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NIEHS/NTP
P.O. Box 12233
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***SUBMITTED ELECTRONICALLY TO:*** masten@niehs.nih.gov

Dear Dr. Masten:

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our over 750,000 members in response to your *Federal Register* notice of July 25, 2001, soliciting public comments on substances nominated to the National Toxicology Program (NTP) for toxicological studies and on the testing recommendations made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC).

## GENERAL COMMENTS

PETA questions both the wisdom and the value of the NTP's active solicitation of chemical nominations for toxicological evaluation. It has been our experience that efforts to fill perceived "data gaps" lead, almost invariably, to a "check-the-box" exercise using an arbitrary series of unvalidated animal tests. This approach not only ignores the many other sources of scientifically relevant data upon which an assessment of potential human health risks could be more appropriately based, but also results in a great deal of unnecessary chemical-testing, at a high cost to both animals and U.S. taxpayers.

This fact has been clearly acknowledged by other U.S. federal agencies, including the Environmental Protection Agency (EPA). In a letter to all participants in its high production volume (HPV) chemical-testing program, former EPA Deputy Assistant Administrator, Susan Wayland, wrote: "In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested." We therefore urge the NTP to follow this example and develop a more "thoughtful" approach to the study of chemicals that does not rely on an arbitrary check-list of animal-based toxicity tests.

In regard to specific categories of substances nominated by the ICCEC for further evaluation, it is remarkable that fully half of the chemicals are natural plant extracts, many of which have been in widespread use for centuries or more without evidence of toxicity. If the objective in soliciting nominations is truly to identify "those substances of greatest concern for public or occupational health based on the extent of human exposure and/or suspicion of toxicity," we strongly

advise the NTP to reevaluate and substantially revise the ICCEC's current set of chemical nominations. As we pointed out in a similar set of comments to the NTP dated January 19, 2001, it is an unconscionable waste of both taxpayer dollars and animal lives to subject natural, plant-derived substances—such as grape seed and pine bark extracts—to check-the-box animal testing for no other reason than a perceived "limited availability of toxicity information." This unnecessary and inappropriate testing proposal should be withdrawn immediately.

We are also very concerned to see that ICCEC has recommended additional testing of three classes of HPV chemicals, despite the fact that these substances are already covered under the EPA's HPV chemical-testing program. The potential for duplicative and unnecessary animal-testing to occur as a result of a parallel NTP evaluation of these chemicals is high, and unacceptable. We therefore call on the NTP to forego the testing of all HPV chemicals until the EPA's HPV chemical-testing program has been completed, and the resultant data are fully analyzed and made available for public review.

## SPECIFIC COMMENTS

- **Bladderwrack** [68917-51-1 + 84696-13-9]

  Bladderwrack is one of numerous varieties of seaweed that has been consumed by human societies for centuries—as far back as 3000 B.C.—without evidence of toxicity. This plant's ability to stimulate the thyroid gland has also been well established. In fact, bladderwrack's anti-hypothyroid properties have been utilized medicinally since the early 1700's, with no known reproductive or other harmful effects, or evidence of adverse drug interactions. We would suggest that the perceived "limited availability of toxicity information" noted by the National Cancer Institute (NCI) and ICCEC is merely a reflection of this plant's inherent lack of toxicity. As such, the proposed evaluation of bladderwrack for subchronic toxicity and reproductive parameters is unnecessary and inappropriate, and should therefore be abandoned.

- **Grape seed and pine bark extracts**

  Grape seed and pine bark extracts are very similar in that they contain a unique type of bioflavonoids called proanthocyanidins (PCO), which are synergistic with ascorbic acid, thereby strengthening the cellular membranes and protecting cells from oxidative damage. As with other herbal extracts, millions of people have used grape seed and pine bark extracts (since at least 1970 in Europe) without any reported adverse health effects. In addition, a literature review revealed that mutagenicity, carcinogenicity and developmental toxicity assays have been conducted on grape seed and pine bark extracts as well as their active ingredient, PCO, and all have been found to be non-toxic, even at extraordinarily high doses.[1] As such, there can be no justification for the conduct of additional animal studies of these already well characterized and inherently non-toxic plant extracts.

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[1] Masquelier J. The fate of total flavanolic oligomers (OFT) extracted from *'Vitis vinifera L.*' in the rat. *European Journal of Drug Metabolism and Pharmacokinetics* 1978;1:15-30.

