



**ALL OF US AT BURDOCK GROUP JOIN IN WISHING
YOU A HAPPY HOLIDAY SEASON AND
A PROSPEROUS NEW YEAR!**

**A SERIES OF
NANOTECHNOLOGY / NANOTOXICOLOGY
ARTICLES WILL BE FEATURED IN THE 2007 ISSUES
OF OUR NEWSLETTER**

NANOTOXICITY

- A QUESTION OF OXIDATIVE STRESS -

By George A. Burdock, Ph.D. and Ray Matulka, Ph.D.

There is general agreement among nanotechnologists that the toxicity of some nanosized particles (NSPs) can be characterized by the specific *in vivo* effects they produce. For example, some nanoparticles may accelerate the rates of absorption of various molecular species, while others change protein conformation to create new allergens or autoimmune symptoms, create subpopulations of enzymes with transport or carrier deficiencies, or new toxic sequelae as a result of NSP entry into previously protected environments (*e.g.*, brain, thyroid, fetus, or gonadal tissue). However, nearly all NSPs produce oxidative stress through the generation of reactive oxygen species (ROS).

Under normal coupled reduction-oxidation (redox) conditions in the mitochondrion, ROS are generated at a relatively low frequency, but are easily neutralized by antioxidant defenses, principally by the glutathione (GSH) defense system. However, under conditions of excess ROS, GSH is depleted and the oxidized form of glutathione (GSSG) accumulates. The ratio of GSH to GSSG is a signal that determines when a cell mounts a protective or a "self-destruct" response.

In the hierarchical oxidative stress model (Nel A, Xia T, Madler L, Li N. *Science* 2006;3:622), the normal state is presided over by a high GSH:GSSG ratio. As soon as the

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KEEPING THE HOLIDAY SEASON JOYOUS FOR PETS, TOO

By Sabine Teske, Ph.D.

During this time of year, many of us are preoccupied decorating our homes and planning for get-togethers with family and friends. While we are busy with holiday traditions, it is important to take certain precautions for our beloved canine and feline family members, because many holiday decorations, plants, and foods can be harmful to our four-legged friends.

For example, among popular holiday decorations, artificial snow can cause respiratory irritation if inhaled or gastrointestinal problems if ingested by dogs or cats. Angel hair and tinsel can lead to intestinal upset and blockages if consumed by your pet. In fact, intake of large amounts of angel hair, which is spun glass, can rupture the intestine and be fatal. In addition to these common holiday decorations, liquid potpourri oils, which are used to infuse our homes with scents typical of the Holiday Season are sometimes consumed by pets and may cause diarrhea and other gastrointestinal problems, and potentially, liver damage.

We know that just as traditional Holiday Season plants, such as poinsettias, American mistletoe, and American holly, can be harmful to children, they may also be poisonous to our furry companions. Although not fatal, ingestion of leaves and flowers from poinsettias (*Euphorbia pulcherrima*) causes gastrointestinal upset, while exposure to the sap of poinsettias irritates eyes and/or the oral cavity (including painful blisters on the tongue and mouth). American mistletoe (*Phoradendron serotinum*) and holly (*Ilex opaca*) can be fatal to dogs and cats; in particular, ingestion of the white mistletoe and red holly berries has proven to be fatal to both canines and felines. Lastly, many holiday treats con-

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IN THE NEWS...



October and November were busy months for several members of Burdock Group. Starting in mid-October, Burdock Group was a sponsor as well as a participant at the Nano4Food Conference in Atlanta, Georgia. Dr. George Burdock provided relevant expertise at the conference speaking on "What are the implications for the food industry when examining the toxicological issues surrounding nanotechnology?" Later in the month, several members flew across country to the SupplySide West show in Las Vegas, Nevada. At the show, the Group had a booth where they displayed their expertise

BURDOCK GROUP AROUND THE GLOBE IN 60 DAYS...

Atlanta...Las Vegas...Xiamen...Shanghai...Frankfurt...Home

on U.S. regulations for food ingredient and dietary supplement safety. Venturing into the month of November, Drs. Griffiths and Matulka hopped on the plane and traveled thousands of miles to another continent – first to Xiamen, China where they spoke at the Food Summit Conference on American regulations for food safety, efficacy and claims; then to Shanghai, China where they were present at the Supply Expo/Nutracon show. Lastly, Drs. Griffiths and Teske jetted around the globe to Frankfurt, Germany where they attended the Health-Ingredients Europe show. December poses to be a quieter month for the Group, but big plans are in the making for 2007. *Stay tuned.....*

To obtain copies of any presentations referenced above, contact Shirley Reul, Client Relations Coordinator at sreul@burdockgroup.com

Regulating Botanical Drugs

By Lonnie Williams, Ph.D.

In the United States, a botanical product can be a food, a dietary supplement, and/or a drug, and botanicals have a long history of use in each of these categories. This article focuses on the gray area where a botanical product crosses from dietary supplement use into the realm of a drug, *i.e.*, where the intended use is for diagnosing, mitigating, or treating or curing disease, and thus is classified as a drug according to the Federal Food, Drug and Cosmetic (FFD&C) Act.

