

# Mycotoxin Testing with Aran Gas: Aflatoxin B<sub>1</sub> Mycotoxin Destruction using Allotropic Oxygen O<sub>4</sub>, O<sub>5</sub>, and O<sub>6</sub>

## Proceedings

Aran gas is called several things. It goes by the names: Molecular Oxygen Plasma, Polyatomic Oxygen Polymer- "POP", Polyatomic oxygen, multimolecular oxygen, allotropic oxygen and even activated oxygen. Oxygen commonly exists as O<sub>2</sub>. The aranizer and other machines like the Octozone produce allotropic forms of oxygen higher than Ozone- O<sub>3</sub>. They produce O<sub>4</sub>, O<sub>5</sub>, O<sub>6</sub> and up. The molecules have 32 and up electrons to create a powerful oxidizer.

The theoretical value of the O<sub>5</sub> molecule to break down a molecule of Mycotoxin Aflatoxin B<sub>1</sub> is 5 O<sub>5</sub> molecules to 1 Aflatoxin B<sub>1</sub> molecule. Therefore, by creating more oxygen than Aflatoxin it is possible to break down the Aflatoxin B<sub>1</sub> molecule into CO<sub>2</sub> and water vapor.

On July 18<sup>th</sup>, 2005 I set up an AJ-1 water aranizer unit made by the now defunct Aran Aqua Pollution Control Systems. It is a unit that pushes the gas into a tubing system used for introduction into water or other solutions and systems. The output of the unit is 2525mg/hr of Aran gas. This gas was tested in previous machines to have a molecular average value of O<sub>6</sub> tested by Campbell and Associates. I also tested this unit on the Shimadzu 2401 UVProbe spectrophotometer. The analysis showed the peak forming below the 190 nm mark. O<sub>4</sub> peaks at 151nm. The limit of my equipment was 190nm.

The Aflatoxin B<sub>1</sub> I purchased was from Sigma Aldrich and is a standard used for the HPLC testing. It is therefore created more stable. Sigma Aldrich has this info on the Aflatoxin B<sub>1</sub>:

"A number of mold species from the genus *Aspergillus* produce fungal metabolites called aflatoxins. Aflatoxins are an interesting example of DNA damaging agents from a natural source. The detrimental effects of aflatoxins are due to their ability to bind covalently to DNA. The DNA damage leads to mutagenesis followed by possible cellular dysfunction. These naturally occurring mycotoxins are highly toxic and exceedingly carcinogenic. Aflatoxins are among the most potent liver carcinogens known. 1-4 At least 13 different types of aflatoxins are produced in nature. Aflatoxins are a particular concern as food contaminants. Aflatoxins are found naturally in plant or animal derived food products with mold growth, particularly when foodstuffs are stockpiled. Their toxic derivatives can also occur as indirect contaminants in animal products. Human exposure is usually the result of food consumption, particularly peanuts.

Aflatoxin B<sub>1</sub> is one of several aflatoxins that can be isolated from the fermentation broth of the mold *Aspergillus flavus*. This mold is common and widespread in nature. The mold is found in soil and grows in any kind of decaying vegetation such as hay or grains stored under warm moist conditions. Although detection of this mold in foodstuffs indicates the potential of aflatoxins, the presence of this ubiquitous mold is not substantiation of aflatoxin contamination. Among the aflatoxins of natural origin, aflatoxin B<sub>1</sub> is the most potent hepatocarcinogen and considered to be the

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most toxic. Aflatoxin B<sub>1</sub> consists of a difurofuran ring system that is fused to a substituted coumarin moiety, with a methoxy group attached at the corresponding benzene ring. Of particular interest is the presence of derivatives of aflatoxin B<sub>1</sub> that can be found in edible animal products obtained from cattle that have consumed sublethal doses of aflatoxin B<sub>1</sub>. Consumed aflatoxins are converted to aflatoxin derivatives in the liver. Aflatoxin B<sub>1</sub> is known to be oxidized by the mixed function oxygenases of the liver cytochrome P-450 system present in the microsomal fraction of liver extracts. This oxidation results in aflatoxin B<sub>1</sub>-8,9-epoxide as the major product. This reactive epoxide seems to preferentially attack certain guanine residues in double-stranded DNA, giving rise to a large guanine adduct dihydro-guanyl-hydroxyaflatoxin B<sub>1</sub>,2

