Biotoxins: Part 3

On June 12, 2002, President George W. Bush signed into law the Public Health and Safety Act of 2002 (PL 107-188) which requires that the Department of Health and Human Services maintain a list of biological agents and toxins which pose a severe treat to public safety. The list of biotoxins, as it appears in the August 23, 2002 Federal Resister, (see also 42 CFR Part 72, Appendix A) is as follows:

- Abrin
- Botulinum neurotoxins
- Clostridium perfringens epsilon toxin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shigatoxin and Shiga-like toxins
- Staphylococcal enterotoxins
- Tetrodotoxin
- T-2 toxin

The U.S. Center for Disease Control (CDC) lumps several of these toxins into a broad classification called "Selected Low Molecular Weight (LMW) Toxins". These include (1) conotoxins, (2) Saxitoxin, (3) Tetrodotoxin, (4) the T-2 toxin, and (5) Diacetoxyscirpenol, as opposed to the other listings which are "Protein Toxins," which have a high molecular weight. Generally speaking, the low molecular weight toxins are not destroyed by cooking and might be potentially used by a terrorist to contaminate surfaces and food as they are more stable in the environment, and can be more potentially inhaled as an aerosol. Four of the LMW toxins are discussed in this Newsletter.

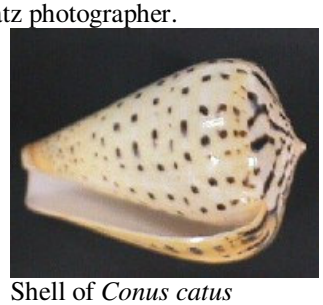
Some other LMW Toxins recognized by CDC but not appearing on the Department of Health and Human short list are brevetoxins, palytoxin, and microcystins.

Conotoxins

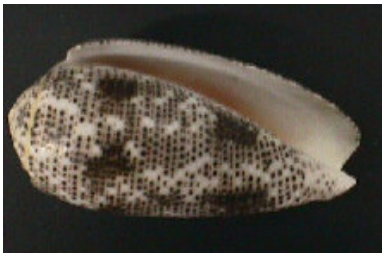
Conotoxins are neurotoxins derived from marine cone snails of the genus *Conus* that occur in the Indian-Pacific Oceans especially off the coast of Australia. Cone snails do not occur naturally off the coast of the United States (Hawaii an exception) or Europe. The conotoxins are in the toxin sacs of these predatory snails. The snails use their venom to immobilize and kill fish, shellfish, and marine worms. Conotoxins are a complex group of chemicals made up of typically 12 to 40 amino acid residues forming compact peptide molecules of which over 2000 different variant combinations are known. There are probably over 50,000 different conotoxins in existence from perhaps 500 different species of cone snails. Any cone snail species can inject a mix of many different conotoxins.

Conus geographus, one of the most deadly cone snails, venomous tube visible, snail can grow up to 10 cm in length; a typical attack takes place in milliseconds with a 70% fatality rate to humans. Photo from National Geographic website, Kerry Matz photographer.

Shell of *Conus geographus*, from www.Biopix.com (J.C. Schon photo)



Shell of *Conus catus*



Shell of *Conus textile*

Images of *C. catus* and *C. textile* shells and other cone shells at http://grimwade.biochem.unimelb.edu.au/cone/images/coneshellspics/cones.html

Human deaths have occurred naturally when divers and fishermen have accidentally stepped on a cone snail, or in the process of harvesting the snails. The shells are very attractive, and some shells are worth a small fortune to collectors. About 30 deaths have been documented and studied. There are probably a lot more deaths that have not been studied or reported. Deaths occur by injection of the venom if the snails are handled or stepped upon, but the venom is also toxic by ingestion of the mollusk.

Conotoxins are classified into six different broad classifications based on their biological activity (Table 1).

Table 1 Biological Activity of Conotoxins

Classification	Biological Activity
Alpha-Conotoxins	Inhibits nicotinic acetylcholine receptors at nerves and muscles. The result is paralysis.
Mu-Conotoxins	Inhibits voltage-graded sodium channels in muscles. The mechanism is similar to that of saxitoxin produced from red tide algae and discussed in an earlier PEAC Newsletter.
Delta-Conotoxins	Inhibits the inactivation of voltage dependent sodium channels ("delta" slows the inactivation of the sodium channel, "mu" inhibits the sodium channel.)
Omega-Conotoxins	Affects the calcium channels associated with nerve impulse transmission at the neuromuscular junction. Calcium channels are related to sensitivity to pain.
Kappa-Conotoxins	Inhibits voltage-graded potassium channels, resulting in tremors.
Conantonkins	Blocks nerve impulses that use glutamic acid rather than acetylcholine as the neurotransmitter.

