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Via e-mail to: masten@niehs.nih.gov

Dr. Scott A. Masten, Director
Office of Chemical Nomination and Selection
NIEHS/NTP
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Dear Dr. Masten:

The following comments are submitted on behalf of the more than 1.3 million members and supporters of People for the Ethical Treatment of Animals (PETA) in response to the nominations of substances to the NTP for study in 2006 (71 FR 18341, April 11, 2006). PETA is the world's largest animal rights organization and is committed to using the best available science to protect animals from suffering and to promoting the acceptance of alternatives to animal testing.

Nominated for study are substances in widespread use for which there is little reason to suspect toxicity (arbutin, gypsum), substances for which toxicity has already been well-characterized (ceric oxide, flame retardants) and substances that are included or structurally similar to chemicals that are included in the EPA High Production Volume (HPV) Challenge Program and Voluntary Children's Chemical Evaluation Program (VCCEP) (tert-butylacrylamide, 3-dimethylaminopropyl methacrylamide, flame retardants). Nevertheless, the NTP has recommended additional animal tests that would result in the poisoning and death of thousands of animals if carried out.

Specific comments are submitted for each of the nominated substances with the exceptions of phenoxyethyl acrylate and trifluoromethylbenzene, for which the NTP has recommended new testing be deferred. In each case, we urge the NTP to thoroughly consider potential human exposure, existing toxicity data, and ongoing testing programs 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,

Joseph Manuppello, MS
Research Associate

Samantha Dozier, PhD
Research Associate

Attach.

Arbutin

Arbutin is a naturally occurring constituent of bearberry (*uva-ursi*) leaves and other botanicals. As such, it has a long history of use in traditional as well as modern western medicine for urinary tract infections and as a diuretic.[1] Infrequent side effects include nausea and vomiting.

The NIEHS nominated arbutin for toxicological study principally because it is hydrolyzed to hydroquinone (HQ) in humans.[2] Arbutin does not appear to be hydrolyzed to HQ in rodents[3] and is not toxic to mice even at extremely high doses (8,000 mg/kg).[4] Previous NTP studies have shown HQ to induce renal tubule adenomas in male rats as well as liver adenomas and thyroid gland follicular cell hyperplasia in mice.[5] It should be noted, however, that each of these tumor types results from a well-characterized, species-specific mechanism. Nearly all male rats in both dosed and control groups in the NTP study suffered from nephropathy, a common condition in this strain of rat. Renal tubular adenomas result from accumulation of α2u-globulin, a protein found only in rats.[6] Liver adenomas result from peroxisome proliferation, another mechanism which appears irrelevant to humans.[7] Thyroid gland follicular cell hyperplasia follows elevation of thyroid stimulating hormone levels in rodents, but not in humans.[8] These observations cast doubt on the importance of HQ toxicity in humans, and, consequently, on the rationale for this nomination.

The NIEHS defines a need for further studies to clarify rodent and human differences in biological disposition of arbutin and conversion to HQ, while citing earlier studies which thoroughly detail these fundamental and relevant differences.[9,10] Likewise, the NTP recommends *in vivo* metabolism and disposition studies, but given these species differences, it is difficult to imagine how these studies will contribute to an understanding of arbutin toxicity in humans, unless such studies are conducted in humans. The NTP also recommends *in vivo* genotoxicity studies. Arbutin has already tested negative for genotoxicity *in vivo*.[11] Arbutin and HQ have also

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[1] American Botanical Council. 2000. Uva Ursi leaf. Excerpt from Herbal Medicine: Expanded Commission E Monographs (published in 1994). Internet address: http://www.herbalgram.org/iherb/expandedcommissione/he098.asp.

[2] Schindler, G., Patzak, U., Brinkhaus, B., von Niecieck, A., Wittig, J., Krähmer, N. Glöckl, I., and Veit, M. 2002. Urinary excretion and metabolism of arbutin after oral administration of Arctostaphylos uvae ursi extract as film-coated tablets and aqueous solution in healthy humans. J Clin Pharmacol, 42(8):920-927.

[3] Jahodar, L., Leifertova, I., and Lisa, M. 1983. Elimination of arbutin from the organism. Pharmazie, 38(11):780-781.

[4] Li, S., Liu, G., Zhang, Y., and Xu, J. 1982. Experimental study on antitussive effect of arbutin (Chin.). Yaoxue Tongbao, 17(12):720-722.

[5] NTP (National Toxicology Program). 1989. Toxicology and carcinogenesis studies of hydroquinone (CAS No. 123-31-9) in F344/N rats and B6C3F1 mice (gavage studies). Technical Report No. 366. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr366.html.

[6] IARC (International Agency for Research on Cancer). 1999. Scientific publications no. 147—species differences in thyroid, kidney, and urinary bladder carcinogenesis. Internet address: http://wwwcie.iarc.fr/htdocs/iarcpubs/pub147/pub147contents.html.

