evaluating these three cases as having been caused by this organism due to its close taxonomical relationship to *A. flavus* and the possibility of incorrect identification. The relative rarity of such cases in light of the commonplace use of *A. oryzae* suggests this species has a low potential for expressing pathogenic traits.

#### 5. Allergic Reactions to *Aspergillus oryzae*

Allergic reactions are not uncommon for aspergilli in general. There is one reported case of an allergic bronchopulmonary aspergillosis due to *A. oryzae* in a 19-year old female (Akiyama et al., 1987). However, the  $\alpha$ -amylase produced by *A. oryzae*, that is used by bakers in bread making, was reported by Birnbaum, et al. (1988) to have caused asthma in a baker. Based on an observation of a case of baker's asthma due to monovalent sensitization to  $\alpha$ -amylase used as an additive to flour, investigators tested 31 bakers who had occupational asthma and/or rhinitis by skin tests and serologic RAST examinations. Thirty-two percent of the bakers had RAST specific IgE to  $\alpha$ -amylase from *A. oryzae*. Baker's asthma is reported to be the most frequent occupational lung disease in Switzerland and West Germany (Wuthrich and Baur, 1990). However, allergic reactions in bread bakers are quite common, both to the flours of various grains, as well as to the flour additives such as fungal amylases. Allergic reactions in bakers are not specific to *A. oryzae*, nor the enzymes produced by *A. oryzae* (O'Neil and Lehrer, 1991). In addition, the exposure scenario of a bread baker to flour and the additives contained therein is quite different from that of workers in a fermentation facility using general worker hygiene and protection practices.

#### 6. Conclusions

There are two possible concerns for human health hazards associated with *A. oryzae*. The first, which is directly tied to *A. oryzae*, is the potential for mycotoxin production with extended fermentation. A variety of toxins can be produced, with the most common being the moderately toxic kojic acid. Other more potent toxins may only be produced by a few strains or in lesser quantities. These mycotoxins seem to be produced only under conditions of extended fermentation, and therefore, their production could be averted under proper fermentation conditions i.e., short fermentation times.

The second issue is the possibility for the production of aflatoxins because of the nearly indistinguishable identity of *A. oryzae* and *A. flavus*. Kurtzman, et al. (1986) have shown that *A. flavus* and *A. oryzae* are essentially the same based on DNA comparisons. *A. oryzae* appears so closely related to its aflatoxin-producing counterpart as to be viewed as consisting of

culture-attenuated strains of *A. flavus* (Kurtzman, 1994; Wicklow, 1984). It has been hypothesized that *A. oryzae* evolves under culture from *A. flavus* strains due to selection for features that would be ecologically detrimental in the wild.

Hypothetically, then, if *A. oryzae* has evolved to non-aflatoxigenic status after centuries in culture, the question remains whether it can revert to the "wild" type. The experience of oriental food production would seem to suggest not, or at least not frequently enough as to be detectable. Recent studies (Payne, 1994; Klich, 1994) suggest homology between parts of the *A. oryzae* genome and structural genes for aflatoxin production. It is conceivable that reintroduction of regulatory genes or their gene products could activate a dormant aflatoxin synthetic potential. There is no evidence to show that the required gene transfer or gene rearrangement that might provide the needed functional sequences for an aflatoxin producing *A. oryzae* strain occurs naturally. The question is, therefore, whether this type of genetic modification is possible in culture. Gene transfer from a toxigenic strain during fermentation is highly unlikely due to the need for maintaining axenic conditions during fermentation. The theoretical possibility of genetic rearrangement occurring in culture resulting in reversion back to the "wild-type" seems unlikely. Anecdotal evidence gathered over centuries suggests that *A. oryzae* commercial food strains do not produce aflatoxins, nor have there been reports of any adverse human health effects from aflatoxin.