Molecular Formula: C<sub>17</sub>H<sub>12</sub>O<sub>6</sub>

Molecular Weight: 312.3

CAS Number: 1162-65-8

Melting Point: 268 - 269 °C

Extinction Coefficient (ethanol): EmM=25.6 (223nm), 13.4 (265nm), 21.8 (363nm)

Fluorescence Emission Maxima: 425nm (ethanol) Synonyms: AFB1, Aflatoxin B, Aflatoxin B1, 6-Methoxydifurocoumarone" (Figure 1 & 2).

[https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product\\_Information\\_Sheet/a6636pis.pdf](https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/a6636pis.pdf)

Based on previous experimental quantities, an unknown portion of aflatoxin B<sub>1</sub> is placed in a sterile Class A 100mL volumetric flask. Distilled water is the matrix for carrying the aflatoxin powder in this experiment. Solvent or other carrier liquids from experimental experience would give unclear results and possibly be oxidized completely before the experiment was finished (Figure 3).

When I started this experiment, I thought the aflatoxin would dissolve in water easily. WRONG. To fix this problem, I placed the solution on a stir plate. There I withdrew the 2-10mL aliquots. These 2 samples are my initial start point samples. The remainder

80mL solution was placed in the glove box.

After 30 minutes of allotropic oxygen treatment, a 10mL aliquot was taken while stirred. After another 30 minutes of treatment another 10mL sample was taken. After an additional 11 hours of Allotropic oxygen treatment the last 10mL sample was taken. Two blanks were taken from the dilution water not treated. 1 Aliquot of water was aranzized for comparison.

I timed this test to correlate with shipping. These details were thoroughly discussed with the veteran analyst PhD Kristine Kurtz at the PK laboratory. The shipping pick up time was 2:30 PM. You can see much to my delight, the 2 start point sample results were identical. The pink and the blue are the initial untreated samples (Figure 4). The test results on a scale (Figure 5):



Figure 1: Neat Powder before powder aranzized. A6636 Neat Powder Sigma Aldrich catalog#.

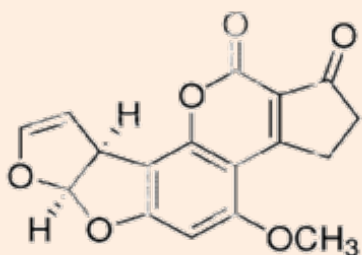


Figure 2: This is an aranzized dry neat powder standard Aflatoxin B1.

The final test results show a reduction of mycotoxin Aflatoxin B<sub>1</sub> using allotropic oxygen with an average molecular weight of O<sub>6</sub>.

The starting Aflatoxin concentration was 26,000 ng/ml. The sample of deionized water and Aflatoxin B<sub>1</sub> neat powder standard from Sigma Aldrich were treated for thirty minutes of the 2525mg/hr allotropic molecular average weight of O<sub>6</sub> output into a 100 ml volumetric class A flask of 80 ml sample. After

30 minutes another sample was taken. This concentration of Aflatoxin B<sub>1</sub> is now 11,000ng/ml, 58% reduction. The treatment is started again with now 60 ml of Aflatoxin B<sub>1</sub> solution. After an additional 30 minutes of treatment with the 2525mg/hr average molecular weight of O<sub>6</sub> into 60ml of sample the concentration is now 4,000 ng/ml Aflatoxin B<sub>1</sub>, 85% reduction. The treatment is continued for an additional 11 hours to make the total treatment 12 hours. The final Aflatoxin B<sub>1</sub> concentration is 520ng/ml. This is a 98% Aflatoxin reduction in 720 minutes. Details of the experiment (Figure 6, 7 & 8):

Where does the Aflatoxin come from?