[7] IARC. 1995. Technical report no. 24—peroxisome proliferation and its role in carcinogenesis. Internet address: http://www-cie.iarc.fr/htdocs/iarcpubs/techrep24/contents.html.

[8] IARC. Scientific publications no. 147. Op cit.

[9] Jahodar, et al. Op cit.

[10] Temple, A., Gal, F., and Reboul, C. 1971. The phenolic glucosides of certain Ericaceae. The elimination of arbutin and hydroquinone (Fr.). Trav Soc Pharm Montepellier, 31(1):5-12.

[11] Müller, L., and Kasper, P. 1996. The mutagenic potential of arbutine, a naturally occurring hydroquinone glycoside. Mutat Res, 360(3):291-292.

been tested for genotoxicity by *in vitro* methods with varying results.[12,13] The OECD has adopted numerous guidelines attesting to the widespread acceptance of *in vitro* methodology for genotoxicity testing.[14,15,16,17,18] We strongly urge the NTP to reconsider subjecting more animals to needless tests on this substance.

[12] Ibid.
[13] NTP. Op cit.
[14] OECD (Organization for Economic Co-operation and Development). 1997. Test Guideline 471: Bacterial Reverse Mutation Test.
[15] OECD. 1997. Test Guideline 473: *In Vitro* Mammalian Chromosome Aberration Test.
[16] OECD. 1997. Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Test.
[17] OECD. 1986. Test Guideline 479: Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells.
[18] OECD. 1986. Test Guideline 479: Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vitro*.

tert-Butylacrylamide and 3-Dimethylaminopropyl methacrylamide (DMAPMA)

tert-Butylacrylamide and 3-dimethylaminopropyl methacrylamide (DMAPMA) are among five high production volume (HPV) chemicals nominated by the NCI that do not meet the criteria for inclusion in the EPA HPV Challenge Program. The NCI cites concerns about the carcinogenic potential of acrylamides as its reason for nominating both of these related chemicals. We support the NCI's recommendation that studies selected for these chemicals be designed in conjunction with ongoing and anticipated tests of acrylamide and related chemicals. Moreover, the NTP recommends coordination with voluntary data development activities of the EPA HPV Challenge Program.

In 2001, the NMA/NBMA Association submitted a test plan and robust summaries to the EPA HPV Program for N-(hydroxymethyl)-acrylamide (NMA) and N-(butoxymethyl)-acrylamide (NBMA).[1] The NMA/NBMA Association categorized these two chemicals together with acrylamide, citing their close structural similarities. In each case, the only structural difference results from a substitution on acrylamide. Environmental Defense agreed with this categorization and noted that the database for acrylamide is "extraordinarily rich."[2] In a 1999 Letter to Manufacturers/Importers specifically addressing animal welfare concerns, the EPA directs HPV Program participants to maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.[3] Had tert-butylacrylamide and DMAPMA been included in the HPV Program, the category proposed by the NMA/NBMA Association could have reasonably been expanded to include them. Both of these chemicals similarly result from substitutions on acrylamide. The NCI specifically identifies acrylamide and N-methyl acrylamide as compounds that are structurally-related to tert-butylacrylamide. The NCI also identifies methacrylamide as being related to DMAPMA. Furthermore, tert-Butylacrylamide and NBMA have nearly identical chemical formulas, differing only in the arrangement of the substituted group.

The NMA/NBMA Association concluded that available data were sufficient to meet the requirements for NMA and NBMA under the HPV Challenge Program without additional testing since: 1) there exists a very extensive body of studies available on this family of compounds; 2) the chemical and biological characteristics of these compounds are very similar; 3) where differences between these compounds have been detected, the acrylamide results are the same as or potentially more problematic than the other two; and 4) the use of data from acrylamide to substitute for missing data for NMA or NBMA provides a good but likely conservative estimate.[4] The same conclusions apply to tert-butylacrylamide and DMAPMA.

The NTP recommends genotoxicity studies, metabolism and disposition studies (to determine if acrylamide is formed), and subchronic toxicity studies for both chemicals. The NCI proposes tests for chromosomal aberrations and the mouse lymphoma assay for assessing genotoxicity,

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[1] NMA/NBMA Association. 2001. Test Plan for N-(methyl)-acrylamides category. Internet address: http://www.epa.gov/chemrtk/nmac/c13168.pdf.

[2] Environmental Defense . 2002. N-(methyl)-Acrylamides Category – Comments of Environmental Defense. Internet address: http://www.epa.gov/chemrtk/nmac/c13168ed.pdf.

[3] EPA (Environmental Protection Agency). 1999. Letter to Manufacturers/Importers. Internet address: http://www.epa.gov/chemrtk/ceoltr2.htm.

