PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Dr. Scott A. Masten, Director
Office of Chemical Nomination and Selection
Environmental Toxicology Program
National Institute of Environmental Health Sciences
P.O. Box 12233, MD A3-07
111 T.W. Alexander Drive
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Dear Dr. Masten,

The following comments are submitted on behalf of the more than 1.4 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 2007 (March 29, 2007; *Federal Register* 72(60):14816-14818). PETA and PCRM are committed to using the best available science to protect animals from suffering and to promoting the acceptance of alternatives to animal testing.

Specific comments are submitted for: aminopyridines; artificial butter flavoring mixture and certain components; asbestos, naturally occurring and atypical forms; diethyl phthalate; nanoscale materials; and *o*-phthalaldehyde. 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 ongoing testing and regulatory efforts along with the application of non-animal test methods in order to avoid unnecessary and duplicative animal tests.

Thank you for your attention to these comments. I can be reached at 610-586-3975 or by e-mail at josephm@peta.org.

Sincerely,

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Aminopyridines

The National Cancer Institute (NCI) has nominated three monoaminopyridines (MAPs), 2-aminopyridine, 3-aminopyridine, and 4-aminopyridine (2-AP, 3-AP, and 4-AP respectively), for study by the NTP based on the chemicals' use in commerce and purported lack of toxicity information. It has been proposed to conduct a 2-year carcinogenicity bioassay, genetic toxicity, and subchronic toxicity studies on 2-AP, comparative mechanistic studies on 3-AP and 4-AP, and comparative neurotoxicity studies on all three chemicals. These proposals are contrary to established risk assessment principles, reflected in their removal from the TSCA Priority Testing List by the TSCA Interagency Testing Committee[1], due to declining use and low potential for occupational exposure. Before these studies are conducted, we urge the NTP to look closely at the available data and evidence for this class of chemicals, as the proposed animal tests can, and should, be avoided.

First, it is unclear why subchronic toxicity studies are proposed, as 4-AP has subchronic data from multiple species, including humans (as the drug Fampridine-SR), available. Enough comparative data exists to reliably bridge data from 4-AP to the other two MAPs discussed here. Regarding genetic toxicity, while the MAPs are not mutagenic in bacterial assays, human cell-based assays could help to confirm suspicions that these chemicals are not toxic to genetic systems.

Overwhelming evidence available for MAPs and similar chemicals make the proposed mechanistic and neurotoxicity studies superfluous and excessive. In addition to the *in vitro* and *in vivo* studies reported in the dossier, a MedLine search reveals over 50 published articles investigating several aspects of the mechanistic basis of action and toxicity for 4-AP, which is not surprising considering its use as a human pharmaceutical.[2,3,4,5] Structurally-similar chemicals, such as Piperidine (CAS RN 110-89-4) and Pyridine (CAS RN 110-86-1) also have information on these effects, found in the Hazardous Substances Data Bank.[6,7] For 2-aminopyridine, information is given for *in vivo* and *in vitro* neurotoxicity investigations in cats, chicks, amphibians, and mice.[8] For 4-Aminopyridine, neurotoxicity studies are listed for porcine tissue, wild birds, cats, rats, guinea pig tissue, and chicks.[9]

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[1] Environmental Protection Agency. 2005. Fifty-sixth report of the TSCA Interagency Committee to the Administrator of the Environmental Protection Agency; Receipt of Report and Comments; Notice. *Fed. Reg.* **69(10)**: 2467-73.

[2] Franciosi s et al. 2006. Braod-spectrum effects of 4-aminopyridine to modulate amyloid beta-1-42-induced cell signaling and functional responses in human microglia. *J Neurosci.* **26(45)**: 116526-64.

[3] Kovacs A et al. 2003. Seizure, neurotransmitter release, and gene expression are closely related in the striatum of 4-aminopyridine-treated rats. *Epilepsy Res.* **55(1-2)**:117-29.

[4] Wang et al. 2003. Block of Na+ K+-ATPase and induction of hybrid death by 4-aminopyrdidine in cultured cortical neurons. *J Pharmacol Exp Ther.* **305(2)**:502-6.