## **B. Environmental Hazards**

### 1. Hazards to Animals

The potential for toxin production is the main environmental hazard issue of concern for *A. oryzae*. If there were a method to distinguish between toxicogenic and non-toxicogenic strains, there would be no environmental concern for *A. oryzae*. Two recent studies that addressed the question of differentiating between toxin producing and non-toxicogenic strains of the related species *A. flavus*, *A. parasiticus* and *A. nomius* were unable to correlate either mitochondrial or chromosomal DNA RFLPs with mycotoxin production (Moody & Tyler, 1990a, 1990b). This again points to differences that may only involve small regulatory regions or that involve differences in structural gene complements that are beyond the detection limit of current DNA typing technologies.

Compounding this is the observation that *A. oryzae* and *A. flavus* are essentially indistinguishable by most molecular techniques. *A. flavus* is believed to be second in frequency only to the frank fungal pathogen, *A. fumigatus*, as a cause of aspergillosis in many species. *A. flavus* is associated specifically with invasive diseases of insects as well as toxicosis (Austwick, 1965). Recently, some insect pathogenic *A.*

*flavus* strains were reclassified into *A. nomius* (Kurtzman et al., 1987). Whether *A. oryzae* is involved depends on how one defines the species of the *A. flavus* group. The effects on livestock of the various toxins that occur after extended koji fermentations, or in contaminated feed, show that the "minor" mycotoxins can still cause economic loss. No anecdotal accounts have been found that demonstrate that these potential effects occur in wildlife outside the agricultural environment.

## 2. Hazards to Plants

No reports of *A. oryzae* effects on living plants have been found. This species does not appear to be pose a hazard to plants.

## 3. Conclusions

The issues for environmental hazards are similar to those for human health hazards. The primary hazard concerns are for toxin production by *A. oryzae* strains. Under usual conditions of culture, well established commercial strains of this species do not seem to produce significant levels of mycotoxins, although certain moderately potent toxins can be produced after extended culture. Aflatoxins appear not to be produced by such cultures. The potential for environmental hazard is dependent on the likelihood that commercial strains could escape and establish themselves in the wild and grow under conditions analogous to those resulting in toxin production in extended culture. The few examples of livestock poisoning associated with the "minor" toxins, b-nitropropionic acid, maltoryzine and cyclopiazonic acid cited above imply that, for a short time at least, strains of *A. oryzae* may be able to survive in the wild.

## **IV. EXPOSURE ASSESSMENT**

### **A. Worker Exposure**

*Aspergillus oryzae* is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986). In Europe, *Aspergillus* spp. are treated as low-risk-class microorganisms, i.e., category 2 of the European Federation of Biotechnology (Frommer et al., 1989) or category 1 on the OECD containment scale. Category 1 of the European Federation of Biotechnology scale includes organisms deemed harmless, which can be grown under good industrial large scale practices (GILSP), while category 2 organisms like *Aspergillus* require more stringent containment.

No data were available for assessing the release and survival specifically for fermentation facilities using *A.*

*oryzae*. 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<sup>3</sup>. 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 Organism

Controversy exists over the ability to isolate *A. oryzae* from the natural environment. Some researchers believe that *A. oryzae* is widely distributed in nature while other maintain that all strains of *A. oryzae* are variants of *A. flavus* which have been modified through years of selection in an artificial environment. Specific data which indicates the survivability of

industrial "domesticated" strains of *A. oryzae* in the environment are not available. The process of domestication may have resulted in lessened survivability in the environment. However, its ability to produce spores suggests that it may survive in the environment (Versar, 1991).

## 2. Releases

Estimates of the number of *A. oryzae* 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 *A. oryzae* Organisms Released During Production

Release Media	Uncontrolled/ Untreated (cfu/day)	Full Exemption (cfu/day)	Release (days/year)
Air Vents	$2 \times 10^8 - 1 \times 10^{11}$	$< 2 \times 10^8 - 1 \times 10^{11}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7 \times 10^{12}$	$7 \times 10^6$	90
Soil/Landfill	$7 \times 10^{14}$	$7 \times 10^8$	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 *A. oryzae* 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  $3.0 \times 10^3$  to  $1.5 \times 10^6$  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, 1991).

#### 4. Water

The concentrations of *A. oryzae* 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 *A. oryzae* 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 *A. oryzae* in surface water for the uncontrolled/untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1991).

