November 6, 2008

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Dear Dr. Masten,

The following comments are submitted on behalf of the more than 2 million members and supporters of People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM) in response to the nominations of substances to NTP for study in 2008 (October 2, 2008; *Federal Register* 73(192):57358). Our organizations are committed to using the best available science to protect animals from suffering and to promote the acceptance of human-relevant methods for risk assessment.

Specific comments are submitted for bisphenol AF, dimethylamine borane, ethylene glycol 2-ethylhexyl ether, L-beta-methylaminoalanine and Triclosan. NTP has recommended additional animal tests for these substances that would result in the poisoning and death of thousands of animals if carried out. In each case, we urge NTP to thoroughly consider potential human exposure, existing toxicity data and the application of non-animal test methods in order to avoid unnecessary and duplicative animal tests. It is long past time for NIEHS and NTP to start applying thoughtful toxicology rather than defaulting to additional animal testing regardless of relevance and applicability.

Thank you for your attention to these comments. I can be reached at (757) 622-7382, ext. 8001, or by e-mail at josephm@peta.org.

Sincerely,

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Bisphenol AF

NIEHS nominated Bisphenol AF (BPAF) for comprehensive toxicological characterization based on its moderate production and use as a crosslinking agent for certain fluoroelastomers and as a monomer for polycarbonate and other polymers and resins.

While NIEHS expresses concern over potential exposure of the general population to BPAF from its use as a monomer and its use in fluoroelastomer gaskets and hoses in food processing equipment, it notes that information on specific uses and potential exposure are not available. Indeed, NIEHS was unable to identify detailed information regarding the use of BPAF in the manufacture of any specific consumer products. Only a DuPont press release touting the company's Viton® fluoroelastomers for containing fluids in "automotive, chemical, petrochemical, pharmaceutical and food processing industries," and a single Cole-Palmer application of this product – Masterflex® FDA-approved Viton® pump tubing – are cited as evidence of potential consumer exposure to BPAF. Information on exposure is a prerequisite for assessing risk, so obtaining this data should precede the development of any research program. Occupational exposure is also identified, with molding and casting machine operators in the plastic products industry accounting for 65% of this exposure. Details were not supplied by NIEHS, but if this exposure is similar to that for bisphenol A (BPA), it is likely to be by the inhalation route. Engineering and workplace controls could reduce the potential for this exposure. In any case, since the exposure route for the proposed studies would be oral, it seems that occupational exposure is not a primary concern.

Given the structural similarity of BPAF to BPA, it seems likely that attempts to assess the reproductive and developmental toxicity of BPAF in animals will be subject to the same problems that have complicated the study of BPA – especially with regard to reproducing endocrine effects that have been observed only at low doses. The NTP-CERHR Expert Panel on the Reproductive and Developmental Toxicity of Bisphenol A (2007) expressed its frustration over the interpretation of data from these low dose animal studies noting that "many members of the panel expected the high dose studies with bisphenol A to detect some manifestation of toxicity." The two-generation study of Tyl et al. (2008), for example, exposed estrogen-sensitive CD-1 mice to a full dose range of BPA by the oral route throughout the animals' life spans. The European Union (2008) called this study – which found no evidence of reproductive or developmental harm – "the gold-standard, definitive study of the reproductive toxicity of BPA" and concluded that there was no need for further testing. Likewise, the only *in vivo* data cited as indicating possible endocrine effects of BPAF were obtained either by intraperitoneal injection or by oral exposure to very high doses that also produced general toxicity (Yamasaki et al., 2003) and far exceeded any possible human exposure. Moreover, the authors of this study note that while the glans penis weight in rats given BPAF increased in the high-dose group, the control values for sex organs in all of the studies varied considerably, and some of the values for the test groups were within the control ranges. They concluded, as a result, that it could not be determined whether the chemicals tested exhibited androgenic properties.