[4] NMA/NBMA Association. Op cit.

and we urge the NTP to limit any genotoxicity testing to *in vitro* methods as adopted by the OECD.[5,6,7,8,9] Given what is known regarding the metabolism of tert-butylacrylamide[10] and other substituted acrylamides, it seems unlikely that formation of acrylamide from tert-butylacrylamide and DMAPMA will be ruled out by metabolism and disposition studies. Consistent with results for other substituted acrylamides, including those reported in a previous NTP study,[11] subchronic studies of both tert-butylacrylamide and DMAPMA show their toxicity to be less than that of acrylamide.[12,13] As a result, it is unlikely that additional subchronic studies will provide information that differs from that which could be estimated from studies of acrylamide and related compounds, and new tests are unnecessary.

[5] OECD (Organization for Economic Co-operation and Development). 1997. Test Guideline 471: Bacterial Reverse Mutation Test.
[6] OECD. 1997. Test Guideline 473: *In Vitro* Mammalian Chromosome Aberration Test.
[7] OECD. 1997. Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Test.
[8] OECD. 1986. Test Guideline 479: Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells.
[9] OECD. 1986. Test Guideline 479: Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vitro*.
[10] Tanii, H. & Hashimoto, K. 1981. Studies on in vitro metabolism of acrylamide and related compounds. Arch. Toxicol., 48, 157-166.
[11] NTP (National Toxicology Program). 1989. National Toxicology Program, Toxicology & Carcinogenesis Studies of N-Methyloacrylamide in F344/N Rats and B6C3F1 Mice. Technical Report Series No. 352. Internet address: http://ntp.niehs.nih.gov/index.cfm
[12] Tanii, H. & Hashimoto, K. 1983. Neurotoxicity of acrylamide and related compounds in rats. Effects on rotarod performance, morphology of nerves and neurotubulin. Arch. Toxicol., 54, 203-213.
[13] Degussa Corporation. 2002. N-3-dimethylaminopropylmethacrylamide (CAS 5205-93-6) combined Repeat Dose and Reproductive/Development Toxicity Screening. Final Report. Vol. 1 and 2 Submitted to EPA November 25, 2002. 8EHQ 1102-15236A, 16 pp.

Ceric Oxide

The NIEHS nominated ceric oxide for inhalation toxicity, chemical disposition and toxicokinetics studies citing both a lack of data on nanoscale ceric oxide, along with its use as a diesel fuel additive (in nanoparticulate form) and as a carrier for otic and ophthalmic compositions. The NIEHS nominated both microscale and nanoscale ceric oxide, even though these endpoints have already been well-characterized for the microscale form. These studies are reviewed in an extensive Health Effects Institute (HEI) report on health risks from cerium.[1]

In regard to inhalation toxicity, the exposure route of greatest concern for humans, HEI concludes that a concentration of cerium oxide in the low $\mu g/m^3$, such as that expected to result from its use in fuel, is unlikely to present a health risk.[2] The chemical disposition and toxicokinetics of cerium have been studied for decades. A 1978 National Council on Radiation Protection and Measurements panel evaluated the physical, chemical, and biological properties of radio-cerium for the purpose of setting guidelines for radiation protection.[3] It is particularly surprising that the NTP added dermal penetration studies to the NIEHS' list of recommendations given that the supporting document specifically notes that skin absorption of ceric oxide is negligible due to poor solubility in water and bodily fluids. Furthermore, the dermal LD_{50} is greater than 2000 mg/kg.[4] NTP also adds the phrase "toxicological characterization" to its recommendations—which may include studies for genotoxicity and chronic toxicity/ carcinogenicity—yet offers no explanation or justification for these additional recommendations.

Although nanoscale chemicals are often more toxic than their microscale counterparts, cost-effective *in vitro* assays are available to measure the toxicity of nanomaterials. In a 2006 review, "Toxic Potential of Materials at the Nanolevel," Nel and colleagues describe many relevant *in vitro* assays and list some of the short-term goals that toxicity studies of nanomaterials should attempt to attain.[5] This study lists the Nrf-2, MAP kinase, and NF- κB cascades as excellent indicators of oxiditative stress, and subsequent toxicity. When human cell culture (including cultures of skin, lung, organ and specialized tissue cells) is coupled with microarray experiments, cellular toxicity can be predicted without conducting studies in animals.