TABLE 2. *A. oryzae* Concentrations in Surface Water

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

\*MLD = million liters per day

Source: Versar, 1991

## 5. Soil

Since soil is a possible natural habitat for *A. oryzae*, long-term survival in the environment, particularly as spores, may occur. Human exposures via dermal contact and ingestion routes, and environmental exposures [i.e., to terrestrial, avian, and aquatic organisms (via runoff)] may occur at the discharge site if there is establishment of *A. oryzae* within the soil (Versar, 1991).

## 6. Summary

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

## **V. INTEGRATION OF RISK**

In the previous sections, information regarding the potential exposures and hazards to workers, the general public, animals, plants and the environment was reviewed. This section serves to integrate this information to evaluate the potential risks associated with the industrial use of *Aspergillus oryzae*.

### **A. Discussion**

The only major concerns identified are associated with human and animal toxicity due to mycotoxin production. *A. oryzae* and *A. flavus* are designations of taxa that represent the extremes of a spectrum of traits associated with a common fungus. Current evidence points to *A. oryzae* as a domesticated derivative of *A. flavus*. The evidence is not complete enough to indicate whether *A. oryzae* represents a unique genotype as well as a stable phenotype. It appears that under prolonged cultivation the phenotype of *A. oryzae* will be exhibited and that aflatoxins will not be produced from such strains. Other toxins such as cyclopiazonic acid and kojic acid may, however, be expressed.

### 1. Aflatoxin Production

Although it is likely that *A. oryzae* held in cultivation for decades or even centuries are likely to represent strains having small, but key, deletions in an otherwise identical genome to *A. flavus*, it is remotely possible that the phenotypic differences between the two species may be due to differences in the arrangement and control of genes rather than the loss or gain of them. If *A. oryzae* strains have had reversible gene modifications that prevent the expression of aflatoxin genes, then environmental control of such rearrangements is possible and reversion can occur. It must be noted that there have been no



reports of workers in the industrial setting suffering from aflatoxin effects.

There is a basic question as to the likelihood that *A. oryzae* exists in the wild. Some researchers (Klich, 1994) indicate that *A. oryzae* can be isolated in nature. Other researchers (Kutzman et al., 1986) contend that *A. oryzae* is a domesticated version of *A. flavus*, with decreased survival characteristics such as reduced sporulation and the lack of sclerotia. Wicklow (1984) has described the competitive disadvantages of *A. oryzae*. These observations suggest that this organism is highly adapted to conditions in the laboratory.

All this points to an incomplete knowledge base for *A. oryzae*. However, it appears that aflatoxin production is not a concern for established *A. oryzae* strains. Although there is a theoretical possibility for reversion to the aflatoxigenic phenotype of *A. flavus*, it has not been observed through hundreds of years of use in food production. In addition, controls on exposure mitigate concerns. While some workers might be exposed to the organism, much of that exposure would presumably be via an inhalation route rather than an ingestion. They would be exposed mostly to spores of *A. oryzae* during large-scale fermentation. Spores that escape the manufacturing site would be unlikely to persist in the environment because of less than optimal conditions for germination and growth. As pointed out in the hazard assessment, *A. oryzae*, lacks many survival features possessed by the related *A. flavus*. Therefore, from the information cited above and using the values in the assessment of exposure, environmental exposure relevant to aflatoxin production appears highly unlikely.

## 2. Other Toxins

There remains some concern for other mycotoxins produced by koji molds. These toxins are less potent than aflatoxins and their production is tied both to strain specificity and culture conditions. However, they can occur even with current domesticated strains, although there are no reports that their production in industrial fermentations have resulted in adverse effects on human health. The most toxic ones, such as cyclopiazonic acid, seem to be produced by a few strains under special conditions. The less toxic ones, such as kojic acid, may be limited by engineering controls on the fermentation process.

The exposure component of the risk for this concern is similar to that described for aflatoxin. Proper conditions of cultivation should limit production of these toxins and limit exposure to workers.

## 3. Other Issues

Allergenicity seems to be related more to the product of the fermentations than to *A. oryzae per se*. Sensitivity to  $\alpha$ -amylase

in particular, is a potential concern, but one that exists for all microorganisms producing this enzyme. There is thus no incremental risk specific to the use of this fungus.