- **Epigallocatechin-3-gallate (green tea)** [989-51-5]

  Green tea is another natural product that has been used for literally thousands of years without evidence of toxicity. Yet once again, the NCI and ICCEC have proposed to subject animals to genotoxicity and subchronic toxicity studies of an innocuous plant product—green tea extract—for no other reason than a perception that there is "limited available toxicity information." This justification is woefully inadequate, and illustrates PETA's previously articulated concern about the wisdom and the value of the NTP's active solicitation of chemical nominations for toxicological evaluation. Moreover, the ICCEC's recommendation for genotoxicity testing contradicts its own acknowledgement of the chemopreventive properties of green tea. It is absurd that a substance that is so widely recognized to exert anti-carcinogenic effects would be nominated for evaluations of genetic toxicity. This frivolous and unnecessary testing being called for by federal agencies in general, and the NCI in particular, would be an unconscionable waste of both taxpayer dollars and animal lives if allowed to proceed. Accordingly, the proposed testing recommendations should be withdrawn.

  However, should the NTP disagree with our assessment and permit the proposed genotoxicity testing to proceed, we trust that such testing would be carried out using the internationally accepted *in vitro* method in lieu of an *in vivo* assay. As you are no doubt aware, the *in vitro* method is not only capable of identifying the effects of genetic toxicity, but has been found to be *more* sensitive to these effects than animal models. For this reason, the *in vitro* assay has become the preferred (and required) genotoxicity screening method in European countries such as the United Kingdom and Germany. You may also be aware that, in an October 1999 agreement with animal protection organizations, the U.S. Environmental Protection Agency stated that companies "are encouraged to use *in vitro* genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use."

- **Cylindrospermopsin** [14345-90-8]

  We concur that the presence of cylindrospermopsin in drinking water poses an unacceptable public health risk in view of the demonstrated high acute toxicity of this bacterial toxin. However, we do not believe that the recommendation by the ICCEC and NIEHS for a complete toxicological characterization of this toxin—including conducting lengthy and non-validated chronic toxicity and carcinogenicity tests in animals—is an adequate or appropriate response to this problem. Public health would be much better served by a proactive water treatment program for cylindrospermopsin elimination. Treatment methods have been investigated in order to degrade the toxin, including chlorination, ozonation and the use of UV photocatalysis. It has been shown that all of these techniques have the ability to degrade cylindrospermopsin.[2]

- **Metalworking fluids**

  A literature review of four randomly selected metalworking fluids—1,1,2-Trichloro-1,2,2-

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[2] Kuiper-Goodman T, Falconer IR & Fitzgerald J. Human health aspects. In: *Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management* (Eds. I. Chorus & J. Bartram). London, UK: World Health Organisation. 1999. pp. 113-153.

trifluoroethane [76-13-1], Polypropylene glycol [25322-69-4], Tetraethylene glycol [112-60-7] and 1,2,3-Benzotriazole [95-14-7]—revealed a wealth of existing human and laboratory data concerning the toxicity of these substances following acute, subacute, subchronic and chronic exposures, as well as data concerning the chemicals' mutagenicity and carcinogenicity (see Table 1). These existing data more than satisfy the ICCEC's recommendations for toxicological studies. In view of this fact, together with the fact that metalworking fluids will also be subjected to a thorough evaluation under the EPA's HPV chemical-testing program, additional testing of these chemicals by the NTP is unnecessary and inappropriate, and the proposed testing recommendations should be withdrawn.

- **2-Ethylhexyl-*p*-dimethylaminobenzoic acid** [21245-02-3]

  A review of the technical literature for 2-Ethylhexyl-*p*-dimethylaminobenzoic acid revealed that considerable research on this substance has been conducted by the World Health Organization's International Agency for Research on Cancer (IARC). These include evaluations of acute toxicity in dogs, subacute and reproductive toxicity in rats, and chronic toxicity and carcinogenicity in mice (see Table 2). These existing data are considerably more extensive than those sought by the ICCEC (which has recommended reproductive, developmental and subchronic toxicity testing via the dermal route of exposure). Moreover, the fact that 2-Ethylhexyl-*p*-dimethylaminobenzoic acid will also be subjected to an evaluation under the EPA's HPV chemical-testing program should preclude any testing by the NTP for the same endpoints as in the HPV program. Finally, with regard to the ICCEC's desire for an assessment of phototoxicity, these data may be obtained *in vitro* using the 3T3 Neutral Red Uptake (3T3 NRU) phototoxicity assay. This test has been thoroughly validated by the European Center for the Validation of Alternative Methods (ECVAM), and is now the default method for phototoxicity testing in Europe. Any assessment of photo-toxicity by the NTP should be carried out using available *in vitro* methods and should <u>not</u> involve the use of animals.

