# SEAALAS Animal Bytes… no blood , just ones and zeroes….

Seaalas.org

**Volume 15, Holiday Edition** **December 2015**

## Fat Mice Bred to Have More Muscle are Healthier… by Toni Baker, GRU (ALN excerpt)

Even without losing fat, more muscle appears to go a long way in fighting off the bad cardiovascular effects of obesity.

That emerging evidence has scientists looking hard for new targets to uncouple the unhealthy relationship between fat and cardiovascular disease.

"If you look at the exercise literature, we understand very well that if you exercise, things get better. What we don't really understand is what about exercise is good; what does it tell us about physiology and how disease starts, and how can you customize it to different populations?" said Dr. David Stepp, vascular biologist in the Vascular Biology Center at the Medical College of Georgia at Georgia Regents University.

Stepp and his colleagues have evidence that an increase in muscle mass — a huge consumer of glucose, a natural energy source that is often elevated in obesity — could mean a healthier ticket for some.

While fat has the unhealthy habit of storing fuel, "muscle is a much more metabolically active tissue, even when it's just sitting there," Stepp said. "It burns more oxygen at rest; it burns more energy at rest; so it burns more calories at rest." Some of things scientists don't know is if muscles secrete something that improves glucose metabolism or if just having more glucose-consuming muscle is the apparent magic.

"We are trying to establish links between the health of skeletal muscles and the circulatory system," said Dr. David Fulton, Director of the MCG Vascular Biology Center and Co-Principal Investigator with Stepp on the grant. "When you eat, most of the glucose ends up in your skeletal muscle. When you are young, most of your body mass is skeletal muscle, so that glucose is efficiently distributed in the places where it should go to get used for energy and work."

Stepp and Fulton were authors on a 2014 study in the Journal of the American Heart Association that showed the benefits of adding muscle when fat is monopolizing the body. They looked at normal mice and mice genetically altered to be obese — mice with voracious appetites that soon doubled their normal weight — as examples

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## Inside this issue:

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## Fat Mice Bred to Have More Muscle are Healthier...Cont'd

human. When they deleted myostatin, a natural, negative regulator of muscle growth, from both, both groups developed bigger muscles. The normal mice also had less fat tissue.

But it was the obese-with-muscles mice that truly benefited in the cardiovascular sense: glucose tolerance and blood vessel dilation went up and insulin resistance and superoxide production went down. More muscle didn't result in these additional changes in the leaner mice.

"If the insulin burner gets bigger and the storage (fat) gets smaller, that's good," Stepp said. "What we have demonstrated is that if the burner gets bigger, no matter what the storage does, it's still good."

When they looked further at the obese mice, minus the muscles, they also found the superoxide-producing gene Nox1 is a major culprit in obesity-related vascular disease. In fact, the gene is over-expressed in the blood vessels of the fat mice, apparently driven by high glucose levels in the blood.

Obese mice with no muscle added but Nox1 removed also experience cardiovascular improvement, an observation that Postdoctoral Fellow Dr. Jennifer Thompson is pursuing further with a new American Heart Association grant.


Meanwhile, Stepp and Fulton are exploring how elevated blood glucose elevates Nox1, acknowledging that while it makes intuitive sense, the science needs to be clear. Because while the search is on for Nox1 inhibitors, there aren't any at this moment. Fulton and Stepp hope their studies will further inspire the search and identify additional points of intervention as well.

"We know that high glucose goes to Nox1 goes to superoxide, and superoxide goes to cardiovascular disease. What we don't know is what is in between glucose and Nox1," Stepp said. A possibility is galectin-3, a receptor for proteins that get coated with glucose when circulating levels of the sugar get too high. At least in culture, when glucose is added to cells, they produce more Nox1. But when the scientists block galectin-3 and add glucose, Nox1 doesn't increase.

While it's known that sugar-coating messes up protein function, the scientists aren't certain what galectin-3 is doing. Is it clearing the dysfunctional proteins, telling them to die, and/or driving up Nox1? So they are looking at the signaling between all of the above. They are also developing additional mice models, where Nox1 and galectin-3 are removed from already genetically fat mice, to further explore their role in vascular dysfunction. They will also explore the cardiovascular impact, such as blood pressure and how well blood vessels dilate in response to stress, in their fat mice models with added muscle as well as the two new knockouts.

The bottom line: they want to know if they can break "the metabolic connection" between fat and cardiovascular disease. "Where is the key event that causes all these bad things to happen?" Stepp said, and, of course, where and how best to intervene.

**"IF THE INSULIN BURNER GETS BIGGER AND THE STORAGE (FAT) GETS SMALLER, THAT'S GOOD," STEPP SAID. "**

ALN EXCERPT

# Congratulations James "Robbie" Champion...Receives AAALAC Fellowship 2015!!!

The AAALAC International Fellowship is meant to promote and reward technicians who "have demonstrated a commitment to a career in laboratory animal science and have shown a strong interest in attaining additional education and training to become more proficient in their vocation. The awardees are individuals who have made (or have the potential to make) significant contributions to the field of laboratory animal care and use." After my own experience, I feel AAALAC has truly accomplished its goal.