[5] Tutka et al. 2002. Nitric oxide and convulsions in 4-aminopyridine-treated mice. *Eur J Pharmacol.* **437(1-2)**:47-53.

[6] HSDB. Piperidine. Accessed at http://toxnet.nlm.nih.gove/cgi-bin/sis/htmlgen?HSDB on 5/5/2007.

[7] HSDB Pyridine. Accessed at http://toxnet.nlm.nih.gove/cgi-bin/sis/htmlgen?HSDB 5/5/2007.

[8] HSDB. 2-Aminopyridine. Accessed at http://toxnet.nlm.nih.gove/cgi-bin/sis/htmlgen?HSDB on 5/5/2007.

[9] HSDB. 4-Aminopyridine. Accessed at http://toxnet.nlm.nih.gove/cgi-bin/sis/htmlgen?HSDB on 5/5/2007.

The available evidence indicates that the MAPs are probably carcinogenic. Not only are some pyridines known carcinogens, but metabolic and SAR analyses for the proposed chemicals indicate carcinogenic potential. One of the chemicals, 4-AP, is used in both an avicide, Avitrol, and an experimental pharmaceutical, Fampridine-SR. The EPA has established a tolerance of 0.1 ppm for use on food crops, the FDA is allowing Phase-III clinical trials to proceed, and several emergency-response authorities, such as the Comprehensive Environmental Response, Compensation, and Liabilty Act (CERCLA), have promulgated policies for its use. In fact, 4-AP is slated for reregistration assessment by the EPA this fiscal year (2007). Waiting to see that docket would be a very acceptable decision. For 2-AP, OSHA, NIOSH, and ACGIH have all promulgated exposure limits for the workplace. These regulatory decisions have not all come about in a vacuum; data likely exists on these chemicals that have led to these decisions. If this data is not publicly available, it is up to the federal government to develop policies that protect confidential business information (CBI) while at the same time ensuring that animal tests are not duplicated simply because the data contains CBI.

The submitted dossier mentions (page 30) that toxicities for 4-AP are similar between mammals and birds. If this is indeed the case, every effort should also be made to extrapolate available avian toxicity information to mammals.

We urge the NTP and NCI to reconsider the proposed studies; based on the information already available, and the lack of new information that will result, additional animal studies will be a waste of resources and animal lives.

Artificial butter flavoring mixture and certain components

Bronchiolitis obliterans, also called "popcorn worker's lung", is a rare, irreversible and life-threatening form of fixed obstructive lung disease. The case reports of eight former workers from the same Jasper, Missouri popcorn manufacturing plant with severe bronchiolitis obliterans syndrome first sparked public interest in May 2000. Since then, the disease has been identified among workers in popcorn, flavoring and chemical manufacturing plants in several states. At least three of the affected workers have died, others are awaiting lung transplants and several have received damage awards against flavoring manufacturers for exposure-related injuries totaling more than $50 million.[1] The United Food and Commercial Workers (UFCW) nominated artificial butter flavoring and its ingredients, especially diacetyl and acetoin, for long term testing for respiratory and other toxicity, and cancer-causing properties, by inhalation exposure. According to NTP's standard protocols, each chronic toxicology/carcinogenicity study can be expected to consume between 1,000 and 1,400 rats and mice.[2] When inhalation exposure is studied, animals are either confined to a gas chamber, squeezed tightly into inhalation tubes, or are restrained with a breathing apparatus over their mouths

Epidemiological studies of occupationally-exposed workers in plants reporting clinical bronchiolitis obliterans cases strongly implicate the volatile organic compound diacetyl as the etiological agent. Diacetyl is used in many food products, including popcorn, pastries, frozen foods, and candy. It is one of the main components in butter flavoring that gives this food its buttery taste. Diacetyl was the predominant compound found in the artificial butter flavoring and the indoor air of the Jasper, Missouri plant. Further, as cumulative exposure to diacetyl increased, the incidence of airway obstruction and abnormal results on spirometry also increased, demonstrating a clear dose-response relationship.[3] Importantly, reports of three bronchiolitis obliterans cases among former workers of a chemical plant manufacturing diacetyl narrow the disease's potential cause, since in contrast to the diverse chemical exposures characterizing flavoring manufacture and use, exposure in the manufacture of diacetyl is limited to diacetyl and low concentrations of acetoin, acetic acid, and acetaldehyde.[4] In addition, NIOSH scientists have already confirmed that diacetyl exposure by inhalation produces necrotizing and suppurative bronchitis in rats.[5] Extrapolation of exposure-response relationships for inhalation exposure from animals to humans is complicated by the anatomical and physiological differences between animals used in laboratories and humans.