- **Polybrominated diphenyl ethers**

  As yet another class of substances to be evaluated under the EPA's HPV chemical-testing program, polybrominated diphenyl ethers should not undergo further assessment by the NTP until all new and/or existing data generated through the EPA program are brought forward and fully analyzed. If, after such a review has been completed, the NTP considers that additional data are still needed, it could at that time issue a more informed set of testing recommendations for public review and comment.

  In the event that future testing of polybrominated diphenyl ethers is deemed to be necessary, we submit the following specific comments and recommendations. Polybrominated diphenyl ethers are all very similar, both structurally and in terms of chemical and toxicological properties, and may therefore appropriately be evaluated as a category of related substances rather than as individual chemicals. We strongly recommend that the NTP follow this approach wherever possible in its testing strategies, not only for the sake of minimizing costs—both financial and in terms of animal suffering and death—but in order to harmonize its testing practices with those of other federal agencies. You may be aware, for example, that the EPA has directed all participants in its HPV chemical-testing program to "…maximize the use of scientifically

appropriate categories of related chemicals and structure activity relationships."

With this in mind, we call your attention to the wealth of existing human and laboratory data concerning the toxicity of polybrominated diphenyl ethers, in general. The data available on these chemicals include evaluations of mutagenicity, carcinogenicity, acute, subacute, reproductive and developmental toxicity, among other endpoints (see Table 3). These existing data are considerably more robust than those sought by the ICCEC (which has recommended subchronic and chronic toxicity testing of selected individual congeners), and should be more than sufficient to permit the NTP to make sound predictions regarding the toxicity of the specific substances identified in the ICCEC's testing recommendations.

With respect to the ICCEC's recommendation that polybrominated diphenyl ethers be further assessed using a developmental neurotoxicity test (DNT), we cannot overstate our opposition to this proposal. As you may be aware, numerous scientists have gone on record stating that the current DNT test guideline has not been validated (i.e., shown to be reliable, reproducible and relevant for its intended purpose), and that its use for regulatory purposes is premature. In fact, the EPA's own Scientific Advisory Panel concluded that "developmental neurotoxicity testing must be further refined to develop more sensitive endpoints which are relevant to significant outcomes in humans" and that "the current form of the DNT guideline is not a sensitive indicator of toxicity to the offspring."[3] In addition, a panel of experts at the 18th International Neurotoxicology Conference—including three EPA officials—acknowledged that they did not know how to interpret the results of the DNT. They also agreed with a National Research Council report that questioned whether the rat was the correct "model" for the DNT.[4] One EPA official even stated that the agency's reliance on rats was "like being in a bad marriage—you know you should get out but you don't because there is so much history there."[5] As such, we strongly object to the inclusion of the DNT among the ICCEC's testing recommendations, and urge the NTP to reject all present and future calls to utilize this flawed and non-validated test method.

- **Methyl tetrahydrofuran** [96-47-9]

The only rationale for the proposed testing of methyl tetrahydrofuran is a stated "lack of toxicity information." However, a literature review revealed an abundance of existing human and laboratory toxicity data, including studies conducted by the NTP itself. These include assessments of acute, subchronic and chronic inhalation toxicity, deveopmental toxicity, mutagenicity (*in vitro* and *in vivo*) and carcinogenicity (see Table 4). These existing data vastly exceed the ICCEC's recommendations for short-term and genotoxicity testing. As such, additional testing of these chemicals by the NTP is unnecessary, and the proposed testing recommendations should be withdrawn.

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3 EPA Scientific Advisory Panel. A set of scientific issues being considered by the agency in connection with the use of FQPA 10X safety factor to address special sensitivity of infants and children to pesticides: Final Report, March 1998.

4 NRC. *Pesticides in the Diets of Infants and Children.* National Academy Press: Washington DC. 1993.

5 Rice D. Public comments at *18th International Neurotoxicology Conference.* Colorado Springs, Colorado, 23-26 September 2000.

## CONCLUSIONS

A thorough review of the ICCEC's current testing recommendations only serves to reinforce the concerns expressed in our opening remarks: that NTP's active solicitation of chemical nominations promotes sloppy toxicology, which results in a great deal of cruel and unnecessary animal-testing. It is clear that neither the parties responsible for submitting chemical nominations, nor the ICCEC itself, have made any meaningful effort to review the technical literature to determine the availability of existing data prior to recommending further chemical-testing. Althouth we trust that our comments have amply demonstrated the inappropriateness of much of the proposed testing in this instance, it is unconscionable that the responsibility for conducting a proper literature review appears to have been foisted upon the public, rather than resting with the ICCEC, where it belongs. In the future, we hope that the ICCEC will be more circumspect in its review of chemical nominations to prevent the submission of inappropriate testing recommendations such as those in its current report.