Botanical drugs have many characteristics that distinguish them from synthetic or highly purified drugs. They may come from a single plant or from a mixture of plants, and have significant variability in their ingredients depending on their origin and time of harvest. They may also contain multiple active constituents that are not easily identified, and be produced using traditional methods that are difficult to standardize. As a result, the United States Food and Drug Administration (FDA) published a draft guidance document to assist industry in the development of botanical drugs. This guidance outlines when a botanical drug may be marketed under an over-the-counter (OTC) drug monograph and when FDA regulations require submission of an approved new drug application (NDA). It also discusses several areas in which FDA finds it appropriate to apply regulatory policies that differ from those applied to synthetic or highly purified drugs.

Because of the unique nature of botanical drugs, the documentation on the chemistry, manufacturing, and controls will often be different from that for synthetic or highly purified drugs, whose active constituents may be more readily identified and quantified. For example, the FDA would expect an NDA for a synthetic or highly purified drug to identify the active ingredient. However, it would not be essential for the sponsor of a botanical drug to identify

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DID YOU KNOW?

- ✦ **Low fat diets can make you depressed. Research has linked diets that drastically cut down on all types of fat with an increase in symptoms of depression.**
www.psychologytoday.com/articles/pto-20030429-000002.html
- ✦ **The FDA allows an average of 30 or more insect fragments and one or more rodent hairs per 100 grams of peanut butter.**
www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg570-300.html
- ✦ **The romantic associations we have with chocolate may be due to the effects on the brain of a naturally occurring substance called phenylethylamine (PEA). Chocolate contains PEA, which often results in enhanced endorphin levels and increased libido.** www.chocolate-chemistry.com/phenylethylamine.php
- ✦ **According to French researchers, Resveratrol a component of red wine, also found in peanuts, raises the metabolism so muscles burn more energy and work more efficiently.** www.sciencedaily.com/upi/index.php?feed=Science&article=UPI-1-20061117-07542100-bc-france-redwine.xml

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GSH:GSSG ratio decreases slightly, a Tier 1 antioxidant defense pathway is initiated, with the Type-2 neurofibromatosis (Nf-2) signaling pathway triggering the antioxidant response, and generation of Phase II enzymes for conjugation and disposal of the miscreants. When the GSH:GSSG ratio decreases further, the Tier 2 inflammation response pathway is set into motion. In inflammation, signaling pathways such as p38 mitogen-activated protein kinase (MAPK) and nuclear factor kappaB (NF kappaB) are activated. These pathways turn on genes within the cell to produce inflammation *via* signaling cytokines and chemokines, both of which are chemicals that alert neighboring cells and processes within the same cell that a critical point in cell viability has been reached. If the amount of GSH continues to drop (Tier 3), the cell enters into a terminal stage. At this point, the cellular environment becomes toxic, and the mitochondria become porous and leak substances that signal the cell to close down in order to protect the rest of the body from its own toxic state. This last stage is called apoptosis, and the cell goes through a process of destroying its own infrastructure through the activation of the caspase cascades, and it prepares to have its remains engulfed by a macrophage, which has already been signaled by cytokines and chemokines to come to this specific site and be ready for a disposal assignment.

Interestingly, there are reports that following addition of empty liposomes to *in vitro* systems, some of the later signals of the oxidative stress models—such as apoptosis and caspase cascades (Tier 3)—were initiated prior to other presumably obligatory steps in the model (Tiers 1 and 2), possibly even prior to a change in the GSH:GSSG ratio. These findings raise the question as to whether some liposomes may, in fact, act directly with cell or mitochondrial membranes to produce an artificial oxidative stress signal that leads to self-destruction of the cell.

NSPs of different chemical elements may behave quite differently in respect to the oxidative stress. For example, in experimental *in vitro* studies, addition of 15 and 45 nanomolar crystalline silica NSPs for 48 hours to cultured human bronchoalveolar carcinoma-derived cells dose-dependently decreased cell viability. Quantitative analysis of typical indicators of oxidative stress and cytotoxicity, including ROS, glutathione, malondialdehyde, and lactate dehydrogenase found that exposure to crystalline silica NSPs increased ROS levels and reduced glutathione concentrations. Malondialdehyde and lactate dehydrogenase were released from the cells, indicating the occurrence of lipid peroxidation and membrane damage [Lin, W; Huang, YW, Zhou, XD, Ma, Y. *Toxicol Appl Pharmacol*, 2006. Oct. 6. (Epub ahead of print)].

In another study, NSPs of selenium, when compared to natural selenium, were reported to be better scavengers of carbon-centered free radicals generated from (a) 2,2'-azo-bis-(2-amidinopropane) hydrochloride, (b) superoxide anion generated from the xanthine/xanthine oxidase system, (c) the relatively stable free radical 1,1-diphenyl-2-picrylhydrazyl, and (d) the singlet oxygen generated by irradiated hemoporphyrin. NSPs of selenium also protected against the oxidation of DNA (Huang, B; Zhang, J; Hou, J; Chen, C. *Free Radic Biol Med*. 2003, 35(7):805-13).