I have worked in research for about 12 years and I have had the honor and privilege of working with a wide variety of animals and people. I have had a fulfilling and exciting career up this point in my life. I am always searching for more experience and knowledge. This fellowship provided exactly what I needed. When I applied for this fellowship, it was my fourth attempt. Prior to my most recent application, I had given up on trying, figuring there were many more qualified and worthy applicants out there. Last year, during my first year of ILAM (Institute for Laboratory Animal Management), one of our presenters was a previous recipient. After hearing of his experiences going into and coming out of the fellowship, I went home and worked on my application.

For me, this experience has molded my view of my path in this field. I will forever be grateful for this experience and I hope I can continue to contribute to our field in a positive way for years to come.

From the minute my feet touched the ground at Heathrow International Airport they started running and didn't stop until I returned to the US. "They drive on the wrong side of the road" is what I have heard about England. Although, I wouldn't call it 'wrong', it took a lot to get used to that difference. Up until my last day, I tried to sit in the driver's seat of vehicles. One of the main intentions for this fellowship is to study the differences between the English system and the American system. Other than the side of the road, accents and dining utensils, there wasn't much difference between our two countries. Although I spent my tea time drinking coffee and the fact that I did not use my knife to eat, not many other differences were exceptionally notable in my day-to-day activities.

Throughout my trip, I saw amazing sites, toured many types of facilities and fully immersed myself in the experience; including eating haggis and blood pudding. Out of everything, I am most enchanted with the people I met. From managers to husbandry staff, everyone I met seemed genuinely proud and excited about working in animal research. Two individuals deserve special attention, Gail Thompson and Margaret Skeoch. Gail, a past president for AALAS and founding member of ILAM, found me the first night of IAT Congress and was a great companion for my last week. Margaret or Mags was my adoptive mum from Scotland. She was gracious enough to take me around Glasgow and out to see Loch Loman and other sites. Both Gail and Mags were a part of small group of IAT attendees that were indoctrinated into the S.O.B.S. (Society of Bridge Spitters). During the conference I had the special honor of being added to the group and we all spit over a bridge on the River Clyde.

As with our driving lanes, the regulations for re-

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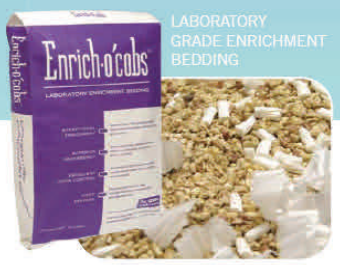


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## Congratulations James "Robbie" Champion...Receives AAALAC Fellowship 2015!!!.....Cont'd Pics from the Road!!!

search in the UK may seem to be different than the US, almost on the opposite side of the road. In reality, we are all on the same road going from point A to point B, we arrive there differently. Left or right side of the road, we are all on the correct side of research and regulations. The main difference lies in culpability. When compliance is a concern, the institute is held accountable in the US but the individual is held accountable in the UK.

It was an honor receiving this fellowship and I encourage everyone to apply, it was truly an amazing experience. More details on the fellowship and the experiences of mine and previous fellowship winners can be located at the AAALAC International web site: (http://www.aaalac.org/about/fellowship.cfm)
Cont'd Next Page...See Robbie's Pics!!!!!

# WHO'S NUTZ? Institutional News and Updates...

**Georgia State University**

ATLANTA—The Southern Association of Colleges and Schools Commission on Colleges (SACSCOC) gave its approval today to the consolidation plans of Georgia State University and Georgia Perimeter College (GPC). Chancellor Hank Huckaby first announced the consolidation plan to create a new, combined institution in January 2015.

Officials from SACSCOC, the regional body for the accreditation of degree-granting higher education in the Southern states, announced the approval of the prospectus submitted for the new institution during the SACSCOC annual meeting in Houston.

SACSCOC's approval represents the final step needed for the Board of Regents to review and grant authorization for the consolidated institution to officially operate as the new Georgia State University. The board is scheduled to review the final recommendation for consolidation at its January 6, 2016 meeting.

"Georgia State is a recognized national leader in improving student success and will be able to apply its best practices to a broad student body from across the state," said University System Chancellor Hank Huckaby. "Combining these attributes with Georgia Perimeter College's leadership in providing access to students across the metro area presents a significant opportunity to improve student success. I thank the Southern Association of Colleges and Schools Commission on Colleges for its thoughtful assessment and approving, once again, a new University System institution."

The new institution will maintain its access mission through Perimeter College, offering associate degrees while the main campus of Georgia State University will continue its research mission.

