Newsletter for the TDM and Toxicology Division of AACC

Summer 2012

Therapeutics & Toxins News

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Dimethylamylamine— a supplement of potential health concern

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Dimethylamylamine (DMAA) has recently received much attention in the international press as a potentially dangerous dietary supplement and as an emerging drug of abuse. While it has been extensively sold on the global market very few human safety studies have been conducted. Published research in 1996 stated that DMAA was a natural component of the edible geranium plant but additional research has unequivocally shown this to be inaccurate. The identification of DMAA as a synthetic compound has caused significant regulatory issues in Canada and the United States. These issues, its ban in competitive sports, and the history of DMAA will be discussed in this short review.

DMAA is a relatively volatile, straight chain aliphatic primary amine ($C_7H_{17}N$) with a molecular weight of 115.2 g/mol. This chemical is also referred to as methylhexaneamine, methylhexamine, 4-methyl-2-hexanamine, 1,3-dimethylpentylamine and by IUPAC 4-methylhexan-2-amine.

DMAA was first developed and patented by Eli Lilly in 1944 based upon its vasoconstrictor action on the nasal mucosa. It was patented again by the same company in 1971 as an inhaled nasal decongestant called Forthane and also for the treatment of hypertrophied or hyperplasic oral tissues (1,2). Other trade names include Floradrene and Geranamine. Shortly after the 2005 ban of the dietary supplement ephedrine, Patrick Arnold of BALCO reintroduced DMAA into the US market (3). The trademarked name Geranamine is owned by the company he founded, Ergopharm, a division of Proviant Technologies, Inc.

Logo for Therapeutic and Toxin Newsletter

The pharmacological profile of DMAA has not been intensively studied since Eli Lilly filed its initial patent (4). They stated that DMAA has CNS stimulant effects that are less than that of amphetamine and ephedrine (5). Further, the systemic toxicity of DMAA in animals is greater than that of ephedrine and less than that of amphetamine (1). DMAA has both a similar structure and mechanism of action to the drugs amphetamine and propylhexedrine. The only structural difference between DMAA and amphetamine is that DMAA lacks the phenyl group, containing only four of the six carbons that form the ring (Figure 1). DMAA is structurally different than propylhexedrine; the active ingredient in the over-the-counter Benzedrex inhaler used for nasal decongestion; in that it lacks two carbons that create a cyclohexyl ring and a methyl group attached to the amine group. Despite these structural differences DMAA has a similar mechanism of action to these drugs, being a stimulant and having norepinephrinergic effects.

Today, DMAA is commonly found in supplements promoted for weight loss, increased energy, bodybuilding, and

"DMAA is commonly found in supplements promoting weight loss."

Dimethylamylamine (continued from page 1)

enhanced athletic performance. Common DMAA products available online and at popular health supplement suppliers include Jack3d, OxyELITE Pro, Code Red, Napalm, HydroxyStim and Lipo-6 (Figure 2). One manufacturer refers to DMAA as being a low side-effect alternative to ephedrine (6). These nutritional supplements may list DMAA in the ingredients as any of the previously mentioned names or as geranium oil extract. Commonly reported anecdotal health benefits include enhanced focus and mood, improved exercise performance and reduced appetite.

Apart from its use as a dietary supplement, DMAA has also recently emerged as a drug of abuse being sold as an active ingredient in over-the-counter party pills. This became prevalent in New Zealand after 1-benzylpiperazine (BZP) became a scheduled drug (7). Soon thereafter New Zealand authorities observed many adverse events associated with DMAA use including seizures and hospitalizations. In November 2009, the New Zealand government moved to restrict sales of DMAA pills (8). Their Expert Advisory Committee on Drugs considered the risk from DMAA and recommended it be scheduled as a restricted substance (9). The first case report involving DMAA was published in December 2010 and described a young male New Zealander who suffered a large cerebral hemorrhage shortly after ingestion of ~576 mg DMAA, 150 mg caffeine and one beer (10). On May 8, 2012 three additional cerebral hemorrhages were reported in adult New Zealanders after ingesting DMAA (11).

"DMAA has also recently emerged as a drug of abuse. DMAA ingestion can cause seizures, heat intolerance, rhabdomyolysis, kidney and liver failure, and heart problems"

Since DMAA is a stimulant that can improve focus and potentially enhance athletic performance, the World Anti-Doping Agency added the chemical to its 2010 prohibited substance list (12). The NCAA and many other professional athletic organizations have also banned DMAA. Since then more than 75 professional athletes that were competing in a variety of sports to include track, cycling, rugby, basketball and tennis have tested positive for DMAA.