Another example of effective non-animal toxicity testing can be found in a study entitled "Nano-C60 cytotoxicity is due to lipid peroxidation." In this study, Sayes et al. report experiments performed to assess cytotoxicity/cell viability, lactate dehydrogenate release, mitochondrial activity, DNA content, plasma membrane permeability, lipid peroxidation, glutathione production, and the ability to prevent oxidative damage with L-ascorbic acid.[6] The authors state

[1] HEI (Health Effects Institute). 2001. Evaluation of human health risk from cerium added to diesel fuel. HEI Communication 9. Internet address: http://www.healtheffects.org/Pubs/Cerium.pdf.
[2] Viau A. 1994. A 13-Week Inhalation Toxicity and Neurotoxicity Study by Nose-Only Exposure of a Dry Powder Aerosol of Ceric Oxide in the Albino Rat. Bio-Research Laboratories.
[3] (NCRP) National Council on Radiation Protection and Measurements. 1978. Physical, Chemical, and Biological Properties of Radiocerium Relevant to Radiation Protection Guidelines. NCRP Report 60.
[4] Hirano S, Suzuki K. 1996. Exposure, metabolism and toxicity of rare earths and related compounds. Environ Health Perspect 104 (Suppl 1):85–95.
[5] Nel, A., Xia, T. et al. 2006. Toxic potential of materials at the nanolevel. Science 311(5761): 622-7.
[6] Sayes, C. M., Gobin, A. M. et al. 2005. Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials 26(36): 7587-95.

that, "in vitro testing provides a cost-effective means for such studies, and as this report illustrates, cell culture experiments are well suited for developing mechanistic models to inform material development." In addition, the authors explain that this study seeks "to set a standard for future efforts to characterize the environmental and health impacts of other classes of engineered nanoparticles." This study clearly shows that the most efficient (and humane) means of toxicity testing lie in modern, high-throughput in vitro assays.

For questions pertaining to the chemical metabolites or the toxic effects of the nanomaterial on skin, organ, or lung cells, the HuREL, a microfluidic device that allows reserchers to test compounds within a matrix of different cell types linked by microfluidic channels, can answer questions regarding how nanomaterials will interact with human tissues. Details are reported in "The Design and Fabrication of Three-Chamber Microscale Cell Analog Devices with Integrated Dissolved Oxygen Sensors."[7]

It is difficult to understand what the NTP believes will be gained by repeating inhalation toxicity, chemical disposition and toxicokinetics studies on microscale ceric oxide. Additional studies recommended by NTP, including dermal absorption, have not been justified, and their rationale is far from obvious. We urge the NTP to limit any dermal absorption and genotoxicity testing to *in vitro* methods as adopted by the OECD.[8,9,10,11,12,13]

[7] Sin, A., Chin, K. C. et al. 2004. The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors." Biotechnol Prog 20(1): 338-45.
[8] OECD (Organization for Economic Co-operation and Development). 1997. Test Guideline 471: Bacterial Reverse Mutation Test.
[9] OECD. 1997. Test Guideline 473: *In Vitro* Mammalian Chromosome Aberration Test.
[10] OECD. 1997. Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Test.
[11] OECD. 1986. Test Guideline 479: Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells.
[12] OECD. 1986. Test Guideline 479: Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vitro*.
[13] OECD. 2004. Test Guideline 428: Skin Absorption: *In Vitro* Method.

Diazonaphthoquinone (DNQ) Derivatives

Four diazonaphthoquinone (DNQ) derivatives, sodium 1,2-naphthoquinonediazide-5-sulfonate, 2,3,4-trihydroxybenzophenone tris(1,2-naphthoquinone-diazide-5-sulfonate), and 2,3,4-trihydroxybenzophenone 1,2-naphthoquinonediazide-5-sulfonate were nominated by the NIEHS for toxicological characterization based on moderate production and use as well as the "lack of adequate toxicological data." Occupational exposure results from the use of DNQ compounds as photoresists in the manufacture of semiconductors for the electronics industry.

We commend the NTP for limiting its testing recommendations for this group of related chemicals to *in vitro* toxicity studies evaluating genotoxicity, immunotoxicity and phototoxicity. In addition, we urge the NTP to use *in vitro* tests, such as those adopted by the OECD[1], to measure dermal absorption and to refrain from adding additional *in vivo* tests in the future. We also urge the NTP to consider a similar approach, consistent with its 21st Century *Vision* statement, for all chemicals nominated for study.

[1] OECD. 2004. Test Guideline 428: Skin Absorption: In Vitro Method.

Flame Retardants

Six chemicals or groups of related chemicals with flame retardant (FR) applications have been nominated for study by the CPSC on the grounds that the CPSC is considering the development of a performance standard to reduce the potential for ignition of upholstered furniture by cigarettes and small open flames. The CPSC believes manufacturers are likely to treat upholstery materials with FR chemicals and seeks to limit additional health risks resulting from exposure to these chemicals. As noted in specific cases below, the potential for human exposure to FR chemicals through treatment of upholstered furniture is frequently extremely low or inadequately determined. Hazard-based tests are premature if they cannot be matched to valid exposure models, and we urge the NTP to prioritize any proposed tests accordingly.