Despite the overwhelming human-based evidence, there are still no specific OSHA standards regulating flavorings-related lung disease or requiring diacetyl exposures to be

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[1] Brown, C. Popcorn Plant Workers Reach Settlement with Flavoring Maker Over Lung Problems. *Chemical Regulation Reporter*. 2005; 29(42): 1054.
[2] National Toxicology Program. NTP 2-Year Study Protocol. *Available at*: http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=36305D16-F1F6-975E-79776DAD38EC101E.
[3] Kreiss, K. et al. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N Engl J Med.* 2002; 347(5): 330-338.
[4] Kreiss, K. Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol.* 2007; 7: 162-167.
[5] Hubbs, A. F., et al. Necrosis of Nasal and Airway Epithelium in Rats Inhaling Vapors of Artificial Butter Flavoring. *Toxicol Appl Pharmacol.* 2002; 185: 128-135.

controlled. In July, 2006, UFCW and the International Brotherhood of Teamsters requested an emergency standard setting an exposure limit for diacetyl of 0.05 ppm and the requirement of respiratory protection and medical surveillance for all employees exposed over the limit, along with mandatory airborne diacetyl monitoring. A letter sent by the Project on Scientific Knowledge and Public Policy at George Washington University to Labor Secretary Elaine L. Chao, and signed by 40 prominent occupational health physicians and scientists, supported the unions' request.[6] OSHA, however, will not even identify diacetyl as a hazard, calling it only a substance of suspicion and noting that flavorings are made of complex mixes of ingredients. OSHA insists that the general duty clause, which regulates all general hazards, offers protection to workers exposed to these ingredients.[7] The agency has only recently announced that it will initiate a National Emphasis Program (NEP) to address hazards in the microwave popcorn industry associated with butter flavorings containing diacetyl. This NEP conspicuously ignores manufacture and other uses of food flavorings and House Education and Labor Committee Chairman George Miller has stated that the announcement "falls far short of what is necessary to prevent deaths and serious illnesses among workers in the food flavoring industry."[8]

In a 2006 Chemical Regulation Reporter interview, Allen J. Parmet, a physician who investigated the initial popcorn-lung cases observed "we've known how to stop this for four years ... there is no reason for another person to get sick."[9] More redundant animal tests will only delay the implementation of engineering and work practice controls that have already been proven to effectively eliminate this health risk to flavorings workers. In California, legislation has been introduced that would ban diacetyl from workplaces in the state by 2010. In addition, California's OSHA draft regulatory standard would reduce employee exposure to diacetyl and other flavoring ingredients by mandating engineering controls, such as local exhaust ventilation and closed transfer of chemicals, as well as work practices such as covering containers and minimizing spills. It would also mandate comprehensive worker respiratory protection for organic vapors and particulates and require companies to conduct spirometry screening. Fast track rulemaking on this standard could begin as early as this summer, and it has already drawn industry support.[10]

We urge UFCW to withdraw its nomination of diacetyl, acetoin and other artificial butter flavoring ingredients for additional, unneeded, and most likely irrelevant animal tests and instead to redouble its efforts to persuade federal legislators and regulatory agencies to follow California's lead in managing the risks posed by these substances. Such an approach is necessary in order to remove this threat to workers' health in a timely manner.