With the completion of the consolidation, the number of institutions in the University System of Georgia will be reduced from 30 to 29.

## 2015 Board Members and Committee Chairs...

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## WHO'S NUTZ? Institutional News and Updates...cont'd


Athens, Ga. - As wireless networks become more crowded with devices and more taxed by the demand for anytime, anywhere access, these networks are susceptible to radio frequency interference and jamming. It's a problem that potentially affects everything from personal smartphones to communications satellites.

An unlikely source—a small South American fish known as Eigenmannia that depends on electrolocation for survival—presents a potential solution, according to researchers in the University of Georgia College of Engineering.

"IF WE CAN BORROW THE JAR CIRCUIT FROM THE EIGENMANNIA AND REPLICATE IT IN OUR COMMUNICATIONS FREQUENCY BANDS, THEN WE CAN CREATE A COMMUNICATIONS SYSTEM THAT ALLOWS AUTOMATED INTERFERENCE MITIGATION."..FOK SAID....

"Eigenmannia (virescens) is a species of glass knifefish, and they locate objects by generating an electric field and detecting distortions in the field," assistant professor Mable Fok said. "They have a neural circuit that can effectively sense the frequency emitted by other fish, and they use this sense to regulate their own emitting frequency so they don't interfere with the others."

In other words, the fish have developed a natural system that prevents them from jamming each other's signals.

Eigenmannia's previously observed "jamming avoidance response," or JAR, can serve as the model for an artificial neural network that improves the efficiency of wireless communications, Fok said.

Graduate research assistant Ryan Toole is a co-author of the article. Fok and Toole will present their research in March at the Optical Fiber Communication Conference and Exposition in Anaheim, California, and their ongoing work was featured recently in the December issue of Photonics Society News, a publication of the Institute of Electrical and Electronics Engineers.

"If we can borrow the JAR circuit from the Eigenmannia and replicate it in our communications frequency bands, then we can create a communications system that allows automated interference mitigation," Fok said.

The IEEE Photonics Society News article is available online at http://www.ieee.org/ns/periodicals/Photo/dec2015/index.html on pages 4-9

## WHO'S NUTZ? Institutional News and Updates…cont'd

The Yerkes National Primate Research Center, Emory University, announced it has donated seven chimpanzees to the Chattanooga Zoo. This is the center's second confirmed donation of chimpanzees.

The 14-acre Chattanooga Zoo, which was established in 1937, is home to more than 600 animals representing more than 100 species, including big cats, reptiles and nonhuman primates. Approximately 170,000 guests visit this Association of Zoos & Aquariums (AZA)–accredited zoo annually.

"We are pleased to announce this second donation of chimpanzees, which involved Yerkes, the Chattanooga Zoo and the Chimpanzee Species Survival Plan,"

> "YERKES DONATES SEVEN CHIMPANZEES TO CHATTANOOGA ZOO!!! ….

says R. Paul Johnson, MD, director of the Yerkes National Primate Research Center. The Chattanooga Zoo is one of 34 U.S. zoos that participates in the Chimpanzee Species Survival Plan (SSP). "The interdisciplinary team of Yerkes employees that has been leading our donation efforts has been working with administrators and staff at the Chattanooga Zoo and the leaders of the Chimpanzee SSP since last year to coordinate the many details leading to today's announcement. We look forward to visiting the chimpanzees at the Chattanooga Zoo given its close proximity to Atlanta," Johnson continues.

In preparation for the arrival of the new chimpanzee group, Chattanooga Zoo staff made a few adjustments to its exhibit that opened in 2001. "We are excited to see how dynamic the exhibit will be with the larger group of chimpanzees," says Dardenelle Long, Chattanooga Zoo president and CEO.

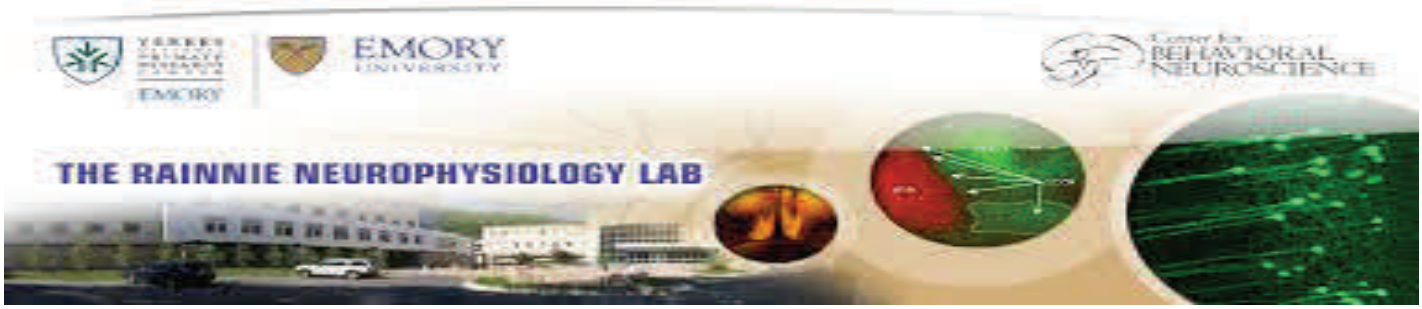
"With multiple viewing windows to indoor and outdoor exhibits, guests will be able to observe our new group from many different angles," she continues.