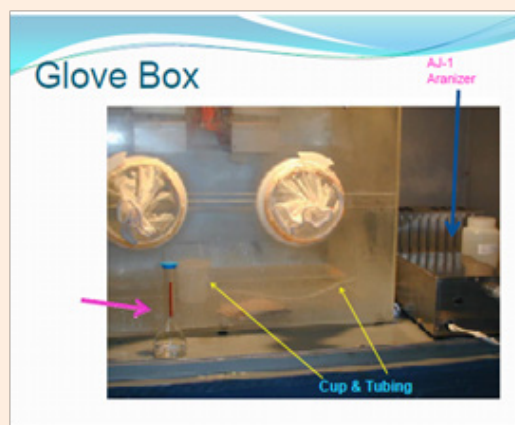


Figure 3

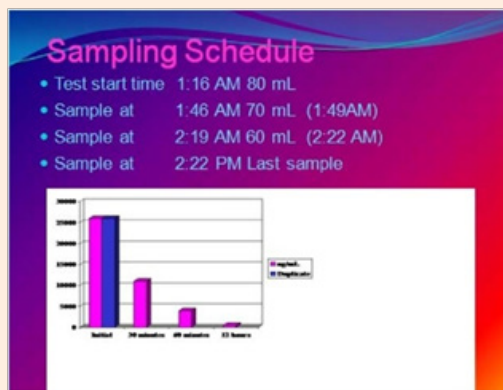


Figure 4

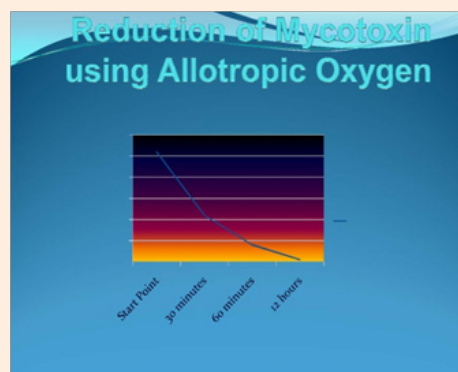


Figure 5

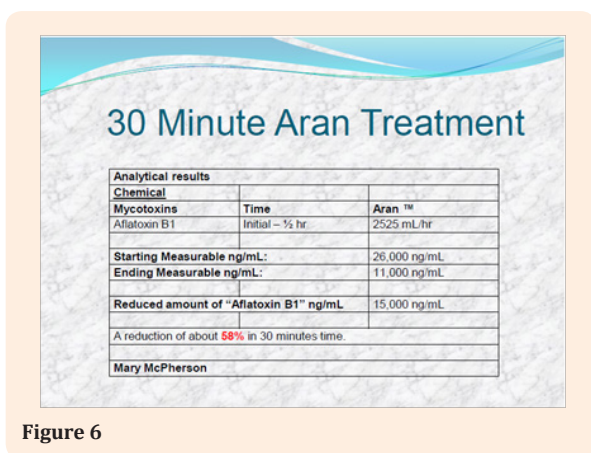


Figure 6

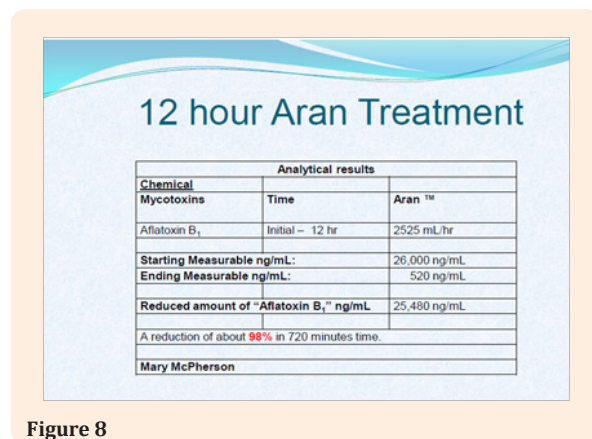


Figure 8

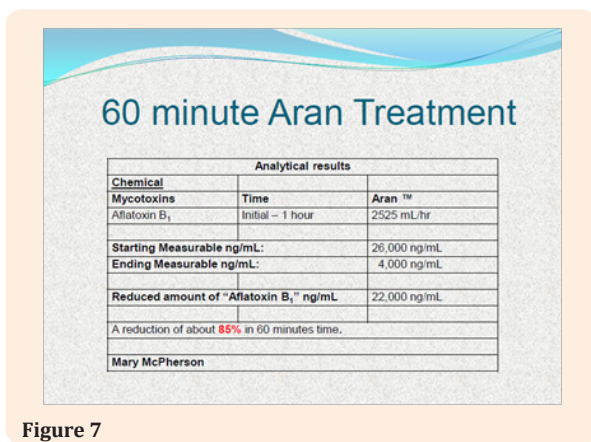


Figure 7

**Liver Cancer and occurrence in foods:** cereals, oilseeds, spices and tree nuts, maize, groundnuts (peanuts), pistachios, brazils, chilies, black pepper, dried fruit and figs, milk, nonfat dry milk, cheese, yogurt, peanut butter, eggs, meat, and just about any other food substance that is grown in warm moist climates.

Irradicating the mycotoxins in the natural environment near and around the farms will prevent introduction to the farms and in the farm fields. We then can prevent it in the storage areas and shipping containments. This will prevent disease worldwide.

Why is this information so important to the science world?

If we can break the benzene ring of this mycotoxin then we can break down many other benzene ring contaminants. The chemical formula is similar to other pollutants such as solvents, formaldehyde, biological agents even biowarfare constituents.

Table 1: *Aspergillus* species capable of producing aflatoxins.

Species	Mycotoxins produced			
	AFB	AFG	CPA	Major sources Geographical distribution
<i>A. flavus</i>	+	-	-	All kinds of foods Ubiquitous in warmer latitudes
<i>A. parasiticus</i>	+	+	-	Peanuts Specific areas
<i>A. nomius</i>	+	+	-	Bees USA, Thailand
<i>A. pseudotamarii</i>	+	-	+	Soil Japan