#### 4. Summary

Thus, the potential risks for *A. oryzae* include the theoretical possibility of genetic rearrangement resulting in the inadvertent production of aflatoxins in *A. oryzae*. Through centuries of use in food production, there are no reports of aflatoxin production. Mycotoxin production can most likely be avoided by properly controlling the fermentation conditions, and human health concerns are mitigated by controls on exposure and worker hygiene practices. *A. oryzae* is not a plant or animal pathogen, and survival in the environment is expected to be limited due to its decreased survival characteristic by years of domestication. The risk of the use of this organism under the specified conditions of this exemption is low.

#### **B. Recommendation**

*Aspergillus oryzae* is recommended for the tiered exemption.

## VI. REFERENCES

### A. Primary Sources

- Akiyama, K., H. Takizawa, M. Suzuki, S. Miyachi, M. Ichinohe and Y. Yanagihara. 1987. Allergic bronchopulmonary aspergillosis due to *Aspergillus oryzae*, Abstract. Chest 91:285-286.
- Austwick, P.K.C. 1965. Pathogenicity of *Aspergillus* species, pp. 82-126. In K.B. Raper and D.I. Fennell, (eds.), The genus *Aspergillus*. Williams and Wilkins, Baltimore, MD.
- Bajpai, P., P.K. Agrawal, and L. Vishwanathan. 1982. Kojic acid: synthesis and properties. J. Scien. Indust. Research 41:185-194.
- Barnes, J.M. and W.H. Butler. 1964. Carcinogenic activity of aflatoxin to rats. Nature 202:1016.
- Beckwith, A., R. Vesonder, and A. Ciegler. 1976. Chemical methods investigated for detoxifying aflatoxin in foods and feeds, Chapter 4. In J. Roderick (ed.), Mycotoxins and other fungal related food problems, Advances in Chemistry Series #149. American Chemical Society, Washington, DC.
- Benkhemmar, O., F. Gaudemer, and I. Bouvier-Fourcade. 1985. Heterokaryosis between *Aspergillus oryzae* cyclopiazonic acid-defective strains: Method for estimating the risk of inducing toxin production among cyclopiazonic acid-defective industrial strains. Appl. Environ. Microbiol. 50:1087-1093.
- Bennett, J.W. 1985a. Molds, manufacturing and molecular genetics. In W.E. Timberlake, (ed.), Molecular genetics of filamentous fungi. Alan R. Liss, Inc., NY.
- Bennett, J.W. 1985b. Taxonomy of fungi and biology of the Aspergilli. In A.L. Demain and N.A. Solomon, (eds.), Biology of industrial microorganisms. Benjamin Cummings Publishing Co. Menlo Park, CA.
- Betina, V. 1989. Mycotoxins, Volume 9. In Bioactive molecules. Elsevier American Publishers, Oxford.
- Bhatnagar, D., J.W. Cary, G.A. Payne, N.P. Keller, and T.E. Cleveland. 1992. Molecular regulation of aflatoxin biosynthetic pathway. Presented at 92nd General Meeting, American Society for Microbiology, New Orleans, LA.
- Birnbaum, J., F. Latil, D. Vervoloet, M. Sneft, and J. Charpin. 1988. The role of alpha-amylase in baker's asthma, Abstract. Rev. Mal. Respir. 5:519-521.

Byard, R. W., R.A. Bonin, and A. Haq. 1986. Invasion of paranasal sinuses by *Aspergillus oryzae*. *Mycopathologia* 96:41-43.

Ciegler, A. 1975. Mycotoxins: Occurrence, chemistry, biological activity. *Lloydia* 38:21-35.

Ciegler, A. and R.F. Vesonder. 1987. Microbial food and feed toxicants: fungal toxins, pp. 19-126. In The CRC handbook of microbiology, Volume VIII. CRC Press, Boca Raton, FL.

Cole, R.J. 1984. Screening for mycotoxins and toxin-producing fungi. In V. Betina, (ed.), *Mycotoxins: Production, isolation, separation and purification*. Elsevier Scientific Publishers, Amsterdam.