Sincerely,

Troy Seidle, B.Sc.
Research Associate
Research & Investigations Department

cc: Dr. K. Olden, NIEHS Director
Ms. E. Stolpe, CEQ Associate Director for Toxics

## Table 1: Availability of Literature for Metalworking Fluids

| Fluid Type | Author | Source | Endpoint(s) |
|---|---|---|---|
| Polypropylene glycol [25322-69-4] | Gosselin *et al*., 1976 | Clinical Toxicology of Conventional Products, 4th ed. | probable oral lethal dose (human) |
| | Patty, 1963 | Industrial Hygeine and Toxicology, Vol II, 2nd ed. New York: Interscience Publishers | acute oral toxicity (human & rodent); acute dermal toxicity; subchronic toxicity; pharmacokinetics & toxicokenetics; dermal & ocular irritation; |
| Tetraethylene glycol [112-60-7] | Bushy Run Research Center, 1987 | EPA Doc. No. 8EHQ-1187-0693 | acute oral, dermal & inhalation toxicity |
| | Bushy Run Research Center, 1987 | EPA Doc. No. 88-870000065, Fiche No. OTS0513409 | mutagenicity (*in vitro* & *in vivo*) |
| | Bushy Run Research Center, 1987 | EPA Doc. No. 8EHQ-0987-0693, Fiche No. OTS0513409 | mutagenicity |
| | Clayton *et al*., 1981/2 | Patty's Industrial Hygeine & Toxicology, Vol. 2A-C. New York: John Wiley Sons. | acute oral & inhalation toxicity; subacute toxicity; |
| 1,2,3-Benzotriazole [95-14-7] | Ciba-Geigy Corp, 1982 | EPA Doc. No. 86-930000383; Fiche No. OTS0538207 | dermal sensitization |
| | Clayton *et al*., 1981/2 | Patty's Industrial Hygeine & Toxicology, Vol. 2A-C. New York: John Wiley Sons. | acute oral toxicity (rat & mouse); chronic toxicity (rodent) |
| | Eastman Kodak Co., 1969 | EPA Doc. No. 86-890000208; Fiche No. OTS0516745 | subchronic toxicity |
| | NTP/NCI, 1978 | Technical Rpt Series No. 88 DHEW Pub No. (NIH) 78-1338 | carcinogenicity |
| | Polaroid Corp., 1989 | EPA Doc. No. 86-890001039; Fiche No. OTS0520182 | acute toxicity; dermal & ocular irritation |
| | Sherwin Williams Co. | EPA Doc.No. 86-890000599, Fiche No. OTS0520638 | subchronic toxicity |
| | | EPA Doc. No. 88-930000385; Fiche No. OTS0538209 | mutagenicity (*in vitro* & *in vivo*) |

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|---|---|---|---|
| 1,1,2-Trichloro-1,2,2-trifluoroethane [76-13-1] | ACGIH, 1971 | Documentation of the TLV for Substances in Workroom Air, 3rd ed. Cincinnati, OH: ACGIH. | acute exposure (human); acute inhalation toxicity (rodent); subacute toxicity (rodent) |
| | ACGIH, 1986 | Documentation of the TLV & Biological Exposure Indices, 5th ed. Cincinnati, OH: ACGIH. | mutagenicity |
| | ACGIH, 1991 | Documentation of the TLV & Biological Exposure Indices, 6th ed. Volumes I, II, III. Cincinnati, OH: ACGIH. | repeat dose exposure (human); subacute toxicity (rodents & dog); developmental toxicity (rabbit); dermal & ocular irritation |
| | USEPA, 1983 | EPA-600/58-82-002F | chronic inhalation toxicity (rodent) |
| | WHO, 1990 | Environmental Health Criteria 113: Fully Halogenated Chloroflurocarbons p.66 | subchronic toxicity (rodent & dog) |

## Table 2: Availability of Literature for 2-Ethylhexyl-*p*-dimethylaminobenzoic acid

| Author | Source | Endpoint(s) |
|---|---|---|
| IARC, 1978 | Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Geneva: WHO/IRAC. p.V16 255. | chronic toxicity/carcinogenicity (mouse); acute toxicity (dog); subacute toxicity (rat); reproductive toxicity (rat); metabolism & pharmacokinetics |