Recently, the US Department of Defense (DoD) urine forensic drug testing laboratories observed a significant decrease in their amphetamine confirmation rates. Many specimens screened positive with both the Roche KIMS and Siemens Syva EMIT II Plus Amphetamine immunoassays but confirmed negative with no presence of amphetamine or related drugs by gas chromatography-mass spectrometry (GC-MS). To investigate this issue, 134 specimens fitting this profile were analyzed at the Armed Forces Medical Examiner System forensic toxicology laboratory (13). Analysis by liquid chromatography-tandem mass spectrometry (LC-MS-MS) revealed a common denominator: over 92% of these specimens contained DMAA at a concentration at or above 2.5 mcg/mL. Spiking DMAA into certified negative drug-free urine showed that false positive responses in amphetamine immunoassays could occur at 7.5 mcg/mL (Roche) and 3.125 mcg/mL (Siemens) of DMAA. These cross-reactivities correlated well to the service member specimens analyzed in the study, which ranged from 2.5 to 67.0 mcg/mL with 6.9 mcg/mL being the lowest DMAA concentration that generated two positive immunoassay results. This data also matched well with the limited human DMAA excretion data published in a 2009 article (14). Two healthy men orally ingested a single 40 mg dose of DMAA and the peak urine concentration for the parent drug was 15-18 mcg/mL at four hours post-ingestion. This level had declined to less than 2.5 mcg/mL by 48 hours. DMAA is not easily detected on GC-MS screens as it elutes very early, often in the solvent front. LC-MS-MS analysis detects DMAA as a double chromatographic peak due to its being sold as a racemic mixture (13,14).

DoD medical authorities have expressed concerns that DMAA ingestion can cause seizures, heat intolerance, rhabdomyolysis, kidney and liver failure, and heart problems (15). They have ordered a review of the ingredient after two soldiers died in 2011 of a cardiac arrest while exercising. Autopsies revealed the presence of DMAA in their systems (16). In December 2011 the DoD banned sales of DMAA containing supplements at all stores and commissaries located on military bases (17).

Lake Texoma Under Blue-green Algae Advisory Ah! Spring and Summer are here again!

Donald Frederick, PhD

This headline from the April 12, 2012 advisory from the Texas Parks and Wildlife department reminds us again that the invasion of "The Green Slime" (1968 film) is returning in force this summer. Previous years stories from around the world announced the invasion of the toxic blue-green algae. A story from the *Independent* (London) last year was entitled, "Call for warnings of algae dangers after dog dies". This year the Oklahoma Department of Environmental Quality issued a two page warning. (1) The Wisconsin Department of Natural Resources issued the following statements "If you think you are experiencing symptoms related to exposure to blue-green algae (e.g., stomach cramps, diarrhea, vomiting, headache, fever, muscle weakness, difficulty breathing), contact your doctor or the Poison Information Hotline (800-222-1222) right away. If your pet displays symptoms such as seizures, vomiting, or diarrhea after contact with surface water, contact your veterinarian right away."

Other areas around the world are hit by blooms of the algae. In Australia early this year there was a large bloom in Gippsland Lakes (2) and an alert for Chifley Dam. Almost every state has issued alerts and warnings about blue green algae toxins. In Ohio researchers have warned that the mild winter could lead to an earlier bloom for toxic blue-green algae in the western basin of Lake Erie.

So what is blue-green algae? Blue green algae more correctly known as *cyanobacteria* are a group of organisms that are among the oldest on the planet with fossils dating beyond 3 billion years. As photosynthetic micro-organisms they are thought to have played a key role in the oxygenation of the earth. The *cyanobacteria* can be many colors including green, red, orange or brown. There are thousands of species in the group that are distributed world wide. Only some of the species are able to produce hepatotoxins, cytotoxins, dermatotoxins and neurotoxins that are responsible for human and animal poisonings. When these organisms multiply in large numbers, a "bloom" is formed. Blooms can form quickly and rise to the water surface appearing most often as the blue-green "pond scum". There is no distinct appearance of a bloom to identify whether it contains toxins or does not.

"Blue green algae more correctly known as cyanobacteria are a group of organisms that are among the oldest on the planet with fossils dating beyond 3 billion years."

Historically the first published report of the potentially lethal effects of microorganisms known as blue-green algae appeared in Nature in 1878. George Francis described an algal bloom that had formed in the estuary of the Murray River, in Australia, as "a thick scum like green oil paint, some two to six inches thick." Blooms are formed as a result of changes water temperature, irradiance and nutrient supply. Over the past decade the incidents appears to have increased with more toxic blooms and the resultant increased human intoxications. Recently genetic basis for toxin production (3) has been identified providing a framework for understanding the environmental conditions the promote toxin production. A nice review of this area has just been published. (4) Although the factors that affect bloom production are complex, the availability of nitrogen and phosphorus promote growth rates while other key factors such as high incident light, iron availability and carbon dioxide levels may regulate the amount of toxin released. General consensus is that run-off from fertilized fields contributes to the increased number of blooms seen in the last decade.