The extreme toxicity results from several different classes of conotoxins acting synergistically by different mechanisms. Some of the toxins by themselves are not lethal but produce tremors or deaden pain. Some alpha-conotoxins by themselves are lethal by injection at 0.025 mg/kg or even 0.01 mg/kg of body weight, from mouse injection tests. No information is available in the public domain on toxicity by inhalation [from http://www.cbwinfo.com/Biological/Toxins/Conotox.html].

On a molecular scale, conotoxins differ from other biotoxins in that they are relatively small, compact peptides made up of 12 to 40 amino acids held tightly together by disulfide bonds. The disulfide bonding network as well as the order of the specific amino acids and how they are configured determine the specifically of conotoxins.

Clinical symptoms (based on interviews by H. Flecher in 1935 of people "stung" by Cone snails and published in the Medical Journal of Australia, and later interviews) include

Non Fatal Case (full recovery)

- Burning pain
- Swollen arm and pain
- Local numbness spreading rapidly to involve the entire body, with some cardiac and respiratory distress
- Progressive weakness, loss of coordination, drooping eyelids, shallow breathing
- Headache, nausea, stomach cramps, shortness of breath

Fatal Cases

- Numbness without pain (some species produce severe pain and spreading numbness)
- Lips become stiff
- Blurred vision
- Paralysis
- Coma
- These symptoms occur almost immediately upon injection
- Death occurs as the result of respiratory and/or cardiovascular collapse.

Supportive care includes artificial respiration

There are severe logistics for a potential terrorist to grow and harvest cone snails for their toxins. Our search using the Internet failed to uncover any use of Conotoxins as a terrorist weapon. There is an interview report on Soviet research using smallpox virus to produce toxic small peptide chains similar to "conotoxins" [see http://www.homelandsecurity.org/newjournal/Interviews/displayInterview2.asp?interview=3].

The potential threat of terrorist use is there because Conotoxins are being studied as a source of potential drugs for treating neurological diseases. In addition, the amino acid sequence forming the peptide chain of several conotoxins have been determined, and synthetic combinations of specific conotoxins have been artificially produced. Patents for producing selected conotoxins or using them for drugs are published in the open literature. The introduction of genes into bacteria, which can be grown to produce the toxins is feasible. The possibility of laboratory theft or someone with the necessary technology and equipment to manufacture the toxins is real.

As an example of medical use, clinical trials are underway in Australia using a conotoxin Vc1.1 (drug called ACV1) derived from *Conus victoriae* to treat neuropathic pain in the treatment of sciatica, shingles, and diabetic neuropathy. The ACV1 also appears to accelerate the recovery of injured nerves and tissues [see B.G. Livett et al, "Therapeutic applications of conotoxins that target the neuronal nicotinic acetylcholine receptor" Toxicon Vol 48(7) 2006. pp 810-829, abstract available on Internet]. Additional examples on the use of LMW Toxins for development of drugs to treat diseases and neurological conditions is at the website, http://www.bentham.org/cpps/contabs/cpps6-3.htm. A synthetic version derived from omega-conotoxin M VII A has found an application in the analgesic drug ziconotide (Prialt®).

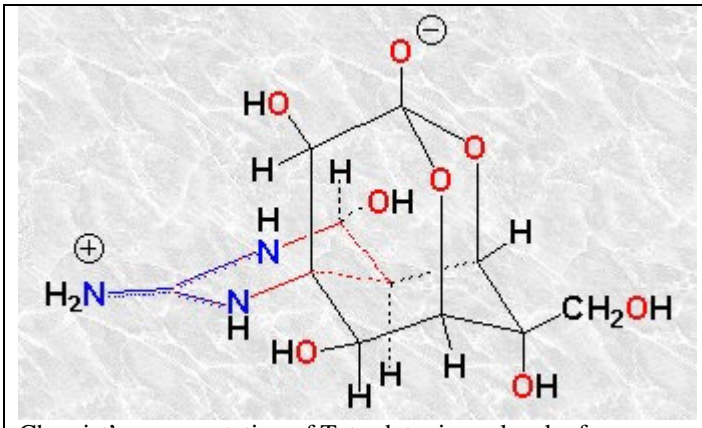
The CDC has issued guidelines on the safe handling on biotoxins including Conotoxins, which can be viewed at http://www.cdc.gov/OD/OHS/biosfty/bmbl5/sections/SectionVIIIG-ToxinAgents.pdf.