The first tier of the proposed testing program is a transgenerational assay in rats by oral exposure from gestation and lactation through sexual maturity to provide a preliminary assessment of the potential for BPAF's reproductive or developmental toxicity. Given past experience with BPA and the limited data available for BPAF, it is extremely unlikely that this

approach will produce clear evidence of adverse effects at doses relevant to human exposure. In addition, differences in metabolism and elimination between humans and rodents call into question the relevance of results in rodent studies generally. In humans, BPA is rapidly absorbed in the intestines and then inactivated in the liver by conjugation with UDP-glucuronic acid to form BPA-glucuronide (BPAG). BPAG is rapidly excreted in the urine, with a half-life of less than 6 hours (Völkel et al., 2002). As a result, availability of BPA in humans following oral exposure is extremely low. In rodents, however, BPAG is excreted from the liver via bile into the gastrointestinal tract, where it is hydrolyzed by bacterial glucuronidases to re-form free, active BPA and subsequently reabsorbed. This enterohepatic recirculation results in slower elimination and consequently higher plasma levels of free BPA given the same dose in rodents compared with humans. Given the structural similarity of BPAF to BPA, it seems likely that BPAF will exhibit similar species differences in metabolism and elimination.

In summary, essentially nothing is known about possible consumer exposure to BPAF. While the results of some *in vitro* studies suggest that BPAF may possess endocrine activity, *in vivo* studies have produced only inconclusive results at very high doses. The structural similarity of BPAF to BPA raises concern that additional *in vivo* studies will also fail to produce clear evidence of adverse effects at doses relevant to human exposure, and species differences observed in the metabolism and elimination of BPA are also likely to complicate the interpretation of any results with BPAF. The proposed research program is premature. We recommend that the potential for human exposure be accurately assessed prior to the development of any research program and that the metabolism of BPAF be studied first *in vitro* in isolated human microsomes or hepatocyte cell culture or in human volunteers as in the study of Völkel et al. (2002) with BPA.

References

Center for the Evaluation of Risks to Human Reproduction (CERHR), National Toxicology Program (NTP), U.S. Department of Health and Human Services. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. November 26, 2007.

Rochelle W. Tyl et al. Two-Generation Reproductive Toxicity Study of Dietary Bisphenol A in CD-1 (Swiss) Mice. *Toxicol Sci.* 2008; 104(2): 362–384.

Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), European Commission Directorate-General Health and Consumer Protection. *Updated European Risk Assessment Report 4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A)*. European Communities, 2008.

Völkel, W et al. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem.Res.Toxicol*. 2002; 15: 1281-1287.

Dimethylamine borane

The reducing agent dimethylamine borane (DMAB) was nominated to the NTP program by the National Institute for Occupational Safety and Health (NIOSH). The nomination is reportedly based on possible allergic reactions and systemic toxicity produced by this compound. The proposed testing of DMAB would include dermal absorption studies, skin sensitization studies, subchronic dermal toxicity tests examining neurotoxicity and behavior, and reproductive and developmental toxicity studies.

Generally recognized safety hazards

According to the Chemical Information Profile provided by NTP, the existing literature and Material Safety Data Sheets, DMAB has been well established to cause eye, skin, and respiratory irritation in both rodents and humans and is toxic if swallowed. While dermal absorption was not the explicit focus of the many previous toxicity studies, it can be inferred from the dermal toxicity of this compound. If additional investigation is required into the nature or extent of dermal absorption, validated *in vitro* and *in silico* dermal absorption methods are readily available and should be used (Stoick *et al*., 2007; Howes *et al*., 1996). In addition, based on previous assessments of DMAB, safety warnings indicating the need for protective goggles, clothing and ventilation are required on container labels and in any associated MSDS. Occupational Safety and Health Association (OSHA) requires that employers communicate these safety precautions to their employees. Therefore, the only eye, skin, oral or respiratory exposure expected from this compound would be the result of an industrial accident or intentional mislabeling or misuse. No expected dermal absorption would occur under normal use conditions and consequently, the results of animal studies would not change the safety precautions already in place for DMAB.