Council for Agricultural Science and Technology. Report No. 116, November 1989. Mycotoxins--Economic and health risks.

Cruickshank, R.H. and J.I. Pitt. 1990. Isoenzyme patterns in *Aspergillus flavus* and closely related species, pp. 250-265. In R.A. Samson and J.I. Pitt, (eds.), *Modern concepts in Penicillium and Aspergillus classification*, NATO Advanced Science Institute Series, Series A: Life Sciences, Volume 185. Plenum Press, NY.

Dickens, F. and H.E.H. Jones. 1964. The carcinogenic action of aflatoxin after its subcutaneous injection in the rat. *Brit. J. Cancer*. 17:691-698. (Cited by Ong, 1975)

Dunkel, V.C. and V.F. Simmon. 1980. Mutagenic activity of chemicals previously tested for carcinogenicity in the National Cancer Institute Bioassay Program, pp. 283-302. In R. Montesano, H. Bartsch, and L. Tomatis, (eds.), *Molecular and cellular aspects of carcinogen screening tests*. IARC Scientific Publication No. 27. International Agency for Research on Cancer, Lyon.

Edwards, J.H. and T.S. Al-Zubaidy. 1977. Medical aspects. In J.E. Smith and J.A. Pateman, (eds.), *Genetics and physiology of Aspergillus*. Academic Press, NY.

El-Hag, N. and R.E. Morse. 1976. Aflatoxin production by a variant of *Aspergillus oryzae* (NRRL strain 1988) on cowpeas (*Vigna inensis*). *Science* 192:1345-1346.

Elis, J. and J.A. DiPaolo. 1967. Aflatoxin B<sub>1</sub> - Induction of malformations. *Arch. Pathol.* 83:53-57.

Fennell, D.I. 1973. Plectomycetes: Eurotiales, Chapter 4, pp. 45-68. In G.C. Ainsworth, F.K. Sparrow, and A.S. Sussman, (eds.), *The fungi: An advanced treatise*, Volume IVa. Academic Press, NY.

Friedemann, T.E. 1934. *Science* 30:34. (Cited by Bajpai, et al. 1982.)

Frisvad, J.C., D.L. Hawksworth, Z. Kozakiewicz, J.I. Pitt, R.A. Samson, and A.C. Stolk. 1990. Proposals to conserve important species names in *Aspergillus* and *Penicillium*, pp. 83-89. In R.A. Samson and J.I. Pitt, (eds.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, NY.

Frommer, W., B. Ager, L. Archer, B. Brunius, C.H. Collins, R. Donakian, C. Frontali, S. Hamp, E.H. Houwink, M.T. Kuenzi, P. Kramer, H. Lagast, S. Lund, J.L. Mshler, F. Normand-Plessier, K. Sargeant, M.G. Tuijnenburg, S.P. Vranich and R.G. Werner. 1989. Safe biotechnology III. Safety precautions for handling microorganisms of different classes. *Appl. Microbiol. Biotechnol.* 30: 541-552

Gilbert, M., A. Penel, F.V. Kosikowski, J.D. Henion, G.A. Maylin and D.J. Lisk. 1977. Electron affinity gas chromatographic determination of b-nitropropionic acid as its pentafluorobenzyl derivative in cheeses and mold filtrates. *J. Food Science* 42:1650-1653.

Giroir, L.E., W.E. Huff, L.E. Kubena, R.B. Harvey, M.H. Elissalde, D.A. Witzel, A.G. Yersin, and G.W. Ivie. 1991. Toxic effects of kojic acid in the diet of male broilers. *Poultry Science* 70:499-503.

Gordon, M.A., R.S. Holzman, H. Senter, E.W. Lapa, and M.J. Kupersmith. 1976. *Aspergillus oryzae* meningitis. *JAMA* 235:2122-2123.

Gould, D.H. and D.L. Gustine. 1982. Basal ganglia degeneration, myelin alterations and enzyme inhibition induced in mice by the plant toxin 3-nitropropionic acid. *Neurpathol. Appl. Neurobiol.* 8:377-393.