Additional Reading on Conotoxins: *BioScience*, Vol. 47, No. 3 (Mar., 1997), pp. 131-134

Tetrodotoxin

Tetrodotoxin is another of what the U.S. Center for Disease Control (CDC) classifies as a "Selected Low Molecular Weight (LMW) toxin. Poisoning usually occurs as the result of eating certain marine fish, in particular organ parts where the toxin is concentrated. Cooking does not destroy the toxin.

Test animals injected (1 to 10 micrograms per kg of body weight) with the toxin develop a rapid onset of excitability, muscle spasm, and respiratory distress. Death may occur within 10 to 15 minutes from respiratory paralysis. Humans ingesting seafood containing tetrodotoxin show similar signs of toxicity, typically preceded by numbness of lips, the face, and extremities. Other symptoms include sweating, weakness, tremor, incoordination, cyanosis, hypotension, nausea, vomiting, diarrhea, and abdominal pain. Cardiac arrhythmias may proceed complete respiratory failure and cardiovascular collapse. The person although paralyzed may be conscious until just before death. Death usually occurs within 4 to 6 hours after ingestion with a range of 20 minutes to 6 hours. The toxin works by inhibiting the sodium channel at the nerves and muscles. [information from CDC website and Wikipedia].



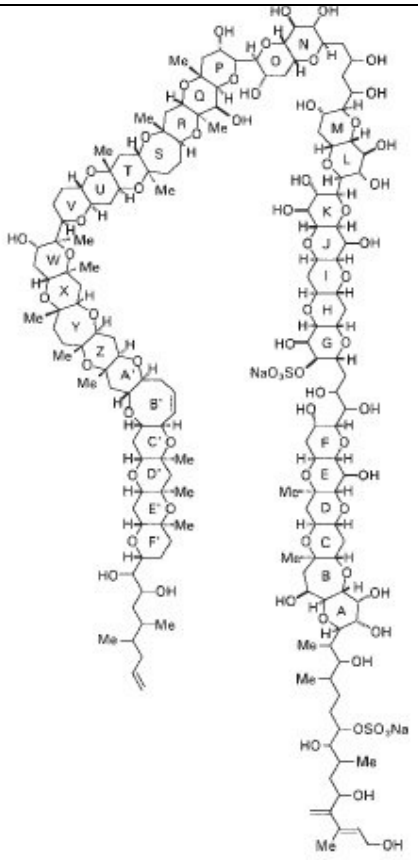
Chemist's representation of Tetrodotoxin molecule, from http://www.chm.bris.ac.uk/motm/ttx/ttx.htm.

Tetrodotoxin

Molecular formula: $C_{11}H_{17}N_3O_4$
CAS Number: 4368-28-9
Molecular Weight: 319.3
Synonym: TTX
Lethal dose (from mouse injection test) is 8 micrograms per kilogram of body weight. Lethal oral dose (mouse) is 334 micrograms per kilogram of body weight.
Note that tetrodotoxin is a non-protein.

Tetrodotoxin poisoning is usually associated from eating pufferfish. The toxin does not come from the fish itself but is produced by certain bacteria, notably *Pseudoalteromonas tetraodonis*, and other bacterial species (e.g. *Vibrio alginolyticus*). Pufferfish grown in a laboratory free from the bacteria do not produce tetrodotoxin unless they are fed food containing the bacteria. The highest concentration of tetrodotoxin in pufferfish is in the ovaries, liver, intestines, and skin; these body parts must be removed before the fish is prepared for eating. The muscular flesh of the pufferfish is considered free of the toxin. Nevertheless, in Japan where pufferfish [Fugu, as it is called in Japan, which is also the genus name for several species of pufferfish] is considered a delicacy, from 1974 through 1983 there were 646 reported cases of pufferfish poisoning with 179 fatalities. Sushi chefs who wish to prepare pufferfish [Fugu] must be licensed by the Japanese government. A technical article on tetrodotoxin paralytic poisoning is available (Ahasen et al, Singapore Med Journal) 45(2) (2004)) at http://www.sma.org.sg/smj/4502/4502a2.pdf. Photos of different pufferfish species are available at http://saltaquarium.about.com/od/porcupinepufferphotos/Porcupine_Pufferfish_Photos.htm.