Existing evidence for skin sensitization

The NTP profile describes the results of multiple skin sensitization assays involving animals that have already been conducted. It is unlikely that the proposed skin sensitization studies will differ significantly from these previous studies or that they will provide pivotal data to inform regulatory decisions. If the issue to be addressed is whether the skin sensitization seen in these animal studies can be extrapolated to humans, then only sensitization studies with human volunteers would be appropriate. It could be argued that the single human exposure case study described in the profile (Tsan *et al.*, 2005; Kuo *et al.*, 2006; and discussed in more detail below) provides evidence that skin sensitization is not a potential outcome of human dermal exposure. Also, after years of industrial use of DMAB, no increased incidence of skin sensitization has been reported. Furthermore, as noted above, required safety precautions are expected to prevent dermal contact with DMAB and therefore, skin sensitization is not a potential hazard. If the recommendation to conduct additional skin sensitization testing is given, however, the "limit dose LLNA" or rLLNA protocol, which uses fewer animals and is more humane than the guinea pig maximization test, should be used (ESAC, 2007).

Weak evidence of neurotoxicity concern
Another key component of the nomination of DMAB was concern over neurotoxicity raised primarily by a single occupational incident in which four workers were splashed with large amounts of DMAB or ingested smaller amounts (Tsan *et al*., 2005; Kuo *et al*., 2006). One of the four failed to follow established decontamination protocols and developed neurological symptoms. We have serious concerns about basing a large-scale animal study on evidence from a single human case study - especially when that single case involved exposures far above any expected normal occupational levels and disregard for established decontamination standards. There is absolutely no indication that use of DMAB under required safety protocols, with appropriate clothing and equipment poses any realistic danger of long-term neurotoxicity. In fact, this incident illustrates that in the case of accidental exposure, established decontamination procedures can completely eliminate that risk. Further toxicological studies in animals are not going to change the recommended safety and decontamination standards nor are they going to protect against the extremely rare accidental exposure described in the case study.

Large epidemiological data set
Given that DMAB has been produced in large quantities in the U.S. for at least 15 years and the likelihood that some facilities produce DMAB exclusively (like the facility described in the case study), ample data should be available to establish if there are any long-term health effects in humans. The fact that no previous occupational poisoning had been reported prior to the Tsan *et al.* case, suggests that accidental high dose exposures are extremely rare. Also the lack of reports of any health effects from chronic occupational exposure supports the assessment that established safety precautions for DMAB are appropriately protective. Targeted epidemiological analysis is the only approach likely to reveal any subtle toxicities resulting from long-term use of DMAB with protective clothing and equipment. The proposed animal studies, in addition to being redundant, are not capable of discerning whether the current safety standards need revision and therefore would not contribute to our understanding of the actual health risk of DMAB.

Based on the abundance of existing animal dermal and systemic toxicity data for DMAB, the weak evidence for human neurotoxicity concern, and the fact that existing occupational safety recommendations virtually remove any potential human health risk, we strongly urge the NTP to assign this nomination a low priority. At a minimum, widely accepted *in vitro* methods based on human skin for confirming the existing dermal absorption data should be employed (OECD, 2004) and human epidemiology should be incorporated into the test plan.

References

ECVAM Scientific Advisory Committee; ESAC Statement on the Reduced Local Lymph Node Assay (rLLNA); 2007. (http://ecvam.jrc.it/publication/ESAC26_statement_rLLNA_20070525-1.pdf).

Howes, D., Guy, R., Hadgraft, J., et al. Methods for Assessing Percutaneous Absorption: The Report and Recommendations of ECVAM Workshop 13. *ATLA* 2006; 2-4, 81-106.

Kuo HC, Huang CC, Chu CC, Chu NS. Axonal polyneuropathy after acute dimethylamine borane intoxication. *Arch Neurol*. 2006; 63(7): 1009-1012.

Organization for Economic Co-operation and Development (OECD), Guideline for the Testing of Chemicals 428, Skin Absorption: *In Vitro* Methods, 2004.

Stoick, K et al. Systemic testing by the dermal route can be precluded by new non-animal percutaneous absorption strategies. *AATEX* 14 2007; Special Issue, 561-564. Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences.