Critical to this donation was the Chimpanzee Species Survival Plan, which helps guide the management of the chimpanzee population.

"The Chimpanzee Species Survival Plan is pleased to have worked with the Yerkes National Primate Research Center to find a new home for these seven chimpanzees at Chattanooga Zoo," says Stephen Ross, PhD, chair of the Chimpanzee SSP. "This collaborative effort and the expertise at Yerkes and the zoo are key components to ensuring the long-term health and well-being of this group of chimpanzees."

Lisa Newbern, 404-727-7709, lisa.newbern@emory.edu

> "WITH MULTIPLE VIEWING WINDOWS TO INDOOR AND OUTDOOR EXHIBITS, GUESTS WILL BE ABLE TO OBSERVE OUR NEW GROUP FROM MANY DIFFERENT ANGLES"….











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## WHO'S NUTZ? Institutional News and Updates…cont'd


The Medical University of South Carolina Gnotobiotic Animal Research Facility is a unique research animal facility that will enable investigators to conduct research with germfree or gnotobiotic animals.

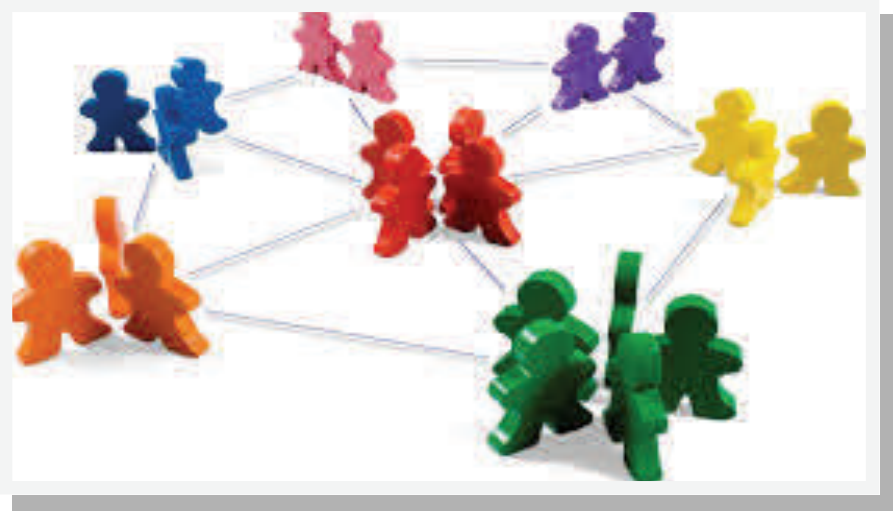
Current research with germ free and gnotobiotic animals is demonstrating that the host's microbial flora has dramatic effects on Morphology, Physiology, Nutrition, Biochemistry, Endocrinology and Immunology. There is increasing evidence that intestinal microorganisms are involved in autoimmune diseases, inflammatory bowel diseases, and tumors of the alimentary tract and bladder. The microbial flora can also have dramatic effects on the pharmacology of orally administered drugs. Germfree animals continue to play a key role in basic research on the etiology, prophylaxis and therapy of caries and periodontal disease.

Recent advances in the genetic manipulation of microorganisms and animal models, via gene knockout or transgenic techniques, provide unique opportunities to study the multifaceted and complex interactions and cross-talk that takes place between a host and the microbial flora. Gnotobiotic technology will not only continue to play a key role in sustaining the health of these valuable immunodeficient, genetically engineered animal models but it will also continue to play an important role in the use of genetically engineered animals for basic research on many diseases that affect humans world wide.

The importance of understanding how the microbial flora can affect the health and well being of a host was recognized over 100 years ago. We still have a great deal to learn about host-microflora interactions. Gnotobiotic research will continue to play a key role in clarifying our basic understanding of host microflora interactions that affect so many facets of our health and well being.

The facility is part of the Center for Oral Health Research (COHR) and is now fully operational. For complete Facility information please visit http://www.musc.edu/gnotobiotic/:
Dr. Caroline Westwater (Director) 843-792-7703 westwatc@musc.edu
Phillip Werner (Supervisor) 843-792-2152 wernerpa@musc.edu

"CURRENT RESEARCH WITH GERM FREE AND GNOTOBIOTIC ANIMALS IS DEMONSTRATING THAT THE HOST'S MICROBIAL FLORA HAS DRAMATIC EFFECTS ON MORPHOLOGY, PHYSIOLOGY, NUTRITION, BIOCHEMISTRY, ENDOCRINOLOGY AND IMMUNOLOGY."