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[6] Couillard, L. Physicians, Unions Ask for Emergency Rule To Limit Worker Exposure to Butter Flavoring. *Chemical Regulation Reporter*. 2006; 30(31): 803.
[7] For Flavorings-Related Lung Disease, General Duty Clause Protects, OSHA Says. *Chemical Regulation Reporter*. 2006; 30(38): 996.
[8] Foulke Defends OSHA's Standards Record, Others Say Rulemaking Has Ground to Halt. *Chemical Regulation Reporter*. 2007; 31(18): 417.
[9] Couillard, L. 2006.
[10] Materna, B. et al. Fixed Obstructive Lung Disease Among Workers in the Flavor-Manufacturing Industry --- California, 2004—2007. CDC. *MMWR Weekly*. 2007; 56(16): 389-393.

Asbestos, naturally occurring and atypical forms

Vermiculite from a mine that operated near Libby, Montana, from the early 1920s until 1990 was contaminated with asbestos and other fibrous amphibole minerals. Several epidemiological studies have documented the toxicity of the amphibole asbestos minerals present in the mine.[1] The Libby amphiboles have been nominated by EPA for subchronic and chronic toxicology/carcinogenicity studies on animals via inhalation; other related atypical asbestos and mineral fibers may be assigned a lower priority for study. According to NTP's standard protocols, each chronic toxicology/carcinogenicity study can be expected to consume between 1,000 and 1,400 rats and mice.[2] In its nomination letter, EPA notes that there is a considerable data set available regarding the adverse health effects and biological activity of commercial grade asbestos materials but claims that there is some question as to the toxicity of the Libby amphiboles relative to commercial asbestos.

Inhalation of asbestos fibers may lead to fibrotic lung disease (asbestosis), as well as cancers of the lung, pleura, and peritoneum. In its Toxicological Profile for Asbestos, ATSDR states that evidence for the role of asbestos in human lung cancer is derived primarily from studies of the cause of death of occupationally-exposed workers. More than 40 epidemiological studies providing reliable dose response information on the inhalation effects of asbestos in humans are summarized in this profile.[3]

ATSDR observes that animal studies provide only supporting evidence for the fibrogenicity of asbestos and cautions that extrapolation of exposure-response relationships for asbestos-induced lung fibrosis in animals to humans is not recommended due to the longer persistence of fibers in humans, the relatively short life-span of animals used in laboratories, and the anatomical and physiological differences between animals and humans that influence rates of lung deposition and clearance of asbestos fibers. In 1995, Rödelsperger and Woitowitz concluded that a significant cancer risk from asbestos exists for humans at a fiber concentration 300 times lower than that needed to produce the same risk in rats, and therefore, that inhalation studies in rats are not sufficiently sensitive to detect risks to humans exposed to other fibers.[4] Muhle & Pott (2000) reached a similar conclusion demonstrating that inhalation studies in rats need fiber concentrations over 100 times higher to produce the same lung cancer risk observed in humans, and about 1000 times higher to produce the same mesothelioma risk.[5] They go on to note that if the current animal protocol for testing synthetic mineral fibers were

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[1] Bandli, B. R. and Gunter, M. E. A Review of Scientific Literature Examining the Mining History, Geology, Mineralogy, and Amphibole Asbestos Health Effects of the Rainy Creek Igneous Complex, Libby, Montana, USA *Inhal Toxicol.* 2006; 18:949–962

[2] National Toxicology Program. NTP 2-Year Study Protocol. *Available at*: http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=36305D16-F1F6-975E-79776DAD38EC101E.

[3] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Asbestos. U.S. Department of Health and Human Services. 2001.

[4] Rodelsperger, K. and Woitowitz, H. J. Airborne Fibre Concentrations and Lung Burden Compared to the Tumour Response in Rats and Humans Eeposed to Asbestos. *Ann Occup Hyg.* 1995; 39(5): 715-725.

[5] Muhle, H. and Pott, F. Asbestos as Reference Material for Fibre-Induced Cancer. *Int Arch Occup Environ Health.* 2000;73(Suppl): S53-S59.

to be applied to asbestos fibers, their very high carcinogenicity, known from epidemiologic studies, would be unlikely to be detected.