Tsan Y, Peng K, Hung D, Hu W, Yang D. Case Report: The Clinical Toxicity of Dimethylamine Borane. *Environ Health Perspect.* 2005; 113(12): 1784–1786.

Ethylene glycol 2-ethylhexyl ether

Ethylene glycol 2-ethylhexyl ether (EGEHE) was nominated by NIEHS for toxicological characterization due to its widespread use, unknown toxicity profile, and structural similarity to other known toxic ethylene glycol ethers (EGE).

EGEHE is structurally similar to other EGEs including ethylene glycol methyl ether (EGME), ethylene glycol ethyl ether (EGEE), and ethylene glycol butyl ether (EGBE), the toxicities of which have been thoroughly characterized. While NIEHS cites this structural similarity as a basis for its nomination, the existing data indicate that it is extremely unlikely that EGEHE will display a similar toxicity profile. Further, in many cases the relevance of the observed toxicities of EGEs to human exposure is questionable.

For example, NTP notes that EGME, EGEE and EGBE are hematotoxic. While the acute toxicity observed in rats, mice and rabbits is consistent with hemolysis, the NTP fails to mention that human erythrocytes are many-fold more resistant to the hematotoxicity of EGEs (OECD, 2004). In fact, an accepted PBPK model demonstrates that even at saturated vapor concentrations of EGBE, the most hematotoxic of the EGEs, it is not possible for hemolytic blood concentrations of butoxy acetic acid (BAA), the metabolite responsible for EGBE's toxicity, to be reached in humans by the inhalation route of exposure (Corley et al., 1994). EGEHE's saturated vapor concentration is lower than that of EGBE.

NTP also notes that gestational exposure to EGME and EGEE results in skeletal and soft tissue malformations in studies with several species of animals. However, an OECD SIDS assessment of a monoethylene glycol ethers category reports that the glycol ethers are not selectively toxic to the reproductive system or developing fetus, but rather that developmental toxicity is secondary to maternal toxicity. In fact, in rats exposed to ethylene glycol hexyl ether (EGHE) and in rabbits exposed to EGHE or ethylene glycol propyl ether by inhalation, no effects on the fetus were noted even at concentrations that did produce maternal toxicity (OECD, 2004). In addition, PBPK model predictions of human blood levels upon simulated inhalation exposure to methoxy acetic acid (MAA), the metabolite responsible for EGME's toxicity, at the 5 ppm threshold limit value set by the American Conference of Industrial Hygienists were approximately 60 μM after 8 hours – well below those causing adverse effects in pregnant mice or rats. At the 0.1 ppm OSHA proposed permissible exposure level, the resulting human maternal plasma levels of MAA were about 1μM, a concentration approximately 1000-fold below the >1mM concentration required to elicit developmental toxicity in animals studies. The concentrations causing developmental toxicity in mice and rats are therefore much higher than those ever anticipated in human occupational settings (Welsh, 2005).

An NTP chronic toxicity and carcinogenicity study with EGBE in rats and mice reported a significant increase in the incidence of liver hemangiosarcomas in male mice and forestomach tumors in female mice. However, it is unlikely that the observed effects are relevant to human carcinogenic risk. Liver hemangiosarcomas found in male mice likely resulted from oxidative stress subsequent to red blood cell hemolysis and iron deposition in the liver (Xue et al. 1999). Since human erythrocytes are resistant to the hematotoxicity of EGEs and have greater hepatic antioxidant capacity compared to rodents, no similar hematotoxic response, increased oxidative stress, or, consequent development of liver hemangiosarcomas is likely. Also, forestomach tumors in mice are unlikely to be relevant

because humans lack an analogous organ. Finally, EGBE is not genotoxic. In addition, the study failed to demonstrate a clear dose response relationship. In its 1999 IRIS review of EGBE, EPA found the results of the NTP study to be of uncertain relevance to human cancer risk (www.epa.gov/iris/subst/0500.htm). In 2000, under the European Commission process for the classification and labeling of dangerous substances, the EU found that EGBE presented no significant hazard for human carcinogenicity (OECD, 2004).