## Sharing is Caring:
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If you or a technician you know is interested in presenting send an email to tbr@seaalas.org for more details.

# Call for Speakers!

The SEAALAS Program Committee is currently looking for volunteers to speak at the 2016 Annual Meeting and Awards Banquet to be held in Charleston, SC January 27-29, 2016.

The theme for this year's conference is

**"What's In It For Me?"**

Please email Michelle Hull at michelle.hull@emory.edu if you would like to participate.

**We hope to see you all there!**

## TECH-Uniques...

ALN magazine

# Considerations for Implementing a Facility-Wide Policy on Carbon Dioxide Euthanasia for Laboratory Rodents

By Les Anderson

**What to think about in order to facilitate safe and humane euthanasia.**

The proper use of $CO_2$ as a euthanasia method is generally considered humane for rodents and safe for personnel. The AVMA guidelines on euthanasia recommend properly used $CO_2$ as a viable methodology. The selection of equipment and development of protocols for $CO_2$ euthanasia need to ensure the humane treatment of the animals and the physical safety and psychological welfare of the technicians performing euthanasia. The protocols also need to consider workflow, possible impact on research parameters, and the cost of implementing and enforcing a facility-wide policy on $CO_2$ euthanasia.

**Welfare Considerations**

Improper use of $CO_2$ causes distress and possibly pain to the animal. To minimize that possibility, there are a number of factors to consider when developing a humane $CO_2$ euthanasia protocol.

***Source of the $CO_2$ Gas***

The only acceptable source of $CO_2$ for euthanasia is a compressed gas cylinder or in house gas line. Dry ice or chemical means of generating $CO_2$ are universally considered unacceptable.

***Euthanasia Chamber***

Ideally, animals should be euthanized in their home cage. This eliminates additional handling and introduction into an unfamiliar environment that can cause distress. The cage can either be treated individually with cage tops that allow direct $CO_2$ input or multiple cages can be treated in a euthanasia chamber. Larger euthanasia chambers should ensure even distribution of gas throughout the chamber via a gas distribution manifold.

If euthanasia in the home cage is not possible or practical, the chamber should have space large enough to allow the animals normal postural adjustments. Avoid obstructions that may interfere with the proper input of $CO_2$ or the animal's exposure to the gas. The chamber should be easy to clean and disinfect. When performing euthanasia outside of the home cage, grouping animals from different cages into a euthanasia chamber is widely considered to be unacceptable.

***Inputting $CO_2$ Gas***

According to the 2007 AVMA guidelines on euthanasia, operators should introduce 100% $CO_2$ into the euthanasia chamber at a rate of at least 20% of the chamber's volume per minute. To comply with these guidelines, a device that meters $CO_2$ gas by volume is required. Typically, a flow meter that outputs in liters per minute (lpm) or cubic feet per hour (cfh) is used for this purpose. Using a $CO_2$ regulator that does not include a flow meter makes it impossible to ensure that the ideal rate of input is being used. Determining the proper inflow is simple:

cont'd next page...

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Multiply the length, width, and height of the chamber in inches and divide by 61 to determine the volume in liters.

Multiply the volume by 20% to determine the proper flow rate (lpm). At a fill rate of 20%, gas should input into the chamber for several minutes to ensure that the $CO_2$ concentration reaches a level high enough for euthanasia. The exact flow rates and times should ultimately be chosen based on close observation of the animals, with the goal of choosing parameters that ensure euthanasia of every animal with the least possible distress.

***High Flow Rates/Extensive Use***

$CO_2$ is stored in cylinders in liquid form. At high rates of flow or prolonged periods of use, the phase change from liquid to gas absorbs a great deal of energy and can result in the freezing of the gas supply equipment and in extreme cases the entire euthanasia system. This freezing shortens the life of the equipment, negatively impacts animal welfare, and can create a dangerous situation for equipment users. With high flow rates or prolonged periods of use, a heated gas source is required.

***$CO_2$/$O_2$***

At one time it was believed that introducing a combination of oxygen and $CO_2$would offset the aversive nature of high concentration $CO_2$, but this has largely been debunked. Adding pure oxygen to the incoming $CO_2$ has been demonstrated to prolong the time to unconsciousness, thereby increasing distress. This could also result in the revival of animals that appeared to be euthanized.

***Pre-Filling vs Slow Fill***

Another practice common at one time was the filling of the euthanasia chamber with $CO_2$ prior to the introduction of animals. This was demonstrated to induce unconsciousness rapidly and thus thought to be more humane. However, a number of studies have determined that such high $CO_2$ concentrations cause increased animal distress. On the contrary, current 'slow-fill' methods are designed to induce unconsciousness prior to $CO_2$ concentrations that are high enough to cause pain.