Hamilton, B.F. and D.J. Gould. 1987. Correlation of morphologic brain lesions with physiologic alterations and blood-brain barrier impairment in 3-nitropropionic acid toxicity in rats. *Acta Neuropathol.* 74:67-74.

Hawksworth, D.L. 1990. Problems and prospects for improving the stability of names in *Aspergillus* and *Penicillium*. In R.A. Samson and J.I. Pitt, (eds.), Modern concepts-in *Penicillium* and *Aspergillus* classification. NATO Advanced Science Institute Series, Series A: Life Sciences, Volume 185. Plenum Press, NY.

Iizuka, H. 1974. Maltoryzine, pp. 405-418. In I.F.H. Purchase, (ed.), *Mycotoxins*. Elsevier, Amsterdam.

Iizuka, H. and M. Iida. 1962. Maltoryzine, a new toxic metabolite produced by a strain of *Aspergillus oryzae* var. *microsporus* isolated from the poisonous malt sprout. *Nature* 196:681-682.

- James, L.F., J. Hartley, M.C. Williams, and K.R. Van Kampen. 1980. Field and experimental studies in cattle and sheep poisoned by nitro-bearing *Astragalus* or their toxins. *J. Am. Vet. Res.* 41:377-382.
- James, L.F. 1983. Neurotoxins and other toxins from *Astragalus* and other related genera, pp. 445-462. In Keeler, R.F. and A.T. Tu, (eds.), *Handbook of natural toxins*, Vol. 1. Marcel Dekker, NY. (Cited by Ludolf et al., 1991.)
- Jong, S.C. and M.J. Gantt, (eds.) 1987. *Catalogue of fungi and yeasts*, 17th edition. American Type Culture Collection, Rockville, MD.
- Kalayanamitr, A., A. Bhumiratana, T.W. Flegel, T. Glinsukon, and N. Shinmyo. 1987. Occurrence of toxicity among protease, amylase and color mutants of a non-toxic soy sauce koji mold. *Appl. Environ. Microbiol.* 53:1980-1982.
- Klich, M.A. and E.J. Mullaney. 1987. DNA restriction enzyme fragment polymorphism as a tool for rapid differentiation of *Aspergillus flavus* from *Aspergillus oryzae*. *Experim. Mycology* 11:170-175.
- Klich, M.A. and J.I. Pitt. 1985. The theory and practice of distinguishing species of the *Aspergillus flavus* group, pp. 211-220. In R.A. Samson and J.I. Pitt, (eds.), *Advances in Penicillium And Aspergillus systematics*. Plenum Publishing, NY.
- Klich, M.A. and J.I. Pitt. 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Trans. Br. Mycol. Soc.* 91:99-108.
- Kozlowski, M. and P.P. Stepien. 1982. Restriction analysis of mitochondrial DNA of members of the genus *Aspergillus* as an aid in taxonomy. *Jour. Gen. Microbiol.* 128: 471-476.
- Kurtzman, C.P., M.J. Smiley, C.J. Robnett, and D.T. Wicklow. 1986. DNA relatedness among wild and domesticated species of the *Aspergillus flavus* group. *Mycologia* 78(6):955-959.
- Kurtzman, C.P., B.W. Horn, and C.W. Hesselstine. 1987. *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. *Antonie van Leeuwenhoek* 53: 147-158.
- LeBreton, C., C. Frayssinet, C. LaFarge, and A.M. DeRecondo. 1964. Aflatoxin - mechanisms de l'action. *Food Cosmet. Toxicol.* 2:675-676. (Cited by Ong, 1975.)
- Liao, W.Q., J.Z. Shao, S.Q. Li, G.T. Wan, L.W. Da, Y.C. Sun, Z.T. Qi, Q.T. Chen, and Z.M. Sun. Mycological identification of pulmonary aspegillioma caused by *Aspergillus oryzae* with proliferating heads, Abstract. *Clin. Med. Jour.* 101:601-604.