NTP notes that the reproductive and development effects of EGEs are inversely related to alkyl chain length, which suggests that EGEHE may not have these effects. NTP also expresses concern over whether EGEHE is metabolized to shorter alkyl ethylene glycol ethers or if the alkoxyacetic acid(s) metabolites are toxic. EGEs are substrates for alcohol dehydrogenase, which catalyzes the conversion of their terminal alcohols to aldehydes. Further conversion of the aldehydes by aldehyde dehydrogenase produces alkoxyacetic acids, which are the predominant metabolites responsible for the toxicities of the EGEs. NTP also notes that subchronic studies of EGME, EGEE, and EGBE showed that EGME and EGEE are testicular toxicants. NTP's Research Concept paper proposes metabolism studies of EGEHE in rodents to determine if shorter chained glycol ethers and/or alkoxyacetic acids are generated *in vivo*. Commendably, NTP observes that if EGEHE is metabolized into shorter alkoxyacetic acid metabolites, this would suggest that EGEHE is a testicular toxicant and further studies may not be needed.

However, there appears to be no reason to suspect that EGEHE will be metabolized to shorter alkyl ethylene glycol ethers or to alkoxyacetic acid(s). Welsh (2005) observes that the term "glycol ethers" has been used indiscriminately to lump together "a wide range of compounds with different physico-chemical properties that are reflected in remarkable differences to elicit adverse effects." We recommend that the metabolism of EGEHE be studied first *in vitro* in isolated human microsomes or hepatocyte cell culture. The investigation of testicular toxicity also lends itself to an *in vitro* approach. Gray et al. (1985) incubated the alkoxy acetic acid metabolites of EGEs with mixed cultures of Sertoli and germ cells. They found a close correspondence between the effects of the four alkoxyacetic acids *in vivo* and their toxicity in the testicular cell cultures. Further, the effects *in vitro* appeared to be specific for the same target cell types, the pachytene and dividing spermatocytes, as *in vivo* and the relative order of toxicity of the four acids was the same. This similarity suggests a similar mode of action in culture and in the intact testis, and points to the potential value of the culture system for investigating the mechanism of EGE induced toxicity.

In summary, it is unlikely that EGEHE will display a toxicity profile similar to EGME, EGEE or EGBE, and there is no reason to suspect it will be metabolized to these shorter alkyl EGEs or to their alkoxyacetic acid metabolites. Further, in many cases the relevance of the observed toxicities of EGEs to human exposure is questionable. Finally, the metabolism, hematotoxicity and testicular toxicity of EGEHE can be assessed *in vitro*, and these options should be thoroughly investigated prior to developing an in vivo test plan.

References

Corley RA, Bormett GA and Ghanayem BI. Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. *Toxicol. Appl. Pharmacol.* 1994; 129: 61-79.

Gray TJB, Moss EJ, Creasy DM, Gangolli SD. Studies on the Toxicity of Some Glycol Ethers and Alkoxyacetic Acids in Primary Testicular Cell Cultures. *Toxicol. Appl. Pharmacol.* 1985; 79: 490-501.

OECD SIDS. Monoethylene Glycol Ethers Category SIDS Initial Assessment Report. UNEP Publications, 2004. (http://www.inchem.org/documents/sids/sids/MonoethyleneGlycolEthers.pdf).

Welsh F. The mechanism of ethylene glycol ether reproductive and developmental toxicity and evidence for adverse effects in humans. *Toxicology Letters 2005;* 156: 13–28.

Xue H, Kamendulis LM and Klaunig JE. A potential mechanism for 2-butoxyethanol (2-BE) induced mouse liver neoplasia. *Toxicologist* 1999; 48: Abstract 1084.