Antimony Trioxide (AT)

For antimony trioxide (AT), the CPSC requests chronic oral studies in rats and/or mice as well as chronic inhalation studies in a second species, such as the hamster. An inhalation reference concentration for AT has already been published by the EPA,[1] and the NTP does not list chronic inhalation studies among its preliminary recommendations. No rationale is offered for repeating chronic studies with an oral exposure route other than the possibility of human oral exposure. Sub-chronic oral exposure to very high doses (2-3% of diet) of AT for as long as 24 weeks resulted most consistently in increased liver weight with few other effects.[2] It seems likely that inhalation is the most sensitive exposure route, since AT is cleared quickly from other organs.[3] Additionally, PBPK data with route-to-route modeling can be used to extrapolate from one exposure route to another. Repeating a chronic study with an oral exposure route is unlikely to produce new toxicity information (e.g., a lower NOAEL) and is therefore unnecessary. The NTP adds consideration for studies of the nanoscale form. For general comments regarding new testing for nanoscale chemicals, please see our remarks on ceric oxide's nomination.

Decabromodiphenyl Oxide (DBDPO)

The toxicity of decabromodiphenyl oxide **(**DBDPO) is especially well-characterized and has already been the subject of chronic and sub-chronic toxicity studies as well as chemical disposition and genetic toxicity studies by the NTP.[4] In addition, DBDPO has been sponsored by the American Chemistry Council Brominated Flame Retardant Industry Panel under the EPA Voluntary Children's Chemical Evaluation Program (VCCEP).[5] The CPSC requests

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[1] EPA (Environmental Protection Agency). 1995. IRIS (Integrated Risk Information System) Summaries. Antimony trioxide (CASRN 1309-64-4). Internet address: http://www.epa.gov/iris/subst/0676.htm.

[2] Hext, P.M., P.J.Pinto, and B.A.Rimmel. 1999. Subchronic feeding study of antimony trioxide in rats. J. Appl. Toxicol. 19(3):205–209.

[3] Newton, P.E., H.F.Bolte, I.W.Daly, B.D.Pillsbury, J.B.Terrill, R.T.Drew, R.Ben-Dyke, A.W.Sheldon, and L.F.Rubin. 1994. Subchronic and chronic inhalation toxicity of antimony trioxide in the rat. Fundam. Appl. Toxicol. 22(4):561–576.

[4] NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No. 1163-19-5) In F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report No. 309. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr309.html.

[5] EPA (Environmental Protection Agency). 2001. Voluntary Children's Chemical Evaluation Program (VCCEP). Decabromodiphenyl ether (CAS No. 1163-19-5). Internet address: http://www.epa.gov/chemrtk/vccep/chem21.htm.

developmental neurotoxicity studies in rats. Concern regarding the risk of developmental neurotoxicity seems largely based on one highly-contentious study in which changes in spontaneous behavior in adult mice are noted following a single, high oral dose of DBDPO received as neonates.[6] This study's critics note that the concentration of DBDPO in the mouse brain associated with neurobehavioral effects is three orders of magnitude greater than the highest levels of polybrominated diphenyl ethers observed in human tissue. These critics also note surprise at the magnitude of the observed effect as it contradicts theoretical expectations based on structure-activity relationships.[7] Another of this study's critics notes evidence for poor absorption of DBDPO reported in the NTP's own earlier chemical disposition studies.[8] In its Data Needs Decision Document for DBDPO, the VCCEP echoes these criticisms and notes that the European Union is requiring a developmental neurotoxicity study of DBDPO. The NTP's preliminary recommendations note that developmental neurotoxicity studies are only to be performed if adequate private sector study is not identified or planned. We urge the NTP to reconsider the necessity of these studies and in particular to avoid duplication of EU and EPA efforts. In a September 2004 petition, in which PETA and the Physician's Committee for Responsible Medicine (PCRM) called upon the EPA to repeal its developmental neurotoxicity (DNT) testing guidelines, it was noted that no DNT testing protocol has ever been validated to confirm its reliability and relevance to neurodevelopmental effects in humans.[26] These general issues regarding DNT testing were also recently discussed at a TestSmart-DNT symposium organized by the Johns Hopkins University School of Public Health.[27]

Tris(chloropropyl) Phosphate (TCPP)

TCPP is a possible substitute for pentabromodiphenyl ether in flexible polyurethane foam (PUF). The CPSC requests sub-chronic and chronic oral studies of TCPP in rat and/or mouse. Numerous acute toxicity studies report oral LD_{50} values in the 2,000 – 5,000 mg/kg range. Sub-chronic studies report minimal evidence of toxicity even at TCPP concentrations as high as 2% of diet. In addition, no clear evidence of genotoxicity has been observed in bacterial or mammalian tests *in vitro*.[9] In 2000, the OECD HPV Chemicals Program recommended no further testing of TCPP due to extensive data showing its low toxicity. Additionally, the OECD noted that since TCPP is expected to be bound up in PUF, consumer exposure is not anticipated. Occupational exposure is also not anticipated since TCPP is produced in a closed system and has low volatility.[10] Likewise, we urge the NTP to consider the low acute and subacute toxicity of as well as the low potential for exposure to TCPP and to refrain from new animal tests. When exposure is expected to be minimal, animal testing should be a low priority.