Not surprisingly, in the six chronic toxicology/carcinogenicity studies of asbestos already conducted by NTP, no evidence of its very high carcinogenicity in humans was found in 17 of the 18 total groups of animals used. In only one group was a result of "some evidence" produced.[6] Nevertheless, EPA is now urging NTP to conduct still more chronic studies of asbestos and related mineral fibers in animals. Given the near total failure of these earlier studies to detect the known adverse effects of asbestos, it is difficult to imagine how new animal studies will address EPA's question regarding the toxicity of the Libby amphiboles relative to commercial asbestos.

Further, there is no reason to suspect that results of studies on the naturally occurring fibers in Libby vermiculite will differ substantively from those obtained using commercial asbestos. Health effects observed in Libby workers have been typical of other groups exposed to amphibole asbestos, including asbestosis and increased risks of lung cancer and malignant mesothelioma.[7] ATSDR notes that available evidence indicates that all asbestos fiber types are fibrogenic and carcinogenic. While there may be some differences in potency among fiber types, fiber size, as opposed to mineral type, appears to be of prime importance.[8] Sullivan (2007) notes that 36% of fibers from the Libby mine had lengths greater than 20 μm in length.[9] Fibers longer than 20 μm have been associated with asbestosis, and it is postulated that since these fibers are longer than the human macrophage, incomplete phagocytosis results. Further, around 65% of airborne fibers collected at Libby were found to be less than 5 μm in length. Fibers shorter than 5 μm may also play a role in fibrosis, particularly under conditions of overload. Sullivan suggests that the high mortality from asbestosis observed among Libby workers may be a function of fiber length. In addition, the potential health effects of winchite and richterite, the two major amphibole contaminants of Libby vermiculite, have been investigated *in vitro*, and an amphibole with similar elemental composition and structure, fluoroedenite, has been linked with asbestos-related mortality in a human population.[10]

We call upon NTP to reject this nomination, yet another example of thoughtless toxicology in which demonstrated human evidence and the lack of relevance of the animal "model" is disregarded. Instead of still more animal studies on asbestos, a mineral class already known from epidemiologic studies to be very highly carcinogenic to humans — but several orders of magnitude less so to animals used in laboratories — a far better approach would be to revise current regulations to include all amphibole asbestos minerals.

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[6] National Toxicology Program. Toxicology and Carcinogenesis Studies TR-246, TR-249, TR-277, TR-279, TR-280, TR-295. *Available at*: http://ntp.niehs.nih.gov/index.cfm?objectid=084801F0-F43F-7B74-0BE549908B5E5C1C

[7] Bandli, B. R. and Gunter, M. E. 2006.

[8] ATSDR. 2001.

[9] Sullivan, P. A. Vermiculite, Respiratory Disease, and Asbestos Exposure in Libby, Montana: Update of a Cohort Mortality Study. *Environ Health Perspect.* 2007; 115(4): 579-585.

[10] Bandli, B. R. and Gunter, M. E. 2006.

Diethyl phthalate

Diethyl phthalate (DEP) is used as a plasticizer in consumer products, including plastic packaging films, cosmetic formulations, and toiletries. The greatest human exposure to DEP comes from the use of DEP-containing consumer products and the ingestion of foods contaminated by DEP leaching from packaging materials.[1]

Concern has been raised for phthalate esters (PE), particularly di-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP),[2] regarding their potential endocrine disrupting properties. The National Institute of Environmental Health Sciences (NIEHS) nominated DEP for reproductive toxicity studies based on what it describes as widespread public exposure and inadequate data to evaluate DEP's potential reproductive hazard. Toxicokinetic studies by oral and dermal routes are also listed among the National Toxicology Program's (NTP) preliminary study recommendations; however, this data point does not appear to be addressed in the chemical information profile submitted by NIEHS in support of the nomination.