L-β-Methylaminoalanine

NIEHS has nominated L-β-Methylaminoalanine (L-BMAA), a non-standard amino acid produced only by cyanobacterial species, for toxicological evaluation based on its prevalence in the environment, its potential presence in dietary supplements containing cyanobacteria (also known as blue-green algae), and its neurotoxicity. The recommended toxicological evaluation involves ADME, neurotoxicity, and biomolecular screening studies (Federal Register Notice, 2008). There are numerous studies of L-BMAA in the literature, including both *in vitro* and *in vivo* animal (Karamyan and Speth, 2008) and human work (Hampton, 2003; Papapetropoulos, 2007)

Proposed Mechanism of L-BMAA toxicity

These papers examine the source(s) of L-BMAA, routes of exposure, its biochemical activity, and its neurotoxic effects. The body of knowledge generated by these studies has led to the hypothesis that upon ingestion, L-BMAA is incorporated into proteins during synthesis. L-BMAA-containing proteins then serve as a reservoir for the unusual amino acid. During the course of normal protein catabolism, L-BMAA is released. Some portion of the L-BMAA pool is (re)-incorporated into new protein and some portion is and available to bind several subtypes of glutamate receptors, resulting in constitutive activation (Murch, et al, 2004). In turn, this constitutive activation, or excitotoxicity, of glutamate receptors can lead to neurological damage. Glutamate-receptor mediated neurotoxicity is associated with the damage seen in amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease (Hampton, 2003; Papapetropoulos, 2007).

This model is the best approximation of the mode of action L-BMAA based on the existing data. Therefore, it seems that the most appropriate way to better understand L-BMAA toxicity would be to test the distinct parts of this hypothesis, many of which could be evaluated with:(1) *in vitro* biochemical approaches, i.e. purified proteins and/or whole (neuronal) cells and (2) human epidemiologic approaches.

In vitro Binding and Activity Studies

A mechanistic, hypothesis-driven approach is the most productive means for answering critical questions surrounding L-BMAA toxicity. We were pleased to see that NTP also embraced this approach with the neurotoxicity studies suggested in the paper "NTP Research Concept: β-N-Methylamino-L-alanine" (Sanders, 2008). This paper refers to *in vitro* assays using L-BMAA and other neurotoxicants including glutamate agonists. Presumably these will include receptor binding and activity assays to address the affinity of human glutamate receptors for L-BMAA and the degree of excitotoxicity elicited. In addition to the assays proposed, some other important biochemical questions to address in greater detail in order to further assess L-BMAA neurotoxicity in humans include the following:

(1) How readily is this unusual amino acid absorbed by the human intestine, that is, what is the affinity/ kinetics of amino acid transport for L-BMAA in human intestinal cells?
(2) Is labeled L-BMAA readily incorporated into proteins of human (neuronal) cells? What is the affinity of human tRNAs and ribosomes for the modified amino acid and what percentage of free L-BMAA is incorporated into proteins?

(3) Does the catabolism of L-BMAA-containing proteins occur with the same kinetics as "normal" proteins? Upon catabolism, what percentage of L-BMAA is captured for protein synthesis and re-incorporated and what percentage is free to bind and activate glutamate receptors?
(4) Does L-BMAA promote truncation of protein prior to completion of translation as seen in neurodegenerative diseases like Alzheimer's disease involving?
(5) Does L-BMAA promote protein misfolding, oligomerization, or aggregation similar to the plaques or neurofibrillary tangles that occur in Parkinson's and Alzheimer's?

Experts in the field have raised questions about the relevance of the existing animal data on L-BMAA to human neurotoxicity. *In vitro* assays based on human cells would yield much more definitive results than the generation of additional animal data, which may not represent the same biochemical mechanisms or manifest the same neurotoxic effects as humans.

ADME Studies

ADME studies are another major recommendation of the proposed research plan. To assure the most accurate data and avoid uncertainty introduced by interspecies extrapolation, we ask NTP to partner with researchers working on human-based ADME technologies. This could include Hμrel circuits, IdMOC plates, or Meta/DataChips , some of these systems are already utilized by NTP. Some ADME or biomonitoring studies should be conducted with humans already taking the blue-green algae supplements identified as an important (potential) route of L-BMAA exposure in the U.S.