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[6] Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., and Eriksson, P. 2003. Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. Toxicol. Sci. 76, 112–120.
[7] Vijverberg, H. P. M. and van den Berg, M. 2004. Letter to the editor. Toxicol. Sci. 79, 205–206.
[8] Hardy, M. L., 2004. Letter to the editor. Toxicol. Sci. 81, 528–529.
[26] Seidle, T., Hall, S. L., Kinburn, D. 2004. Petition to compel the U.S. EPA to repeal its test guidelines for developmental neurotoxicity. Internet address: http://www.stopanimaltests.com/feat/dnt/DNTpetition.pdf.
[27] The Johns Hopkins Center for Alternatives to Animal Testing (CAAT). 2006. TestSmart DNT. Internet address: http://caat.jhsph.edu/dnt/index.htm.
[9] OECD (Organization for Economic Co-operation and Development). 2004. SIDS Initial Assessment Report. Tris (1-chloro-2-propyl) phosphate. CAS No. 13674-84-5.
[10] Ibid.

Phosphonic acid, (3-{[hydroxymethyl]amino}-3-oxopropyl)-, dimethyl ester (PA)

The CPSC requests sub-chronic oral studies of PA in rat and/or mouse, and depending on the outcome of these, possibly chronic studies as well. Acute toxicity studies show PA to be essentially non-toxic with an oral LD_{50} value greater than 10,000 mg/kg.[11] A 28-day sub-chronic study reported no treatment-related effects or clinical signs.[12] Additionally, the level of exposure to PA by the oral route is anticipated to be exceedingly small (0.00075 mg/kg/d), even assuming worst-case parameters.[13] Again, we urge the NTP to consider TCPP's low inherent toxicity and low potential for human exposure and to refrain from new animal tests. The preliminary recommendations listed by the NTP also include dermal penetration studies. We urge the NTP to use *in vitro* tests, such as those adopted by the OECD to measure dermal penetration.[14] If there is no dermal penetration, then there is no risk from systemic dermal toxicity.

Tris(hydroxymethyl) phosphine oxide (THPO) (1067-12-5)

Tris(hydroxymethyl) phosphine oxide (THPO) is reported to be a metabolite of Tetrakis(hydroxymethyl) phosphonium chloride (THPC), which may be released when THPC-treated fabrics are extracted with aqueous solutions. The CPSC requests sub-chronic oral studies of THPO in rats and/or mice, and depending on the outcome of these, possibly chronic studies as well. Although few data are publicly available regarding the toxicity of THPO, the toxicity of THPC is well-characterized, including previous studies by the NTP,[15] and it is regarded as the most toxic of this class of flame retardants.[16] The estimated worst-case oral exposure is still well below the calculated reference dose, however.[17] The CPSC cites no reason to suspect that THPO will prove more toxic than the parent compound. As a result, the toxicity of THPO can and should be evaluated based on the higher toxicity of the parent compound, THPC. The preliminary recommendations listed by the NTP also include dermal penetration studies. Again, we urge the NTP to use *in vitro* tests, such as those adopted by the OECD to measure dermal penetration.[18]

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[11] Suzuki, Y., K.Naito, and M.Tobe. 1983. Acute toxicity of chemicals used in the household [English abstract]. Eisei Shikensho Hokoku 101:152–6.
[12] RCC (Research & Consulting Company AG). 1992. Subacute 28-Day Oral Toxicity (Gavage) Study With FAT 80'001/I in the Rat. RCC Project 316732.
[13] National Research Council. 2000. Toxicological Risks of Selected Flame-Retardant Chemicals. National Academy Press. Washington, D.C. 291-306.
[14] OECD. 2004. Test Guideline 428: Skin Absorption: In Vitro Method.
[15] NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Tetrakis(hydroxymethyl)phosphonium sulfate (THPS) (CAS No. 55566-30-8) and Tetrakis(hydroxymethyl)phosphonium chloride (THPC) (CAS No. 124-64-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies). Technical Report No. 296. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr296.html.
[16] National Research Council. Op cit. 417.
[17] Ibid. 435, 436.
[18] OECD. Op Cit.