The numerous types of toxins of the cyanobacteria have been reviewed in 2010. (5) One group of toxins produced by various species of cyanobacteria is classified as neurotoxins because they target the cholinergic synapses as potent agonists of muscular and neuronal nicotinic acetylcholine receptors. Anatoxin-a and homoanatoxin-a are neurotoxins produced by several of the genera of cyanobacteria. LCMS has been used to detect these neurotoxins at the nanogram and pictogram levels. These have been responsible for the deaths of both domestic and wild animals. Recently with the media attention on food supplements, cyanobacteria have been used in food supplements. A report in 2009 found anatoxin-a in 7.7% of the supplements tested. Although there have been no human deaths proven to be caused by anatoxin-a exposure there was one possible death reported in 2003 but was not proven and occurred 48 hours after exposure. In animals the deaths are very quick from minutes to a few hours.

Blue-Green Algae (continued from page 4)

Another group of toxins are saxitoxin and its analogs; reviewed in 2010. (6) This group of toxins are also know as the paralytic shellfish toxins (PST) and are associated with dinoflagellates (marine) as well as cyanobacteria (freshwater). In the marine environment these toxins are passed through shellfish, crustaceans and molluscs before entering the human food chain. There have been documented human poisoning and a few deaths to paralytic shellfish poisoning (PSP). The toxin is very strong with only 1 mg fatal to humans and is considered Schedule 1 of the Chemical Weapons Convention. The consequences of PST are more economic where vast fisheries have had to be closed for a toxic bloom. There are at least 57 analogs of saxitoxin which makes identification more difficult. Many of these are identified with in the 2010 review.

Hepatotoxins are the most abundant of the toxins of cyanobacteria. Microcystins (MC) are the most abundant of this class and have 80 different variants. Contamination of drinking water is the primary mechanism for human exposure. Increased incidence of liver cancer in China has bee associated with chronic ingestion of sub lethal doses. The main target of MC is the hepatocyte where protein phosphatases are inhibited and phosphorylase b is activated resulting in excessive phosphorylation triggering apoptosis. Nodularin another hepatotoxins is a pentapeptide produced by only Nolularia spumigena. Nodularin is a potent tumor promoter althought not much is known about its mechanisms.

Although cyanobacteria is the most important group of prokaryotes producing toxins there are other bacteria present in the water that are important to human and animal health. *Vibrio* species belong to gamma-proteobacteria are abundant and have special relevance due to their ability to attach to the exoskeletons of crustaceans and other marine organisms. *Vibrio cholerae* of course fits into this class as the serious human cause of cholera. *Vibrio vulnificus* is associated with sever fulminant systemic infections associate with consumption of seafood especially raw oysters. *Aeromonas hydrophila* cause both intestinal and systemin infections in humans and associated with travel's diarrhea.

So this summer just when you thought it was safe to go into the water. BEWARE!!!

"If you think you are experiencing symptoms related to exposure to blue-green algae (e.g., stomach cramps, diarrhea, vomiting, headache, fever, muscle weakness, difficulty breathing), contact your doctor or the Poison Information Hotline (800-222-1222)."

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   Ref Type: Internet Communication

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Dimethylamylamine (continued from page 2)

The DoD is not the only agency with concerns. While at present the FDA considers DMAA to be a safe food additive, its Canadian counterpart, Health Canada, in July 2011 reclassified DMAA as a drug that requires authorization to be legally sold (18). DMAA was previously considered a minor natural plant product of the geranium (*Pelargonium graveolens*) based upon a 1996 publication reporting the extraction of DMAA from this plant (19). However this study in the obscure Journal of Guizhou Institute of Technology has been disregarded by new research from multiple groups. All additional studies concluded that there is no credible scientific evidence that DMAA is detectable in plant isolates and hence cannot be classified as a natural health product (18,20).

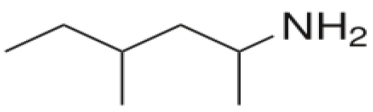
On April 27, 2012 the FDA stated that synthetically produced DMAA is not a dietary ingredient and hence not eligible to be used as an active ingredient in dietary supplements as defined by the Food and Cosmetic Act (21,22). US manufacturers of DMAA-containing products will need to prove that it is a natural ingredient or file the chemical as a new dietary ingredient and illustrate the safety of the product.