<i>A. bombycis</i>	+	+	-	Silkworm frass Japan, Indonesia
<i>A. ochraceoroseus</i>	+	-	-	Soil Africa
<i>A. australis</i>	+	+	+	Soil, peanuts Southern hemisphere

A little history of not so long ago... bit scary... [1-21].

Just prior: Hats off to Khrushchev! Heads still on now!

Khrushchev in 1939 was faced with horses dying on farms all over the western parts of the Ukraine. Khrushchev wrote, "I can't believe that science is absolutely helpless here..." "We had won more than just a victory for our agriculture. It was a moral and political victory as well. But how many collective farm chairmen, cattle raisers, agronomists, animal husbandry specialists, and scientists had lost their heads as saboteurs before I stepped in and took charge of the situation".

Korneev (1948) fed white mice with oats infested with *Stachybotrys alternans* strains.

According to Gajdusek (1953) cases of human stachybotryotoxicosis have been found mainly in regions where the equine disease has also been reported.

Forgacs et al. (1958) studied the toxicity of 40 strains.

The Role of *Stachybotrys* in the Phenomenon Known as Sick Building Syndrome EEVA-LIISA HINTIKKA Finnish Institute of Occupational Health.

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England, early 1960's - 10,000 turkeys and ducks die due to Turkey X disease found to be *Aspergillus flavus* abbreviated A. fla and toxin hence aflatoxin. Cause- *Aspergillus flavus* contamination of peanut meal.

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