The reproductive and developmental toxicity of PEs is extremely well-studied. NIEHS' chemical information profile for DEP cites 20 relevant studies, including two recent multigeneration studies in mice and rats. Nevertheless, NIEHS calls for yet another multigeneration reproductive toxicity study in rats. Such a study would cause the deaths of approximately 2000 animals. Although NIEHS describes the 2005 multigeneration study in rats by Fujii et al. to be well-conducted and notes that few developmental effects and no effects on reproduction were observed, NIEHS claims that limitations in the design of this study leave the question of the potential reproductive hazard of DEP unanswered. These "limitations" are not identified; however, NIEHS notes that the design parameters of the proposed study are to include the assessment of the androgen status of F1 male offspring as measured by anogenital distance (AGD) and retaining a minimum of two males and females per litter in the F1 generation. It is unclear how the inclusion of these design parameters would address supposed limitations of the Fujii et al. (2005) study, since AGD was among the endpoints measured and was found to be unaffected. In addition, the number of F1 weanlings selected from each litter in this study was one or two per sex.[3] No further justification is offered for this apparent duplication.

The results of Fujii et al. (2005) are in general agreement with those of other studies cited and fit the structure–activity relationship observed by Foster et al. (1980) in young male rats and Gray et al. (2000) in rats *in utero*.[4,5] That is, PEs with ester side chains four to six

[1] International Programme on Chemical Safety (IPCS). Diethyl Phthalate. Concise International Chemical Assessment Document 52. WHO, Geneva, Switzerland. 2003; *Available at*: http://www.inchem.org/documents/cicads/cicads/cicad52.htm.
[2] Main K. M., et al. Human Breast Milk Contamination with Phthalates and Alterations of Endogenous Reproductive Hormones in Infants Three Months of Age. *Environ Health Perspect.* 2006; 114(2): 270-276.
[3] Fujii, S., et al. A Two-Generation Reproductive Toxicity Study of Diethyl Phthalate (DEP) in Rats. *J Toxicol Sci.* 2005; 30(Special Issue):97-116
[4] Foster, P. M., et al. Study of the Testicular Effects and Changes in Zinc Excretion Produced by Some N-Alkyl Phthalates in the Rat. Toxicol. Appl. Pharmacol. 1980; 54: 392–398.

carbons in length in the ortho configuration are antiandrogenic (e. g. DEHP and DBP), while others, such as DEP with its two-carbon ester side-chain, are not. Only members of the former group are metabolized to the active monoester. Of particular relevance, Gray et al. (2000) observed that DEHP, benzyl butyl phthalate (BBP) and diisononyl phthalate (DINP) administered to rats during pregnancy through three days post-delivery altered sexual differentiation in male pups as determined by shortened AGD, reduced testis weights, and the presence of female-like areolas and nipples. In contrast, male pups born to dams administered DEP, dimethyl phthalate (DMP), and dioctyl tere-phthalate (DOTP) did not differ from controls in these respects. Gray et al. described their confidence in the DEP and DMP data from this study as high.

Similar reasoning was employed by the American Chemistry Council (ACC) in its test plan for phthalate esters, a category spanning 18 chemicals, submitted to EPA's High Production Volume Chemical Challenge Program.[6] ACC concluded that existing data on DEP indicated that it would not cause reproductive effects and proposed no additional testing. ACC noted that a complete health effects SIDS data set is available for DEP and cited the absence of effects on male reproductive development observed by Gray et al. (2000) as well as the absence of effects on reproductive organs observed in a 1995 NTP study. EPA accepted ACC's conclusions without dispute.

Finally, it should be noted that among the studies cited as raising concern over the endocrine disrupting properties of PEs, particularly DEHP, is that of Swan et al. (2005).[7] This study reported an inverse correlation between maternal urinary monoethyl phthalate and AGD in male offspring; however, the validity and methodology of this study have been questioned.[8,9] Additionally, estimated human phthalate exposures are 1,000 to 10,000-fold lower than the experimental NOEL for DEHP.[10]

In summary, the weight of existing evidence renders it extremely unlikely that DEP will produce reproductive or developmental effects in the proposed, clearly duplicative, study. We call upon NTP to reject this nomination thereby sparing the lives of approximately 2000 animals.