Aromatic Phosphates

The CPSC requests sub-chronic/chronic studies in rats and/or mice as well as neurotoxicity and/or developmental neurotoxicity for one or more representatives from a group of six aromatic phosphates. The toxicity of one of the listed chemicals, tricresyl phosphate (TCP), has been thoroughly characterized, including acute, sub-chronic, chronic and continuous breeding studies by the NTP.[19] Exposure to TCP and related compounds has long been associated with neuropathy in humans.[20] The NTP's previous evaluations extend to neurological effects including measurement of hind-limb grip strength. Repeating these studies or conducting new developmental neurotoxicity studies is unlikely to provide additional information. Another aromatic phosphate, isodecyl diphenyl phosphate (IDDP), has been sponsored in the EPA's HPV Challenge Program.[21] The NTP recommends coordinating with the EPA to pursue testing with the manufacturer. The EPA and PETA have identified reliable studies accounting for all required toxicity endpoints for this substance, and no new testing has been proposed in the sponsor's revised test plan.[22] The studies cited include acute and sub-chronic toxicity, developmental toxicity and neurotoxicity, and demonstrate only minor effects for IDDP even at very high dose levels.[23,24,25] We urge the NTP to consider the extensive existing data on these representative aromatic phosphates and to refrain from new testing. In September 2004, PETA and the Physician's Committee for Responsible Medicine (PCRM) petitioned the EPA to repeal its developmental neurotoxicity testing (DNT) guidelines noting that no DNT protocol has ever been validated to confirm its reliability and relevance to neurodevelopmental effects in humans.[26] The general issues raised by PETA and PCRM were recently discussed at a TestSmart-DNT symposium organized by the Johns Hopkins University School of Public Health.[27]

[19] NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Tricresyl Phosphate (CAS No. 1330-78-5) in F344/N Rats and B6C3F1 Mice (Gavage and Feed Studies). Technical Report No. 433. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr433.html.
[20] IPCS (International Programme on Chemical Safety). 1990. Environmental Health Criteria 110: Tricresyl Phosphate. World Health Organization, Geneva.
[21] Ferro Corporation. 2002. High Production Volume Challenge Program (HPV). Test plans for isodecyl diphenyl phosphate. Internet address: http://www.epa.gov/oppt/chemrtk/isodecyl/c14216tc.htm.
[22] Ibid.
[23] Robinson, E.C., et al. 1986. Teratogenicity studies of alkylaryl phosphate ester plasticizers in rats. Fundamental and Applied Toxicology 7: 138-143.
[24] Robinson, E.C., et al. 1983. Teratology studies of alkaryl phosphates. The Toxicologist 3: 30.
[25] Johannsen, F.R., et al. 1977. Evaluation of delayed neurotoxicity and dose-response relationships of phosphate esters in the adult hen. Toxicology and Applied Pharmacology 41: 291-304.
[26] Seidle, T., Hall, S. L., Kinburn, D. 2004. Petition to compel the U.S. EPA to repeal its test guidelines for developmental neurotoxicity. Internet address: http://www.stopanimaltests.com/feat/dnt/DNTpetition.pdf.
[27] The Johns Hopkins Center for Alternatives to Animal Testing (CAAT). 2006. TestSmart DNT. Internet address: http://caat.jhsph.edu/dnt/index.htm.

Gypsum

Gypsum is described as the most common natural fibrous mineral found indoors.[1] In addition to its primary use to manufacture wallboard and plaster for the construction industry, it is also used as a soil additive, as a food and paint filler, and as a component of blackboard chalk, medicines, and toothpaste.

Gypsum was nominated for toxicological studies by the Mount Sinai-Irving J. Selikoff Center for Occupational and Environmental Medicine and the Operative Plasterers' and Cement Masons' International Association of the United States and Canada. The nominators cite little evidence to suggest that gypsum is more than a nuisance irritant. In fact, they cite considerable evidence to suggest that it is not toxic to humans. Unlike other fibers, gypsum is very soluble in the body and is rapidly cleared from the lungs.[2] In studies of gypsum industry workers, the occurrence and symptomology of any observed adverse health effects occur with exposure to silica rather than gypsum.[3,4,5] NIOSH, OSHA and ACGIH have set exposure limits for gypsum in the range of 5-15 mg/m^3.[6,7] Additional studies with animals will neither affect how gypsum is handled nor result in further limits on worker exposure and risks since these are already controlled with worker protective measures.