Tetrodotoxin is also produced by the bacteria inhabiting other marine and some terrestrial animals. The list of animals include the blue-ringed octopus, triggerfish, goby, anglefish, parrot fish, ocean sunfish, porcupine fish, seastars, starfish, certain species of crabs, flatworms, sea squirts, several marine snails, ribbon worms, arrow worms, some poisonous frogs, and some salamanders. The blue-ringed octopus uses tetrodotoxin as venom for injecting its prey (the venom contains both the bacteria and toxin). With all the different kinds of bacteria inhabiting different hosts, one would expect different kinds of tetrodotoxin. There are different biotoxins produced by different bacteria, but the name "tetrodotoxin" is reserved for just one molecule. Other toxins have been given different names, such as anhydrotetrodotoxin, palytoxin, manitoxin, etc. Two of them (palytoxin and maitotoxin) have potencies 100 times that of tetrodotoxin. Palytoxin has been isolated from small marine organisms of the genus P*alythoa.* Maitotoxin has been found in certain fishes associated with ciguatera poisoning.



Chemist's representation of Maitotoxin (from J. Chandrasekar, Resonance, May 1996, pages 68-70, Me is an abbreviation for CH_3)

Maitoxin

Molecular Formula: $C_{164}H_{256}O_{68}S_2Na_2$
CAS Number: 59392-53-9
Molecular Weight: 3422

This molecule holds the record of being the largest natural and most lethal non-protein, non-peptide product made in nature yet discovered.

The lethal dose is (from mouse injection tests) is 50 nanograms per kilogram of body weight.

The toxin is produced by the red tide algae *Gambierdiscus toxicus*, but human poisoning is associated with eating tropical reef fish which are contaminated with the red tide algae. The condition is called "Ciguatera Fish Poisoning".

Paralysis and death may occur upon ingestion. Recovery time among survivors may take weeks, months, or even years.

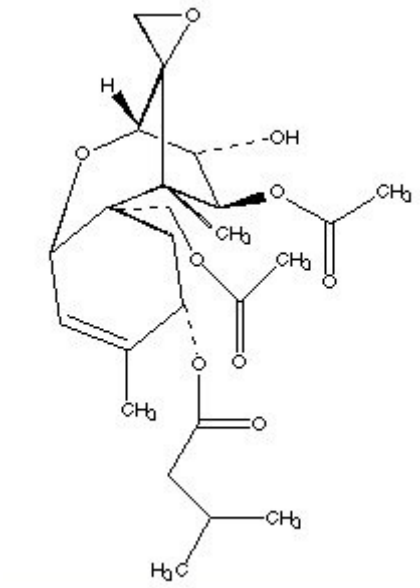
Tetrodotoxin has been blamed for "zombie" poisons in Haiti [see W.H. Anderson, "Tetrodotoxin and the zombie phenomenon", Journal of Ethnopharmacology vol 23 (1) pages 121-126 (1988)].

Tetrodotoxin can be synthesized. The papers are in the open literature. [e.g. Kishi, Y. et. Al., Journal of American Chemical Society, vol 94, 1972]. For a general survey of methods of tetrodotoxin synthesis (2004) see http://www.princeton.edu/~orggroup/supergroup_pdf/SuperGroupMeetingJune2nd.pdf.

Tetrodotoxin in many respects is similar to Saxitoxin, which is discussed in the December 2007 PEAC Newsletter (see http://www.aristatek.com/Newsletter/0712December/TechSpeak.aspx0). The toxicity is about the same. Both are sodium ion channel blockers. The difference is saxitoxin poisoning occurs through eating shellfish and tetrodoxin poisoning usually occurs through eating fin-type fish, in particular pufferfish. Cooking does not destroy the toxins. Both poisons can be artificially synthesized. Both have the same potential to be mass produced and used as a terrorist weapon, to be disseminated as an aerosol or in food.