Epidemiologic Studies

Concerns regarding the neurotoxicity of L-BMAA stem from the high incidence of amyotrophic lateral sclerosis/Parkinsonism-dementia complex (ALS-PDC) observed in the Chamorro people of Guam, who consume foods rich in L-BMAA. The Chamorros consume flour made from cycad palm seeds which concentrate the L-BMAA produced by cyanobacteria in the roots of the tree. Chamorros also eat flying fox bats which feed on the same seeds and bioaccumulate L-BMAA as a result. In fact, several studies have demonstrated the accumulation of L-BMAA in the brains of Chamorro patients with ALS-PDC and Canadian patients with Alzheimer's disease. Further strengthening the correlation between L-BMAA and human neurotoxicity, a declining flying fox bat population has coincided with an 18-fold decrease in the rate of ALS-PDC in Chamorros from its peak in the mid-1900s (Hampton, 2003). Large scale post-mortem studies of individuals with progressive neurodegenerative disorders and specifically of individuals known to consume L-BMAA-containing foods, such as the Chamorro people of Guam and certain Peruvian peoples (Johnson, 2008), would be useful for further investigation.

Conclusion

Given the correlation between L-BMAA and progressive neurodegenerative disorders such as ALS-PDC and Alzheimer's disease, the most intelligent approach for evaluating the risk and human health effects of L-BMAA requires directly testing specific hypotheses based on our existing knowledge of seemingly related neurodegenerative diseases.

References

Federal Register Notice V. 73, No. 192. p 57358-57360, Oct 2, 2008. (http://ntp.niehs.nih.gov/files/73_FR_192bsc_508.pdf)

Hampton, T. Food Chain of Evidence Points to Brain Toxin. *JAMA*. 2003; 290(21): 2788-2789.

Johnson HE, King SR, Banack SA, Webster C, Callanaupa WJ, Cox PA. Cyanobacteria (Nostoc commune) used as a Dietary Item in the Peruvian Highlands Produce the Neurotoxic Amino Acid BMAA. *J. Ethnopharmacology*. 2008; 118(1): 159-65.

Karamyan VT and Speth RC. Animal Models of BMAA Neurotoxicity: a Critical Review. *Life Sci*. 2008; 82(5-6): 223-46.

Murch SJ, Cox PA, Banack S.A. a mechanism for Slow Release of Biomagnified Cyanobacterial Neurotoxins and Neurodegenerative Disease in Guam. *PNAS*. 2004; 101(33): 12228-12231.

National Toxicology Program (NTP), U.S. Department of Health and Human Services. Chemical Information Review Document for L-β-Methylaminoalanine. September 2008.

Papapetropoulos S. Is There a Role for the Naturally Occurring Cyanobacterial Toxins in Neurodegeneration? The beta-N-methylamino-L-alanine (BMAA) Paradigm. *Neurochem Int*. 2007; 50 (7-8): 998-1003.

Sanders, M. NTP Research Concept: β-N-Methylamino-L-alanine. Draft, prepared for the NTP Board of Scientific Counselor Meeting, November 20, 2008. (http://ntp.niehs.nih.gov/files/L-BMAA_Concept_final_100908_508.pdf)

Triclosan

Triclosan was nominated by the U.S. Food & Drug Administration (FDA) and a private individual to the NTP for dermal toxicity and carcinogenesis studies due to its widespread use as a topical antimicrobial agent.

In its Research Concept paper, NTP states that the major data gap with the use of triclosan is the long term dermal safety profile and notes that no acceptable dermal carcinogenesis studies have been conducted. Perhaps one reason that few dermal carcinogenesis studies have been conducted on this substance, which has been safely used in a wide variety of health-care and consumer applications such as deodorants, soaps and dentifrices for over 30 years, is that there is little reason to suspect that it might, in fact, be a dermal carcinogen. In 2001, Ciba Specialty Chemicals Corporation (Ciba) submitted a position paper, *Triclosan: Adequacy of Data to Support the Lack of Potential for Dermal Carcinogenicity*, in support of a petition regarding the Category I safety and long-term use status of triclosan (Ciba, 2001). This paper followed completion of a long-term hamster study and was prepared in response to a suggestion from FDA that data regarding dermal irritation together with data from the hamster study might be sufficient to remove the lifetime dermal study requirement. Ciba noted that all test vehicles examined were too harsh for conducting a long-term dermal carcinogenicity study. The petition formally requested that FDA waive its request for a chronic dermal carcinogenicity study based on information contained in the position paper demonstrating that the existing database on the carcinogenic potential of triclosan is adequate and that a dermal carcinogenicity study is not necessary.