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[5] Gray, et al. Perinatal Exposure to the Phthalates DEHP, BBP, and DINP, but Not DEP, DMP, or DOTP, Alters Sexual Differentiation of the Male Rat. *Toxicol Sci.* 2000; 58: 350–365

[6] ExxonMobil Biomedical Sciences, Inc. for the Phthalate Esters Panel HPV Testing Group of the American Chemistry Council. High Production Volume (HPV) Chemical Challenge Ptogram Test Plan for the Phthalate Esters Category. 2001. *Available at:* http://www.epa.gov/chemrtk/pubs/summaries/benzene/c13467tc.htm.

[7] Swan, S. H., et al. Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure. *Environ Health Perspect.* 2005; 113(8): 1056-1061.

[8] McEwen, G.N. and Renner, G. Validity of anogenital distance as a marker of in utero phthalate exposure. *Environ Health Perspect.* 2006; 114: A19-A21.

[9] Ott, M. G. and Pallapies D. Technical Critique of Swan et al. (2005). *In:* Comments of The American Chemistry Council Phthalate Esters Panel On the Draft NTP-CERHR Expert Panel Update On The Reproductive And Developmental Toxicity Of Di(2-Ethylhexyl) Phthalate. 2005; *Available at:* http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP-Monograph.pdf

[10] CDC. Second (2003) and Third (2005) National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention, Atlanta, GA. 2003, 2005; *Available at:* http://www.cdc.gov/exposurereport.

Nanoscale Materials

Nanoscale Gold

Colloidal nanoscale gold has been studied thoroughly in cellular cytotoxicity assays. The outcome of these assays illustrates that colloidal gold is often toxic to cells. For this reason, there is no advantage to spending tax-payer dollars on additional toxicity testing in animals.

Tiered testing should be employed by the NTP. This is a logical, efficient system by which nanochemicals of interest are tested in a series of human-cell-based toxicity tests. Examples of this testing plan can be found in the following references: Sayes, C. M. et al. (2005);[1] Nel, A. T. et al. (2006);[2] Panessa-Warren, B. et al. (2006);[3] Sayes, C. M. et al. (2006).[4] This testing methodology should be used in all cases of toxicity testing and animal experimentation should only be considered as the last step before regulatory acceptance. Because colloidal nanoscale gold has already been tested in cell-based assays, and found to be toxic to cells, no *in vivo* testing should occur.

Because changes in the coating of colloidal gold changes the toxic potential of colloidal nanoscale gold, each of the prospective non-toxic coated types of colloidal nanoscale gold should undergo a tiered testing approach and only if found to be *non-toxic* to human cells should testing for regulatory approval be carried out.

In addition, because each coating type has changed the biodistribution of nanoscale colloidal gold in animals, it is quite likely that animal studies will not produce accurate biodistribution data for coated colloidal nanoscale gold as human-specific physiology and pharmacokinetics will determine the distribution of the coated nanoscale gold.
An alternative, potentially more accurate and relevant test system would be an in vitro microfluidic circuit, such as the HuREL microchip. This technology allows toxicity testing in multiple cell types linked via microfluidic channels. Since both targeting and toxicity can be effectively tested using human cells, this technology offers a human-relevant alternative to answer biodistribution questions.[5]

At this time, no additional studies on colloidal nanoscale gold are warranted. We urge NTP to institute logical, efficient, tiered testing matrices so that ineffective animal studies are avoided.

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[1] Sayes, C. M., et al. Nano-C60 Cytotoxicity is Due to Lipid Peroxidation. *Biomaterials.* 2005; 26(36): 7587-95.
[2] Nel, A., T., et al. Toxic Potential of Materials at the Nanolevel. *Science.* 2006; 311(5761): 622-7.
[3] Panessa-Warren, B., et al. Biological Cellular Response to Carbon Nanoparticle toxicity. *J Phys: Condens Matter* 2006; 18(33): S2185-S2201.
[4] Sayes, C. M., et al. Correlating Nanoscale Titania Structure with Toxicity: A Cytotoxicity and Inflammatory Response Study with Human Dermal Fibroblasts and Human Lung Epithelial Cells. *Toxicol Sci.* 2006; 92(1): 174-85
[5] Sin, A., et al. The Design and Fabrication of Three-chamber Microscale Cell Culture Analog Devices with Integrated Dissolved Oxygen Sensors. *Biotechnol Prog* 2004; 20(1): 338-45.