T-2 toxin

T-2 Toxin is one of several trichothecene myotoxins which occur naturally in moldy grains (grains infected with *Fusarium* mold). The CDC also classifies it as a "Selected Low Molecular Weight (LMW) Toxin". The CDC also implements T-2 toxin as a potential biological warfare agent [based on a report, Wannemacher R, Wiener SL. Trichothecene mycotoxins. In: Sidell FR, Takafuji, ET, Franz DR, editors. Medical aspects of chemical and biological warfare. Vol.6. Textbook of military medicine, part 1: warfare, weaponry, and the casualty.
Washington, DC: Office of the Surgeon General at TMM Publications, Borden Institute, Walter Reed Army Medical Center; 1997. p. 655-76].



Chemist's representation of T-2 Toxin, from http://www.cbwinfo.com/Biological/Toxins/T2.html

T-2 Toxin

Molecular Formula: $C_{24}H_{34}O_9$
CAS Number: 21259-20-1
Molecular Weight: 466.6
Synonyms: T 2 mycotoxin; Fusariotoxin T 2; Insariotoxin; Mycotoxin T-2; T-2

Related Compound: HT-2 Toxin, $C_{22}H_{32}O_8$, CAS Number 64943-87-2, a metabolite of T-2 Toxin

T-2 Toxin is a powerful natural blister agent which works by inhibiting protein synthesis. About 50 nanograms of T-2 Toxin on skin produces the same blistering effect as 20 micrograms (=20,000 nanograms) of sulfur mustard.

The manner in which T-2 Toxin inhibits protein synthesis has been studied by many researchers (see summary paper on *Fusarium* toxins published by the European Commission, in 2001; *Fusarium* is the name of the mold that produces the toxin), paper at http://ec.europa.eu/food/fs/sc/scf/out88_en.pdf. Specifically, T-2 toxin attacks a critical site on the ribosomal RNA. Ribosomes are the structures within the cell where proteins are made.

Toxicity Data for T-2 Toxin

A detailed summary on toxicity of T-2 Toxin and other trichothecene myotoxins can be found by visiting the website, [website citation from *Textbook of Military Medicine*] http://www.cbwinfo.com/Biological/Toxins/TriToxicol.html.

T-2 Toxin is toxic by inhalation, skin absorption, injection, and ingestion. The chemical is not as toxic by injection or ingestion compared with tetrodotoxin (mouse lethal dose, injection, LD_{50} = 1.6 to 3.8 mg/kilogram of body weight, a mouse weighs about 20 grams). By inhalation, LD_{50} (mouse) = 0.24 to 0.94 mg/kg.

Symptoms of Exposure

Symptoms for skin injury are similar to mustard gas but appear at about a 400 times lower dose. These symptoms include blistering of the skin and irritation of the eyes and throat. The dose required to produce blistering and eye damage is still well below the lethal dose. Inhalation toxicity is comparable to that of other blistering agents (Lewisite, Mustard). Symptoms of inhalation exposure include nasal discharge, throat pain, cough, shortness of breath, and chest pain; the victim spits blood as a result of pulmonary and bronchial hemorrhage. Severe poisoning results in prostration, weakness, jerky movement, shock, collapse, and death. Onset of symptoms occurs between seconds up to about 20 minutes of exposure. Treatment includes decontamination with soap and water.

If ingested as in contaminated grain products, symptoms appear between 8 and 12 hours. These include vomiting and internal hemorrhages in the alimentary track. The intestines, bone marrow, lymph nodes, spleen, and thymus are particularly affected. Severe poisoning results in prostration, weakness, jerky movement, shock, collapse, and death. Treatment includes supportive care including removal of ingested toxin with adsorbents such as superactivated charcoal. The term “alimentary toxic aleuka”, or ATA, is used to describe the poisoning.