***Alternatives or Secondary Methods***

There are circumstances that warrant an alternative or secondary method of euthanasia. Neonatal mice and rats, as well as mouse and rat fetuses, are resistant to hypoxia and require prolonged exposures to $CO_2$ to ensure euthanasia. When neonates are to be euthanized with $CO_2$, exposure times of upwards of 60 minutes may be required to ensure euthanasia. Euthanasia must be verified after exposure in each case. The AVMA guidelines on euthanasia recommend that for neonates, a secondary method of euthanasia be used in conjunction with $CO_2$. A policy specific to neonates must be devised and should be based upon close observation of the animals.

Inhalation of $CO_2$ affects specific physiological parameters and can negatively impact certain types of research. A list of the parameters impacted by $CO_2$ is beyond the scope of this article, but a useful resource is the Report of the ACLAM Task Force on Rodent Euthanasia.

Recently there has been interest in isoflurane as an anesthetizing agent to be used prior to euthanizing with $CO_2$. cont'd next page...

Cont'd from pg. 12…

A newly published study has demonstrated that introducing isoflurane into the process does not lead to less stress for the animal. It, in fact, significantly increases behavioral and neuromolecular signs of stress compared to a pure 20% $CO_2$ induction.

**Workflow and Efficiency**

An advantage of $CO_2$ is its potential to be applied to multiple small laboratory animals at a time with minimal labor. With a well-designed system and good technique, it is possible to humanely and efficiently euthanize hundreds of animals in one treatment cycle. Large-scale systems allow technicians to spend less time performing euthanasia. This not only frees them to spend time on other important functions, but also reduces the negative emotional and psychological impact of performing euthanasia. This emotional component of euthanasia has been linked to increased employee absenteeism and turnover.

***Compliance***

Even when proper euthanasia techniques are incorporated into a well-designed protocol, the responsibility is on the person performing euthanasia to execute proper technique.

When euthanasia is performed in individual labs or in multiple areas throughout the facility, it can be extremely difficult for managers to ensure that euthanasia is being performed properly. Increasing the flow of gas to save time is an especially widespread problem, which negatively impacts animal welfare and is a waste of $CO_2$ that can add up quickly. Even on a small scale, mistakes can turn into major problems for the facility. An operator that neglects to turn off the gas once finished can drain entire tanks and cause gross pollution of the room. In facilities that have piped in gas throughout the building, errors can result in draining the entire building's supply of $CO_2$.

When euthanasia is centralized, overseeing the process becomes more manageable, but the potential problems are greater. Mistakes made are now applied to tens or hundreds or animals at a time. If large volumes of gas are used, safety can be seriously compromised, even by minor mishaps.

Eliminating as many user-controlled variables as possible is the most effective method of ensuring that proper technique is followed. Using factory pre-set cylinder regulators and flow controls, electronic timers and standardizing the size and shape of the euthanasia chamber used throughout the facility are solid steps to achieving this goal. However, the ultimate solution to ensuring compliance is total automation. Advances in equipment design now offer complete systems that automate the entire euthanasia process. Whether euthanasia is decentralized or offered as a core service, automated euthanasia systems ensure compliance with the mandated protocol. Such systems precisely control the input of gas at the correct flow rate for the correct amount of time and if required, exhaust the gas. Most important, a standardized, automated euthanasia system assures facility management that the process is being done the same way every time.

**Advancement of $CO_2$ Euthanasia**

The evolution of $CO_2$ euthanasia methodology has advanced to the point of high capacity automated systems that can ensure humane treatment of animals with maximum efficiency and minimal labor. Facilities now have the option of treating animals with assurance that protocols are precisely followed. Human error variables are virtually eliminated. cont'd next page...

Cont'd from pg. 14...

**Additional Reading**

Artwohl, J., et. al. (2005, August). Public statements: Report of the aclam task force on rodent euthanasia. http://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf.

Guidelines on euthanasia of rodents. (2001, February). http://web.ncifcrf.gov/rtp/lasp/intra/acuc/fred/guidelines.

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- Valentine, H., Williams, W., and Maurer, K. Sedation or Inhalant Anesthesia before Euthanasia with $CO_2$ Does Not Reduce Behavioral or Physiologic Signs of Pain and Stress in Mice. Journal of the American Association for Laboratory Animal Science (JAALAS), January 2012.

***Les Anderson**, President of Euthanex Corp, Trading as EZ-Systems. 610-559-0159, www.euthanex.com.*

## AVMA Guidelines are Available at:

## https://www.avma.org/KB/Policies/Documents/euthanasia.pdf


# LEGAL NEWS...USDA Animal Care Creates New Process for Appealing Animal Welfare Act Inspection Reports...

USDA Animal Care has established a new process for licensees/registrants regulated under the Animal Welfare Act who wish to appeal something that has been cited on their USDA inspection report.