## Table 3: Availability of Literature for Polybrominated Diphenyl Ethers

| Chemical | Author | Source | Endpoint(s) |
|---|---|---|---|
| Decabromobiphenyl Ether [1163-19-5] | EPA, 2000 | IRIS Substance File List http://www.epa.gov/ngispgm2/iris | carcinogenicity |
| | IARC, 1999 | Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Geneva: WHO/IRAC. p.71 1368. | carcinogenicity |
| | Norris *et al*., 1975 | Environ Health Perspect;11:153-61 | acute oral toxicity; dermal absorption; reproductive toxicity |
| | NTP, 1986 | Technical Report Series No. 309, NIH Pub. No. 86-2565, p.19 | Acute oral toxicity (rat); subacute toxicity (rat); developmental toxicity (rat); mutagenicity (*in vitro* & *in vivo*); carcinogenicity (rat & mouse); dermal irritation (rat & rabbit) |
| Pentabromophenol [608-71-9] | Clayton *et al*., 1994 | Patty's Industrial Hygeine & Toxicology. 4th ed. New York: John Wiley & Sons Inc., 1617. | subacute toxicity |
| | Geiger *et al*., 1988 | Acute Toxicities of Organic Chemicals to Flathead Minnows. Vol IV. Superior Wisconsin: University of Wisconsin-Superior. | ecotoxicity (acute) |
| | Szymanska *et al*., 1995 | Int J Occup Med Environ Health; 8(3):245-54 | acute toxicity; subacute toxicity |
| Hexabromobenzene [87-82-1] | Carlson, 1978 | Biochem Pharmacol;27(3):361-3 | subacute toxicity |
| | Courtney *et al*., 1984 | J Environ Sci Health;19(1):83-94 | developmental toxicity |
| | Dupont De Nemours, 1970 | EPA Doc. No. 86-870001063, Fiche No. OTS0514966 | acute inhalation toxicity |
| | Mendoza *et al*., 1977 | Toxicol Appl Pharmacol; 41(1): 127-30 | subchronic toxicity |

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| | Yamaguchi, 1988 | Archives of Environ Contam & Toxicol;17(6):807-12 | acute toxicity |
| 2,4,5,2',4',5'-Hexabomo-biphenyl [59080-40-9] | Cook *et al*., 1978 | Environ Res;15(1):82 | reproductive toxicity |
| | Dent *et al*., 1979 | Toxicol Appl Pharmacol;38(2): 237 | acute toxicity |
| | IARC, 1972-Present | Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Geneva: WHO/IRAC. p.V18 114-17 | subchronic toxicity; developmental toxicity; chronic toxicity & carcinogenicity; neurotoxicity; immunotoxicity |
| | Lucier *et al*., 1978 | Dev Toxicol Energy-Relat Pollut; 188 | developmental toxicity |
| | McCormack *et al*., 1979 | Drub Metab Dispos;7(5):252 | subchronic toxicity |
| 2,3,4,5,6-Pentapromo-toluene [87-83-2] | Zeiger *et al*., 1987 | Environ Mutagen;9:1-110 | mutagenicity (*in vitro*) |

## Table 4: Availability of Literature for Methyl tetrahydrofuran

| Author | Source | Endpoint(s) |
|---|---|---|
| ACGIH, 1991 | Documentation of the TLV & Biological Exposure Indices, 6th ed. Volumes I, II, III. Cincinnati, OH: ACGIH. | acute inhalation toxicity (rabbit); subchronic inhalation (rat); dermal irritancy (rabbit); developmental toxicity (rodent) |
| Browning, 1965 | Toxicity & Metabolism of Industrial Solvents. New York: American Elsevier. | acute oral toxicity (cat); acute inhalation toxicity (rodent & dog) |
| Gosselin *et al*., 1984 | Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams & Wilkins, p.II-408. | acute oral toxicity (rabbit) |
| Horiguchi *et al*., 1981 | Seikatsu Eisei;25(4):176-7 | subchronic inhalation toxicity (rodent) |
| Katahira *et al*., 1982 | Japanese Journal of Industrial Health;24(4):379-87 | subchronic inhalation toxicity (rodent) |
| | Sangyo Igaku;24(4):373-8 | acute oral toxicity (rodent) |
| Mast *et al*., 1992 | Fundam Appl Toxicol;18(2):255-65 | developmental toxicity (rat & mouse) |
| Mortelmans *et al*., 1986 | Environ Mutagen;9:1-119 | mutagenicity (*in vitro*) |
| NTP, 1984 | Fiscal Year 1984 Annual Plan, p.82; NTP-84-023 | mutagenicity (*in vivo* & *in vitro*) |
| NTP, 1998 | NIH Publication No. 98-3965 | chronic inhalation toxicity & carcinogenicity |