Alimentary toxic aleuka occurred in the USSR during 1941-47 and again in 1952, 1953, and 1955 killing thousands of people; the ATA was traced to the people eating over-wintered wheat. Symptoms included vomiting, abdominal pain, diarrhea followed by leucopenia, bleeding from the nose and throat, depletion of the bone marrow, and fever. Extractions of the suspected wheat showed toxic dermal effects when applied to the skin of test animals. The ATA poisoning was not conclusively linked to T-2 Toxin, but the presence of *Fusarium* fungus species was established in the over-wintered wheat, and T-2 toxin and HT-2 toxin was found in later fungal cultures.

Other outbreaks of ATA occurred in China and India. In one Chinese location, 165 subjects became ill after consuming rice infected with two species of *Fusarium*. An ELISA assay of the suspected rice for T-2 Toxin showed a level of 180 to 420 micrograms per kilogram of rice (see European Commission paper, cited earlier).

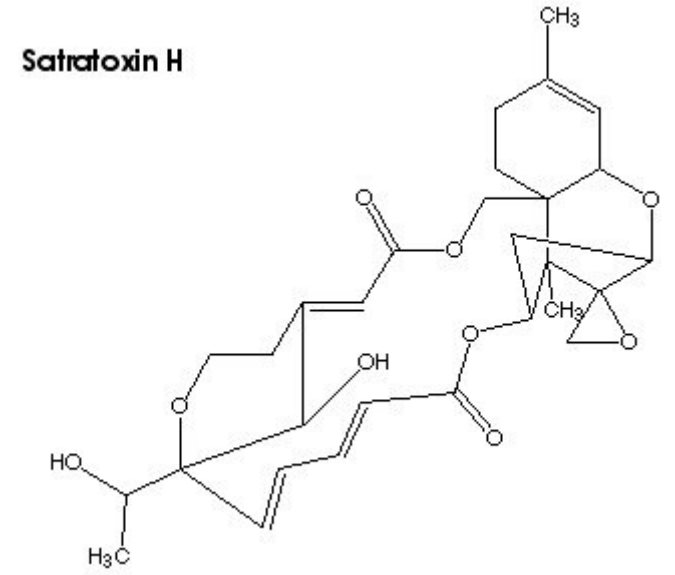
Potential for Terrorist Use

T-2 Toxin and other trichothecene myotoxins are relatively easy to manufacture. The *Fusarium* molds can be grown in large fermentation vessels using grains, barley, rice, maise, or corn as food. Fusarium molds are found in the soils in which the grain crops are grown, or the grain could be inoculated with a particular mold such as *Fusarium sporotrichioides*. The yield of T-2 toxin may be several grams per kilogram of grain material. The T-2 Toxin could be harvested and spread as an aerosol. The target might be people or agriculture (livestock, food crops).

The trichothecene myotoxins including T-2 Toxin are in general stable compounds which are not destroyed during processing or cooking of food, and they do not degrade at high temperatures (from Eriksen, G.S., 1998, cited in European Commission paper).

Diacetoxyscirpenol and other Trichothecene Myotoxins

There is a fairly long list of toxic chemicals produced from molds, which can affect grain products or the air quality in buildings. One of them, diacetoxyscirpenol, is on the Department of Health and Human Services list of biological agents and toxins, which pose a severe treat to public safety. Two other trichothecene myotoxins are also discussed below.



Chemist's representation of Saratoxin H, from http://www.cbwinfo.com/Biological/Toxins/Satra.html

Satratoxin H
Molecular Formula: $C_{29}H_{36}O_9$
CAS Number: 53126-64-0
Molecular Weight: 528.6

Produced by mold *Stachybotrys chartarum*; the mold may contaminate water-damaged homes and other buildings (wallboard, fiberglass, cellulose products, etc.).

LD_{50} (mouse, injection) = 1.0 to 1.4 mg/kg.

If mold spores, fungal fragments, or toxin is inhaled, it can cause nosebleeds, chest pain, and pulmonary hemorrhage. Contact with skin may cause rash. May be fatal if ingested or inhaled in large quantity. A cause of infant deaths (pulmonary hemorrhage) by chronic exposure to mold spores in contaminated houses. Other symptoms headache, fatigue, elevated body temperature.