Our goal is threefold: to bring about quicker appeals resolutions; to ensure consistency in the appeals process; and to ensure that subject matter experts are involved in reviewing each appeal.

All facilities regulated under the Animal Welfare Act undergo routine, unannounced inspections. During each inspection, our inspectors cite on the inspection report anything that is not in compliance with the standards set forth in the Animal Welfare Act regulations. These inspection reports are made available on our website as public information. Sometimes, however, a regulated facility will disagree with one or more of the noncompliant items that were cited on a particular report, and they will file an appeal with us.

**The revised appeals process, effective immediately, is as follows:**

...If, during an inspection, a facility operator has questions or concerns about any of the noncompliant items cited by the inspector, the facility operator should bring the issue up during the inspection and/or exit briefing. If the matter is resolved at that time, the inspector will modify the citation, remove it altogether or leave it as originally written.

...If the facility operator and the inspector are unable to resolve the matter, or if the facility later decides to question the report, the facility operator should send a detailed, written appeal to the regional director in the appropriate Animal Care regional office. We must receive this appeal within 21 days of the facility receiving the finalized inspection report. If the appeal is received after the 21-day period, it will be rejected.
If no appeal is filed, we will make the inspection report publicly available on our website 21 days from the date it is finalized.

...If the inspection report is appealed, the inspection report will not be publicly available until a final decision on the appeal is made.
An Animal Care appeals team will review each appeal. Each team consists of a director from one region and an assistant director from the other region – plus an Animal Care field or staff veterinarian who serves as a subject matter expert, based on the specifics of the appeal.

...Within three weeks of receiving an appeal, the assigned team will either make a final decision or request more information. All decisions made by the appeals teams are final and represent USDA's final determination of compliance. If the inspection report is amended, only the amended report will be made available online.

We realize that disagreements are a natural part of regulatory oversight, and our inspectors understand that regulated facilities have the right to appeal inspection findings. We are committed to ensuring that the appeals process is objective and thorough, while not resulting in reprisal against any facility. The new appeals process is a way to streamline and improve decision making so that we can better serve the regulated community, general public and the animals.

http://www.aphis.usda.gov/publications/animal_welfare/2014/appeals_process.pdf

"OUR GOAL IS THREEFOLD: TO BRING ABOUT QUICKER APPEALS RESOLUTIONS; TO ENSURE CONSISTENCY IN THE APPEALS PROCESS; AND TO ENSURE THAT SUBJECT MATTER" EXPERTS ARE INVOLVED IN REVIEWING EACH APPEAL.
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# LOOK BOTH WAYS….SAFETY FIRST!

## Workplace Exposure Limits for Halogenated Anesthetic Agents...by Brett Field (ALNMAG)

In the past 25 years, halogenated anesthetic agents, primarily isoflurane and sevoflurane, have become indispensible tools in laboratory animal science. With the proliferation of rodent specific equipment, virtually every animal laboratory today makes use of these drugs.

Since the beginning of their widespread clinical use in the 1960's, the health effects of halogenated agents have been the subject of numerous studies and by now, the importance of monitoring and limiting operator exposure has become common knowledge. However, what is considered an acceptable level of exposure continues to be the subject of debate and discussion. In the United States, the recommended exposure limit (REL) for isoflurane is often based upon a decades old report published before the drug was introduced. Shifting attitudes and new information are leading to updated policies on workplace exposure to the most commonly used agents in this industry.

**The NIOSH Report**

When setting policy for workplace exposure to isoflurane, the most often cited REL is from NIOSH, the National Institute for Occupational Safety and Health. In a report published in 1977, NIOSH recommended that no worker be exposed to greater than two parts per million (ppm) of any halogenated anesthetic. This document still guides workplace exposure policies today, though it is increasingly considered outdated as the report only covers older halogenated agents that have now fallen out of widespread use, namely halothane. Though the report refers to all halogenated agents, isoflurane was introduced years later and is arguably not covered by this recommendation.

One important takeaway from the NIOSH report is how they arrived at the two ppm limit. NIOSH was unable to determine a safe level of exposure based upon the information available at the time. Thus, the report recommends that "exposure be controlled to levels no greater than the lowest level detectable using the sampling and analysis techniques recommended by NIOSH in this document," which happens to be two ppm. It does not imply that below two ppm is safe or above two ppm unsafe, it is essentially saying that any exposure is unacceptable. In light of the situation, this was the only sensible policy.

The inability to precisely determine the health risks of chronic exposure to halogenated agents is the reason that this 'zero tolerance' approach to exposure has persisted. Though these drugs have been administered to thousands of patients over the course of several decades, solid data on risks from chronic exposure is difficult to obtain. Workers are often exposed to a variety of anesthetics and in many cases halogenated agents are administered with nitrous oxide, making it difficult to pinpoint the effects of specific drugs.
Cont'd on Pg. 24….