Key issues from this position paper include:

- Triclosan does not have the profile of biological activities of any known human skin carcinogen or skin cancer risk factor;
- Triclosan is nongenotoxic and is unlikely to be a skin carcinogen since these agents appear to be predominantly genotoxic;
- Triclosan does not cause skin hyperproliferative changes such as acanthosis at typical use levels;
- Dermal carcinogenicity studies with other compounds demonstrate that there is no simple association between chronic skin irritation and skin carcinogenesis;
- The available data from the rat, hamster and mouse cancer bioassays with oral dosing of triclosan are adequate to assess the carcinogenic potential of triclosan;
- Extensive human experience with triclosan through both controlled clinical studies and over 30 years of safe product use support the dermal safety of this material; and
- The conduct of a dermal carcinogenicity evaluation of triclosan is unnecessary and is unlikely to add significant additional information to the assessment of the safety of this chemical.

NTP agrees that triclosan is not mutagenic or genotoxic, has a low level of toxicity in acute studies with very high LD50 values, and notes that no maternal or fetal toxicities were observed in mice, rats or rabbits up to the highest doses tested in a battery of reproductive toxicity studies. The only concern noted is that from signs of severe dermal irritation observed in a 1998 Colgate-Palmolive subchronic study in rats. Surprisingly, in its supporting information for the nomination, FDA asserts that this is the only dermal

data that exists to date, apparently ignoring extensive human experience over 30 years as well as studies submitted by Ciba including another 90-day subchronic dermal toxicity study and three 14-day repeated-dose dermal studies in rats (Ciba, 2003). Likewise, FDA finds fault with each of five carcinogenicity studies reviewed including an 18-month dermal study in mice. FDA concluded that this study was not valid due primarily to the presence of test material in control animals; nevertheless, no toxicities or carcinogenesis were reported. FDA has taken no further action on the dermal carcinogenicity assessment of triclosan since 2001, despite Ciba's submission of 27 additional studies in 2003.

It is imperative that FDA reconsider its call for dermal carcinogenicity testing of triclosan. Such testing would consume approximately 800 mice per study, but it is likely to be problematic considering previous difficulties identifying appropriate vehicles. These studies would be extremely unlikely to produce evidence of carcinogenicity for triclosan given the abundance of existing data and extensive human experience with this substance. This is an egregious example of a checklist approach to toxicology. The preliminary phases of the proposed research program would determine triclosan's dermal penetration and steady state levels in the skin of treated mice along with the kinetics of its photodecomposition focusing on photodecomposition products and formation of dichlorodibenzo-p-dioxins. If these data are perceived to be required, we urge FDA to consider human-relevant approaches for obtaining them such as the use of excised human skin or a reconstituted human skin model. OECD's Test Guideline 428 specifies the use of fresh, metabolically active skin to simultaneously measure diffusion and skin metabolism. In addition, various reconstituted human skin models have been optimized for the study of phototoxicity and percutaneous absorption.

References

Ciba Specialty Chemicals Corporation (Ciba). Triclosan: Adequacy of Data to Support the Lack of Potential for Dermal Carcinogenicity. FDA Docket 1975N-0183H, 2001. (www.fda.gov/ohrms/dockets/dailys/01/Sep01/091701/cp00009.pdf).

Ciba Specialty Chemicals Corporation (Ciba). Comments on Topical Antimicrobial Drug Products for Over-The-Counter Human Use. FDA Docket 1975N-0183H, 2003. (www.fda.gov/ohrms/dockets/dailys/03/Sept03/090303/75n-0183h-c000085-01-vol170.pdf).