Nanoscale Silver

Bulk-sized ionic silver has a long antimicrobial history and has been used to fight and prevent infection for more than a thousand years. More recently, nanoscale silver has become a commodity of interest, despite the fact that many studies show the deleterious effects of nanoscale silver on human cells.[6,7,8] Nanoscale silver has also been studied in animals and has been shown to bioaccumulate in organs and muscle of the animals studied.

A burn victim who applied nanosilver topically for six days developed argyia, elevated serum leves of aspartate aminotransferase, alanin amintransferase, and gamma galactosyl transferase and had elevated levels of silver in both urine and serum. Interestingly, preclinical studies with this exact same formulation on pigs did not show any of the adverse reactions reported after its use on humans.[9] It therefore seems illogical for NTP to prescribe a series of animal studies for nanoscale silver. It is clear from this elegant illustration above, and many other examples that animal studies are insufficient for studying nanoparticles.

Nanoscale materials should undergo toxicity testing with human-relevant methods. We now have a large repertoire of reliable *in vitro* methods that are the best candidates to test the safety of nanoscale materials.

Because there is evidence that nanosilver can bioaccumulate and cause harmful effects, an efficient testing strategy consisting of Tier I *in vitro* test methods should be carried out.[10] Tier I level testing is illustrated in the following references and should consist of human cell-based cytotoxicity assays.[11,12,13] Because nanoscale silver has already shown to have dangerous effects and, in contrast, ionic, bulk silver has been used safely for centuries, it is logical and prudent to approve products containing bulk silver and not nanosized silver.

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[6] Lok CN, H.C., et al. Proteomic Analysis of the Mode of Antibacterial Action of Silver Nanoparticles. *J Proteome Res*. 2006; 5(4): 916-924.
[7] Zhang YY, Sun J., A Study of the Bio-Safety for Nano-Silver as Anti-Bacterial Materials. *Zhongguo Yi Liao Qi Xie Za Zhi*. 2007; 1(36): 36-38.
[8] Zhang FQ, et al. Comparison of the Cytotoxicity *in vitro* Among Six Types of Nano-silver Base Inorganic Antibacterial Agents. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 2005; 40(6): 504-507.
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o-Phthaladehyde

The National Institute for Occupational Safety and Environmental Health (NIOSH) has nominated *o*-Phthaladehyde (OPA) for study by the NTP based on its main use, as an ingredient in hospital sanitizers such as Cidex-OPA and Ucarcide P200 Antimicrobial. The submitted dossier states that virtually no published information exists on the toxicity of the chemical, despite pages of references and many studies given in the Data Availability Checklist. Furthermore, the dossier states that animal toxicity studies have been submitted to EPA and FDA but are protected by confidential business information. We strongly recommend that the proposed studies, including genetic toxicity, chronic and/or carcinogenicity studies, subchronic toxicity, dermal irritation and toxicity, sensitization, and asthmagenic potential, are NOT conducted until this submitted information is reviewed. Practices to protect confidential business information, while vital, should never lead to the duplication of toxicity studies.

The submitted dossier reviews the available data, and indeed cites data for genetic toxicity (multiple *in vitro* and *in vivo* studies), subchronic toxicity (conducted orally in rats) as well as dermal toxicity (conducted in rabbits). Furthermore, proposed studies to assess dermal sensitization and asthmagenic potential are repetitive and redundant in the face of volumes of evidence indicating that OPA is indeed a dermal and respiratory sensitizer in humans. Evidence provided by SAR analysis supports this indication, as does information provided for one of OPA's metabolites, Phthalic anhydride (CAS RN 85-44-9), which is also, like OPA, asthmagenic in humans but not a sensitizer in guinea pigs[1]. Additionally, we found another structurally-similar chemical, p-Anisaldehyde (CAS RN 123-11-5), which was found to be moderately irritating to rabbit skin[2]. Patient and worker protection measures can be put into place that will mitigate these risks without further animal studies. NTP nominations are intended for chemicals or classes of chemicals for which there are significant gaps in knowledge—clearly this is not the case with OPA.

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