More research is needed to expand our knowledge of the effects of NSPs on the various cellular and organ systems of the body.

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the specific active constituents, although the FDA recommends that this be done if feasible. Because many botanical products are legally available in the United States as dietary supplements, there is often very little new animal toxicological data needed to initiate clinical studies in humans, as long as there are no known safety issues associated with the product and it is to be used at approximately the same dose as those currently recommended. However, the Investigational New Drug (IND) application for a botanical product that has a previous marketing history in the United States should include the common or usual name of the plant, alga, or macroscopic fungus, the name of the family, genus, species, and variety, and the chemical class of the active constituents for each of the botanical raw materials. The sponsor should also provide information from the historical and scientific literature on the use of the botanical product, as well as information on its current marketed use. For a botanical product that has been previously marketed in a foreign country, but not in the United States, the sponsor must provide sufficient information to assist the FDA in determining the safety of the product for use in initial clinical studies. This supporting information should include a signed certificate of authenticity of the plant and plant parts, data that support safe human use—including the rate of adverse effects—an estimate of the size of the exposure population, and the annual sales volume.

Because of the complex nature of a typical botanical drug, the FDA relies on a combination of tests and controls to ensure the identity, purity, quality, strength, potency, and consistency of its constituents. These include multiple tests for the drug components (*e.g.*, spectroscopic and/or chromatographic fingerprints, chemical assays of characteristic markers, and biological assays), strict quality controls for the botanical raw materials, and adequate in-process controls. Despite differences in procedures, the regulatory pathway for marketing botanical drugs is simplified compared with that for synthetic or highly purified drugs, and there has been increased interest in the development of botanical drugs as evidenced by the increased number of IND submission over the past ten years. The guidance document is available at <http://www.fda.gov/cder/guidance/1221dft.htm>

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taining chocolate, macadamia nuts, and/or raisins should be kept away from dogs and cats. For example, dogs and cats metabolize the cacao bean-derived natural stimulant theobromine, which is present in chocolate, at a slower rate than humans. Rising theobromine levels stimulate the central nervous system and heart, as well as relax bronchial smooth muscles. Depending on the amount and type of chocolate (*e.g.*, dark chocolate contains higher levels of theobromine), symptoms such as restlessness, panting, vomiting, urinary incontinence, and diarrhea are observed within two to four hours following ingestion. If left untreated, toxic levels of theobromine induce a fatal increase in heart rate, seizures, and/or coma. Consumption of raisins or macadamia nuts may cause vomiting and kidney failure in dogs.

Keeping the Holiday Season a happy one for all of the family requires only a few simple measures, including placing harmful decorations, potpourri, plants, and foods out of reach of our four-legged friends.



2001 9th Avenue
Suite 301
Vero Beach, FL 32960-6414



WHAT HAS YOUR CONSULTANT PUBLISHED LATELY?

Select Recent Publications

G.A. Burdock, I.G. Carabin and J.C. Griffiths (2006). **Toxicology and pharmacology of sodium ricinoleate.** *Food and Chemical Toxicology* 44, 1689-1698.

G.A. Burdock and R.A. Isbrucker (2006). **Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin.** *Regulatory Toxicology and Pharmacology* 46, 167-192.

R. Matulka, O. Noguchi and N. Nosaka (2006). **Safety Evaluation of a Medium-and Long-Chain Triacylglycerol Oil Produced from Medium-Chain Triacylglycerols and Edible Vegetable Oil.** *Food and Chemical Toxicology* 44 (9): 1530-1538.

G. A. Burdock, I. G. Carabin and J. C. Griffiths (2006). **The Importance of GRAS to the Functional Food and Nutraceutical Industries.** *Toxicology* 221, 17-27.

G.A. Burdock and B.A. Magnuson (October 2006). **Nanotechnology Nudges into Nutrition.** *Nutritional Outlook*.

G.A. Burdock and B.A. Magnuson (November 2006). **Small Threat? The World of Food Ingredients.**

S. Teske (July 2006). **Adding Product Crunch.** *Asia Food Journal*.

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UPCOMING MEETINGS & SYMPOSIA

December
FDA Functional Foods Meeting
College Park, Maryland

January
ILSI N. America Annual Meeting
Cancun, Mexico

March
Supply Expo West
Anaheim, CA

Food Ingredients Asia-China
Shanghai, China

Society of Toxicology
Charlotte, NC

This newsletter is prepared by:

Shirley A. Reul, Client Relations Coordinator

James C. Griffiths, Ph.D.

For inquiries regarding the contents of the newsletter or if you are interested in receiving an electronic copy, contact us at:

+01-772-562-3900

1-888-6-BURDOCK

sreul@burdockgroup.com