- Ludolf, A.C., F. He, P.S. Spencer, J. Hammerstad, and M. Sabri. 1991. 3-nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. *Canad. J. Neurol. Sci.* 18:492-498.
- Manabe, M., K. Tanaka, T. Goto, and S. Matsuura. 1984. Producing capacity of kojic acid and aflatoxin by koji mold, pp. 4-14. *In* H. Kurata and Y. Ueno, (eds.), *Toxigenic Fungi - their toxins and health hazard*, Proceedings of the mycotoxin symposia held at the Third International Mycological Congress, Tokyo, 1983. Elsevier, Tokyo.
- Moody, S.F. and B.M. Tyler. 1990a. Restriction enzyme analysis of mitochondrial DNA of the *Aspergillus flavus* group: *A. flavus*, *A. parasiticus*, and *A. nomius*. *Appl. & Environ. Microbiol.* 56:2441-2452.
- Moody, S.F. and B.M. Tyler. 1990b. Use of nuclear DNA restriction fragment length polymorphisms to analyze the diversity of the *Aspergillus flavus* group: *A. flavus*, *A. parasiticus* and *A. nomius*. *Appl. & Environ. Microbiol.* 56:2453-2461.
- Morton, H.E., W. Kocholaty, R. Junowicz-Kocholaty, and A. Kelner. 1945. Toxicity and antibiotic activity of kojic acid produced by *Aspergillus luteovirescens*. *J. Bacteriol.* 50:579-584.
- Nishie, K., J.K. Porter, R.J. Cole, and J.W. Dorner. 1985. Neurochemical and pharmacological effects of cyclopiazonic acid, chlorpromazine and reserpine. *Research Communications in Psychology, Psychiatry and Behavior* 10:291-302.
- Nuehring, L.P., G.N. Rowland, L.R. Harrison, R.J. Cole, and J.W. Dorner. 1983. Cyclopiazonic acid mycotoxicosis in the dog. *Am. J. Vet. Res.* 46:1670-1676.
- O'Neil, C.E. and S.B. Lehrer. 1991. Occupation reactions to food allergens. *In*: D.D. Metcalfe, H.A. Sampson, and R.A. Simon (eds.). *Food Allergy: Adverse Reactions to Foods and Food Additives*, pp. 207-236. Blackwell Scientific Publications, Oxford.
- Ong, T.M. 1975. Aflatoxin mutagenesis. *Mutation Res.* 32:35-53.
- Orth, R. 1977. Mycotoxins of *Aspergillus oryzae* strains for use in the food industry as starters and enzyme producing molds. *Ann. Nutr. Alim.* 31:617-624.
- Penel, A.J. and F.V. Kosikowski. 1990. Beta-nitropropionic acid production by *A. oryzae* in selected high protein and carbohydrate-rich foods. *J. Food Protect.* 53:321-323.
- Pitt, J.I. and R.H. Cruickshank. 1990. Speciation and synonymy in *Penicillium* subgenus *Penicillium* -- Towards a definitive

taxonomy. In R.A. Samson and J.I. Pitt, (eds.), Modern concepts in *Penicillium* and *Aspergillus* classification, NATO Advanced Science Institute Series, Series A: Life Sciences, Volume 185. Plenum Press, NY.

Purchase, I.F.H. 1971. The acute toxicity of the mycotoxin cyclopiazonic acid to rats. *Toxicol. Appl. Pharmacol.* 18:114-123.

Rao, B.L. and A. Husain. 1985. Presence of cyclopiazonic acid in Kodo millet (*Paspalum serobiculatum*) causing Bodua poisoning in man and its production by associated fungi. *Mycopathologia* 89:177-180.

Raper, K.B. and D.I. Fennell. 1965. The genus *Aspergillus*, p. 686. Williams and Wilkins Company, Baltimore, MD.

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

Saito, K. 1907. *Bot. Mag. (Tokyo)* 21:240. (Cited by Bajpai et al. 1982.)

Semeniuk, G., G.S. Harshfield, C.W. Carlson, C.W. Hesseltine and W.F. Kwolek. 1971. Mycotoxins in *Aspergillus*. *Mycopathologia* 43:137-152.

Sinhuber, R.O., D.J. Lee, J.H. Wales, and J.L. Ayers. 1968. Dietary factors and hepatomas in rainbow trout (*Salmo gaidneri*). II. Carcinogenesis and the effects of grosypol and altered lipids on aflatoxin-induced liver cancer. *J. Nat. Cancer Inst.* 41:1293-1299. (Cited by Ong, 1975.)