There currently exists only scant research on the effect in humans of orally ingesting DMAA. There have been only two published clinical trials involving orally ingested DMAA, both from the University of Memphis. The first study (23) involved healthy men and women at rest who received a single oral dose of 50 or 75 mg DMAA with and without caffeine (250 mg). DMAA is typically sold in combination with caffeine and less frequently as a single supplement. During the two hour post-ingestion observation period the subject's heart rate was unaffected but their blood pressure increased in a significant and dose-dependent manner with DMAA. The blood pressure peaked at 60 minutes post-ingestion. Unexpectedly, plasma norepinephrine and epinephrine levels appeared to be unaffected by treatment. Subsequently this same group published a study on the effect of caffeine and DMAA alone and combined on exercise performance in healthy men and women (24). One hour prior to exercise healthy subjects orally consumed placebo, caffeine (4 mg/kg), DMAA (1 mg/kg) or caffeine plus DMAA. They concluded that caffeine, DMAA or their combination did not improve exercise performance as measured by 10 km run times.

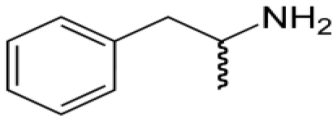
"It is postulated that DMAA-induced hypertension could result in an increased myocardial workload that could precipitate a cardiovascular event ."

It is critical to the public that the overall safety of DMAA be further explored. In the US, the FDA has had 42 adverse events reported that involved products containing DMAA. It is postulated that DMAA-induced hypertension could result in an increased myocardial workload that could precipitate a cardiovascular event (22). While the Army Public Health Command is planning a safety study on DMAA it is incumbent upon the supplement manufacturers to conduct thorough pharmacological safety studies. It will be interesting to watch this scientific regulatory process unfold over the next year.

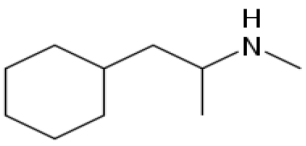
Figure 1: Relevant Chemical Structures



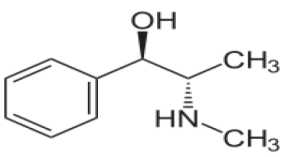
Dimethylamylamine



Amphetamine



Propylhexedrine



Ephedrine

Dimethylamylamine (continued from page 5)

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FROM THE HOT SEAT:

Greetings Division Members!

I am very excited to see and visit with you at the upcoming annual meeting in Los Angeles. This is a great opportunity for us to acknowledge our Division members, network, and share.

Please let me know if there are any events, accomplishments, announcements or topics you would like me to raise at our lunch-time business meeting!!! If you want to speak yourself, I am happy to put you on the agenda.

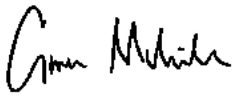
The meeting details:

Monday July 16, 2012: 12:00 noon - 2:00pm
Diamond Ballroom – Salon 10, in the JW Marriott Hotel

We will allow time between noon and 12:30pm for attendees to visit with one another, and also visit the buffet lunch table! The program will start at approximately 12:30pm. The room (and food) will be available until 2:00pm for anyone who wants to stay after the program.
The agenda will include an overview of the Division activities and status, general announcements, presentation of our 2012 Young Investigator award and abstract award winners, and a scientific presentation from the 2011 Young Investigator award winner, Dr. Matt Krasowski.

I hope that everyone also plans to attend the lunchtime meeting we will host in Los Angeles!

With pleasure,



"Please attend the TDM/Tox Division Lunchtime meeting at the 2012 AACC Annual Conference in Los Angeles."

UPCOMING MEETINGS OF INTEREST

THE INTERNATIONAL ASSOCIATION OF FORENSIC TOXICOLOGISTS (TIAFT)
Annual Meeting
June 3-8, 2012, Hamamatsu, Japan
www.tiaft.org

SOCIETY OF FORENSIC TOXICOLOGISTS (SOFT)
Annual Meeting
July 1-6, 2012, Boston, MA
www.soft-tox.org

AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY (AACC)
Annual Meeting
July 15-19, 2012, Los Angeles, CA.
www.aacc.org

THE AMERICAN ACADEMY OF CLINICAL TOXICOLOGY
North American Congress of Clinical Toxicology
October 1-6, 2012, Las Vegas, NV
Www.clintox.org

American Association for Clinical Chemistry
Improving healthcare through laboratory medicine

DRUGS IN THE NEWS

Drug Shortages:

Desmopressin injection
Diazepam injection
Diphenhydramine-HCL injection
Diltiazem injection
Etomidate injection
Fentanyl Citrate injection
Hydromorphone-HCL injection
Lidocaine Hydrochloride injection
Magnesium Sulfate injection
Methotrexate
Metoclopramide injection
Midazolam injection
Morphine Sulfate injection
Naloxone injection
Pancuronium Bromide injection
Propofol injection
Zinc injection

http://www.fda.gov/Drugs/NewsEvents/ucm130958.htm

Please contact Dr. Kamisha Johnson-Davis at kamisha.johnson-davis@aruplab.com if you are interested in joining the editorial board or if you have ideas or article contributions for this newsletter.