LD_{50} values have been measured in rodents and are high, ranging from 4415 mg/kg to 9934 mg/kg.[8] Furthermore, subchronic inhalation studies in rats produced only non-pathological effects dependent on the shape of the gypsum fibers rather than the chemical composition.[9,10] In a chronic inhalation study, gypsum produced only minor effects in the lungs of guinea pigs.[11]

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[1] Hoskins, J.A. 2001. Mineral fibres and health. Indoor Built Environ, 10(3-4):244-251.
[2] Ibid.
[3] Burilkov, T., and Michailova-Dotschewa, L. 1990. Dangers of exposure to dust extraction and production of natural gypsum. Wiss Umwelt, 0(2):89-91. Abstract from EMBASE 91094689.
[4] Einbrodt, H.J. 1988. The health risks by dusts of calcium sulfate (Ger.). Wiss Umwelt, 0(4):179-181. Abstract from EMBASE 89261036.
[5] Oakes, D., Douglas, R., Knight, K., Wusteman, M., and McDonald, J.C. 1982. Respiratory effects of prolonged exposure to gypsum dust. Ann Occup Hyg, 26(1-4):833-840.
[6] NIOSH. Undated-c. NPGD0308-NIOSH Pocket Guide to Chemical Hazards. Gypsum [CAS 13397-24-5]. Internet address: http://www.cdc.gov/niosh/npg/npgd0308.html. Last accessed on July 28, 2005.
[7] IPCS (International Programme on Chemical Safety). 2004a. Gypsum. International Cargo Safety Card (ICSC) No. 1215. Internet address: http://www.inchem.org/documents/icsc/icsc/eics1215.htm. Last accessed on July 28, 2005.
[8] Khodykina, T.M., Arkhangel'skii, V.A., and Kozeeva, E.E. 1996. Experimental studies on the effects of the dust of phosphogypsum and its derivatives (Russ.). Gig Sanit, 0(4):10-12.
[9] Clouter, A., Houghton, C.E., Bowskill, C.A., Hibbs, L.R., Brown, R.C., and Hoskins, J.A. 1997. Effect of inhaled fibers on the glutathione concentration and gamma-glutamyl transpeptidase activity in lung type II epithelial cells, macrophages, and bronchoalveolar lavage fluid. Inhal Toxicol, 9(4):351-367. Cited by Health Council of the Netherlands, Committee on Updating of Occupational Exposure Limits (2002).
[10] Clouter, A., Houghton, C.E., Hibbs, L.R., and Hoskins, J.A. 1998. Effect of inhalation of low doses of crocidolite and fibrous gypsum on the glutathione concentration and gamma-glutamyl transpeptidase activity in macrophages and bronchoalveolar lavage fluid. Inhal Toxicol, 10(1):3-
14. Cited by Health Council of the Netherlands, Committee on Updating of Occupational Exposure Limits (2002).
[11] Schepers, G.W.H., Durkan, T.M., and Delahant, A.B. 1955. The biological effects of calcined gypsum dust. AMA Arch Ind Health, 12:329-347.

The NTP's preliminary study recommendations include short-term pulmonary toxicity studies and comparative studies of intratracheal versus inhalation routes of administration—endpoints for which no scientifically validated toxicity study currently exists. It is also noted that the proposed studies are of relatively low priority given the low suspicion of toxicity. While the proposed tests would result in suffering and death for hundreds of animals, it is clearly very unlikely that they would yield any evidence of toxicity. We strongly urge the NTP to reconsider subjecting more animals to needless tests on this ubiquitous substance.

N-Methyl-3-oxobutanamide

N-Methyl-3-oxobutanamide is among five high production chemicals nominated by the NCI that do not meet the criteria for inclusion in the HPV Challenge Program. NCI cites concern that this chemical may undergo metabolic transformation to more reactive species and recommends *in vitro* and *in vivo* genotoxicity studies as well as absorption, distribution, metabolism, and excretion (ADME) studies. We support the NTP's decision to drop ADME studies from their preliminary recommendations; however, *in vivo* genotoxicity studies are listed among the recommendations. We urge the NTP to limit any genotoxicity testing to *in vitro* methods as adopted by the OECD.[1,2,3,4,5] The supporting document for this nomination cites negative results in Ames tests both with and without S-9 activation.[6] In addition, the related chemical, N,N-dimethylacetoacetamide, is sponsored by the Color Pigment Manufacturer's Association in the Extended HPV (EHPV) Program. The proposed test plan includes bacterial Ames' and chromosomal aberration tests.[7] We urge the NTP to change its preliminary recommendations to defer proposed tests pending voluntary data submission through the EHPV Program.

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[1] OECD (Organization for Economic Co-operation and Development). 1997. Test Guideline 471: Bacterial Reverse Mutation Test.
[2] OECD. 1997. Test Guideline 473: *In Vitro* Mammalian Chromosome Aberration Test.
[3] OECD. 1997. Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Test.
[4] OECD. 1986. Test Guideline 479: Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells.
[5] OECD. 1986. Test Guideline 479: Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vitro*.
[6] European Commission (2000a) N-Methyl-3-oxobutyramide 20306-75-6]. IUCLID Dataset. [http://ecb.jrc.it/IUCLID-Data-Sheet/20306756.pdf] Searched October 19, 2004
[7] Color Pigments Manufacturers Association, Inc. 2003. Test Plan For N,N-Dimethylacetoacetamide. Internet Address: http://www.epa.gov/chemrtk/dmaa/c14993.pdf