Stoloff, L.. 1982. Mycotoxins as potential environmental carcinogens, Chapter, 9, pp. 97-120. In H.F. Stich, (ed.), *Carcinogens and mutagens in the environment*, Volume I: Food products. CRC Press, Boca Raton, FL.

Thom, C. and K.B. Raper. 1945. A Manual of the *Aspergilli*. Williams and Wilkins Company, Baltimore, MD.

Turner, B. 1970. Workbook of atmospheric dispersion estimates. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Ueno, Y. and I. Ueno. 1978. Toxicology and biochemistry of mycotoxins. In K. Uraguchi and M. Yamazaki, (eds.), *Toxicology, biochemistry, and pathology of mycotoxins*. John Wiley and Sons, Halstead Press, NY.

Umezawa, H., (ed.) 1967. Index of antibiotics from actinomycetes. Univ. Tokyo Press, Tokyo.



U.S. Department of Health and Human Services. 1986. Guidelines for research involving recombinant DNA molecules; Notice. 51 FR 16958, May 7, 1986.

U.S. Department of Health and Human Services. Undated. Proposed biosafety guidelines for microbial and biomedical laboratories. Office of Biosafety, Center for Disease Control.

Voss, E.G., et al. 1983. International code of botanical nomenclature adopted at the Thirteenth International Botanical Congress, Sydney, August 1981. *Regnum Vegetabile* 111:1-472. (1)

Wei, D.L. and S.C. Jong. 1986. Production of aflatoxins by strains of the *Aspergillus flavus* group maintained in ATCC. *Mycopathologia* 93:19-24.

Werch, S.C., Y.T. Oester, and T.E. Friedemann. 1957. Kojic acid-- A convulsant. *Science* 126:450-451.

Wicklow, D.T. 1983. Taxonomic features and ecological significance of sclerotia. In U.L. Diener, R.L. Asquith and J.W. Dickens, (eds.), *Aflatoxin and Aspergillus flavus* in corn. Proc. Symp. held in Atlanta, GA, Jan.26-27, 1982. *Southern Cooperative Extension Service Bulletin* 279:6-12.

Wicklow, D.T. 1984. Conidium germination rate in wild and domesticated yellow-green *Aspergilli*. *Appl. & Environ. Microbiol.* 47:299-300.

Wicklow, D.T. and O.L. Shotwell. 1983. Intrafungal distribution of aflatoxins among conidia and sclerotia of *Aspergillus flavus* and *A. parasiticus*. *Can. J. Microbiol.* 29:1-5.

Wicklow, D.T. and P.F. Dowd. 1989. Entomotoxigenic potential of wild and domesticated yellow-green *Aspergilli*: Toxicity to corn earworm and fall armyworm larvae. *Mycologia* 81:561-566.

Wuthrich, B. and X. Baur. 1990. Baking ingredients, especially alpha-amylase, as occupational inhalation allergens in the baking industry, Abstract. *Schweiz-Med-Wochenschr.* 120:446-450.

Yamatoya, K., J. Sugiyama and H. Kuraishi. 1990. Electrophoretic comparison of enzymes as a chemotaxonomic aid among *Aspergillus* taxa (2) *Aspergillus* section *flavi*, pp. 395-406. In R.A. Samson and J.I. Pitt, (eds.), *Modern concepts in Penicillium and Aspergillus classification*, NATO Advanced Science Institute Series, Series A: Life Sciences, Volume 185. Plenum Press, NY.

Yokotsuka, T. and M. Sasaki. 1986 Risks of mycotoxin in fermented foods, Chapter 15, pp. 259-287. In C.W. Hesseltine and H. Wang, (eds.), *Indigenous fermented foods of non-western origin*. *Mycologia Memoirs* No. 11. J. Cramer, Berlin.

**B. Secondary Sources**

- (1) Versar. 1991. Screening level exposure assessment of *aspergillus* species for 5(h)(4) exemption under the proposed biotechnology rule. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.