Additional reading on inhaling fungal fragments inside buildings:

1 Bush R.K., Portnoy J.M., Saxton A., Terr A.I., Wood R.A.: The medical effects of mold exposure. J Allergy Clin Immunol 117. 326-333.2006

2 Brasel T.L., Douglas D.R., Wilson S.C., Straus D.C.: Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. Appl Environ Microbiol 71. 114-122.2005

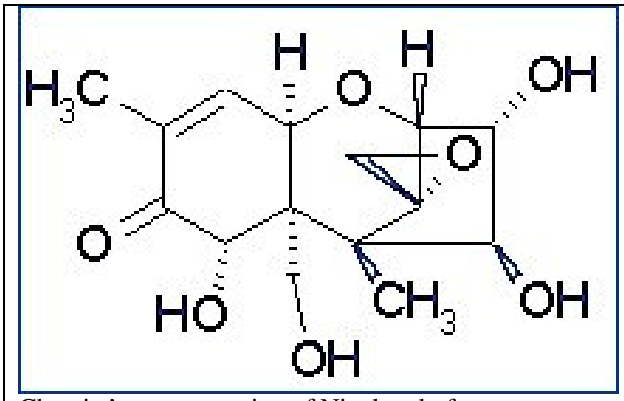
3 Cho S.-H., Seo S.-C., Schmechel D., Grinshpun S.A., Reponen T.: Aerodynamic characteristics and respiratory deposition of fungal fragments. Atmos Environ 39. 5454-5465.2005;

4 Murphy W.K., Burgess M.A., Valdivesio M., Livingston R.B., Bodey G.P., Freireich E.J.: Phase I clinical evaluation of anguidine. Cancer Treat Rep 62. 1497-1502.1978

5 Scheel C.M., Rosing W.C., Farone A.L.: Possible sources of sick building syndrome in a Tennessee middle school. Arch Environ Health 56. 413-417.2001

6 Brasel T.L., Martin J.M., Carriker C.G., Wilson S.C., Straus D.C.: Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. Appl Environ Microbiol 71. 7376-7388.2005

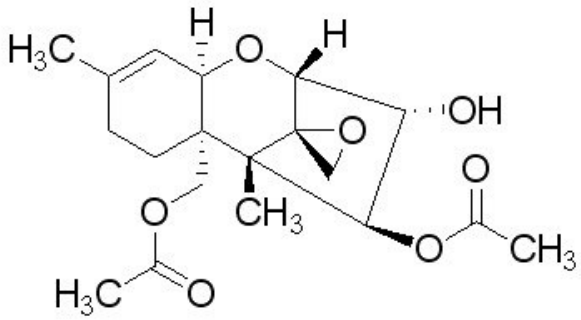
7 Brasel T.L., Campbell A.W., Demers R.E., Fergusen B.S., Fink J., Vojdani A., et al: Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. Arch Environ Health 59. 317-323.2004



Chemist's representation of Nivalenol., from http://www.biopure.at/biopure-index/datasheets/mdc/NIV.htm.

Nivalenol
Molecular Formula: $C_{15}H_{20}O_7$
CAS #: 23282-20-4
Molecular Weight: 212.3
Isolated as a white powder, m.p. 222°C

Produced from mold *Fasarium nivale*, which contaminates grains. Like T-2 Toxin, it inhibits protein synthesis via binding to the ribosome but is less toxic.
LC_{50} (mouse, oral): 39 mg/kg (from European Commission paper, cited earlier).



Chemist's representation of diacetoxyscirpenol, from Sigma-Aldrich website, http://www.sigmaaldrich.com

There is potential for a terrorist to mass produce diacetoxyscirpenol by fermentation using a starch-rich grain or potatoes as a food source.

Diacetoxyscirpenol
Chemical Formula: $C_{19}H_{26}O_7$
CAS#: 2270-40-8
Molecular Weight: 366.4

Produced from molds of species *Fasarium* such as Fasarium sambucinum, *F. moniliforme*, equiseti, *F. graninearum*, etc., which can contaminate grains, potatoes, peas, soybeans, and is toxic if the food is consumed by people or livestock. Diacetoxyscirpenol also has been detected in crude building materials.

LD_{50} (mouse, intravenous injection)= 12 mg/kg. LD_{50} (rat, intravenous injection) = 1.3 mg/kg; LD_{50} (rat, oral) = 7.3 mg/kg, from http://www.cbwinfo.com/

Primarily a concern with livestock fed moldy food, with symptoms similar to the T-2 Toxin

The same contaminated grain products may contain diacetoxyscirpenol, T-2 Toxin, and Nivalenol.