# LOOK BOTH WAYS….SAFETY FIRST!

## Workplace Exposure Limits for Halogenated Anesthetic Agents…cont'd….

**Cont'd from Page. 22...**

However, since the publication of the original NIOSH report in 1977, a growing body of evidence has begun to allow for a reassessment of exposure limits. In 2006, NIOSH published a request for information on the toxicity of isoflurane, sevoflurane, and desflurane with the intent to review and establish RELs for these drugs. An in depth look at the research on the health effects of halogenated agents is beyond the scope of this commentary, but looking at other guidelines published in the United States and abroad can give an idea of what to expect from a new NIOSH standard.

**ACGIH's Threshold Limit Values**

In the late 1980's, the American Conference of Governmental Industrial Hygienists (ACGIH) published recommended Threshold Limit Values (TLV) for halothane and enflurane at 50 ppm and 75 ppm respectively. Last updated in 2001, these values are recognized by OSHA and are cited in several workplace policies around the world.

Enflurane is very structurally similar to isoflurane, shares the same molecular weight and is generally assumed to have similar properties. For this reason the enflurane TLV is often applied to isoflurane. The ACGIH also makes a point to note the relative safety of enflurane vs halothane, stating: "all studies in humans and animals indicate that enflurane's adverse effects are more rare than those of halothane…" The ACGIH cites this rationale for recommending a higher exposure limit for enflurane.

It should be briefly noted that OSHA does not have RELs for any halogenated agent and performs a purely advisory role at this time. OSHA's Anesthetic Gases: Guidelines for Workplace Exposures provides an excellent summary of available information for anyone interested in anesthesia safety.

**Worldwide Guidelines**

Since the worldwide proliferation of halogenated anesthetics, other countries have worked to set acceptable exposure limits (Table 1).

The UK standards, introduced in 1994, outline exposure limits of 10 ppm halothane and 50 ppm isoflurane, highlighting a trend towards setting lower limits for halothane than newer drugs. The relative safety of isoflurane and enflurane vs halothane is often used as a gauge for setting limits, as seen in the ACGIH report for enflurane.

Another notable European example is that of the Dutch Expert Committee on Occupational Standards (DECOS), which set an REL of 20 ppm for isoflurane in 1998. Faced with a lack of information, DECOS relied on the similarities between enflurane and isoflurane when determining this value. This conjecture has been the justification for several policies on isoflurane. Cont'd on Page 26...

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## Workplace Exposure Limits for Halogenated Anesthetic Agents…cont'd….

**The Future of Revised Standards**

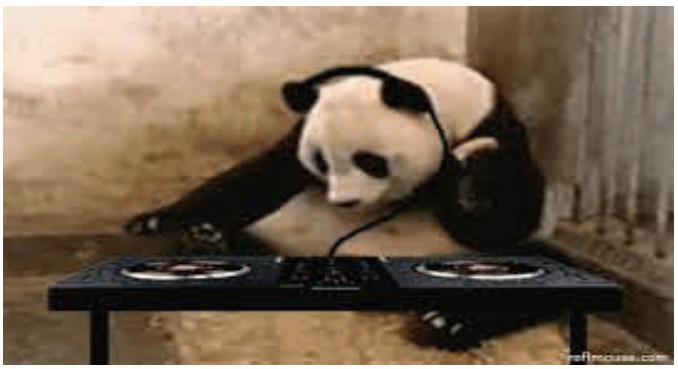
Though NIOSH has yet to publish a new guideline for isoflurane, sevoflurane, and desflurane, institutional polices are already changing in anticipation of revised standards. In 2012, the NIH adopted the ACGIH TLVs of 75 ppm enflurane and 50 ppm halothane as part of their Waste Anesthetic Gas (WAG) surveillance program. This shows that the biomedical research industry is moving away from the original NIOSH standard. A cursory web search reveals other institutions that have adopted either the ACGIH TLVs, recommendations from abroad, or a combination thereof. Considering increasing evidence and current trends it is likely the industry will continue to move away from the two ppm REL for isoflurane and sevoflurane.

**Conclusion**

The need for strict safety protocols when using halogenated agents is without question. In spite of any uncertainties stemming from flawed research or inadequate information, the weight of evidence for potential health risks dictates that exposure to these agents should be controlled to the lowest practical level.

It is tempting to reason that until a 100% definitive safe level of exposure has been determined for isoflurane or sevoflurane that RELs should remain very low. However, there are negative costs associated with a zero tolerance approach to exposure that should be considered, the most important being compliance.

With thorough training, good technique, and quality equipment, it is possible to reduce user exposure to reasonable levels, even when using a basic tabletop anesthesia system, but it can be costly and time consuming to reduce exposure to near zero in many cases. In laboratories with limited resources or oversight this often results in an apathetic attitude towards exposure. If the RELs are perceived to be impractical or not based on reality then they are easier to ignore.



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