PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

September 11, 2002

Chair and Members
NTP Board of Scientific Counselors
c/o Dr. Mary Wolfe
National Institute of Environmental Health Sciences
P.O. Box 12233, MD A3-07
Research Triangle Park, NC 27709

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), a nonprofit animal protection organization with over 750,000 members and supporters, for consideration by the National Toxicology Program (NTP) Board of Scientific Counselors at its meeting of September 17-18, 2002.

NTP Draft Strategy for Using Genetically Altered Animals in Carcinogen Identification

Pursuant to the ICCVAM Authorization Act of 2000, 42 U.S.C. § 2851 *et seq*, PETA objects to the NTP's decision to "refine its testing paradigm to formally include [transgenic mouse models] in the battery of tests used to evaluate agents for carcinogenicity," and strongly urges the NTP Board of Scientific Counselors to recommend against the use on the grounds that these test methods have not undergone adequate or successful scientific validation for this purpose.

As the agency that houses the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), it is untenable that the NTP would not be aware of the ICCVAM Act's requirement that, "each federal agency ... shall ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method." 42 U.S.C. § 2851-4(c). As discussed below, the transgenic rodent "models" identified by the NTP in its June 1, 2002 draft document, "The Use of Genetically Altered Animals in Carcinogen Identification by the National Toxicology Program," (1) clearly fall within the scope of the ICCVAM Act's definition of a "new or revised acute or chronic toxicity test method," and (2) do not satisfy internationally accepted criteria for "validated" test methods.

1. The *p53*+/-, *Ras*H2, Tg.AC, and all other transgenic rodent assays for carcinogenicity, clearly qualify as "new or revised" test methods under the ICCVAM Act in that no standardized and internationally accepted protocol or test guideline exists for any one of these methods (OECD 2002; ICH 1997), and efforts to standardize and refine these methods continue to this day (ILSI, unpublished data). Moreover, the NTP's current proposal comes well after the passage of the ICCVAM Act in 2000, which therefore places these test methods well within the Act's jurisdiction.

2. Validation is the process by which the reliability and relevance of a test method are established for a specific purpose. Reliability refers to the

extent of reproducibility of a test's results within and among laboratories, when performed using the same protocol, and relevance is the extent to which a test correctly measures or predicts the biological effect of interest in the species of interest (ICCVAM 1997). Criteria for test method validation that have achieved international acceptance include those of ICCVAM (1997) and its sister organization in Europe (ECVAM), and the Organization for Economic Cooperation and Development (OECD 1997). To date, the transgenic assays proposed for use by the NTP fail to satisfy even the minimal criteria set forth by U.S. federal agencies represented on ICCVAM (van Zeller & Combes 1999). As but a few examples:

- *p53*+/-: Despite the belief that loss of expression, or function, or both *p53* alleles plays a critical role in neoplasia, heterozygous *p53*-deficient mouse "bioassays" appear to be unresponsive to non-genotoxic carcinogens such as *N*-methyl-*o*-acrylamide and reserpine, and mutagenic non-carcinogens such as *p*-anisidine (Tennant et al. 1995). This suggests that their utility may be limited to the detection of genotoxic carcinogens, for which more rapid, economical and internationally accepted *in vitro* assays are already available and in widespread use.

- *Ras*H2: As many as six copies of the human *ras* gene and its promoter are integrated into the genome of transgenic *Ras*H2 mice. This can lead to a statistically significant increase in spontaneous tumors in these mice — ranging from 10 to 50 percent, according to the International Life Sciences Institute (unpublished data). Such increases in tumor incidence hinder the assessment of the carcinogenic potential where borderline results are obtained, which seriously limits the assay's reliability. The sensitivity and relevance of this assay is also questionable, given its reported inability to detect the known human carcinogen, cyclophosphamide (Yamamoto et al. 1998). In addition, the U.K. Committee on Carcinogenicity of Food, Consumer Products and the Environment concluded that there does not appear to be a mechanistic rationale behind the use of this assay for screening potential human carcinogens, since the relevance of integrating multiple copies of the *ras* gene into the CB6F1 strain with respect to the carcinogenic process in humans is questionable (CCFCPE 1997).

- Tg.AC: The insertion of a mutated v-Ha-*ras* gene into the FVB/N mouse strain has yielded a transgenic rodent with genetically initiated skin that is responsive to tumor promoters in the absence of a mutagenic initiating event (Leder et al. 1990). Although it was initially reported that animals would remain morphologically and physiologically normal in the absence of chemical induction, subsequent reports indicate that the incidence of spontaneous tumor formation is a significant confounder, both in homozygous (Wright et al. 1995) and hemizygous (Holden et al. 1998) mice. Tennant and colleagues (1995, 1999) further observed that the Tg.AC assay is incapable of distinguishing genotoxic complete carcinogens from agents that have only tumor promoting capability, which renders it invalid for these purposes.

- Similar observations and conclusions have been made with respect to other transgenic rodent carcinogenicity assays, such as the *XPA*-deficient model (DeVries et al. 1997; VanSteeg et al. 1998).

Data obtained from any assay must, at the very least, be reproducible. However, the transgenic rodent carcinogenicity assays proposed for use by the NTP are known to produce unpredictable responses, the mechanisms of which are not understood. The examples cited above are but a handful of the growing number of published studies that reinforce this finding. Similarly, the relevance of transgenic rodent assays to human health risk assessment is another open question. Genetic manipulation does nothing to resolve the ever-present scientific limitations concomitant to extrapolation between species, dose levels, exposure routes and time frames (Combes 1997; Clewell et al. 2002). The NTP's own studies have found that rat and mouse bioassays yield concordant results (both positive and negative) only 70 percent of the time (Zeiger 1987), and since these species are more biologically similar to one another than either is to humans, one must assume that rodent-human concordance would be well below 70 percent. A case in point is an analysis by Marselos and Vainio (1991) of 182 pharmaceutical agents — including 20 known human carcinogens — of which rodent bioassays correctly identified only seven (35 percent sensitivity). In view of these findings, it is not surprising that data from toxicity studies conducted in mice rarely alter regulatory assessments of human risk from chemical exposure (Battershill & Fielder 1998).

The NTP's decision to include clearly non-validated test methods in its battery of assays used to evaluate agents for carcinogenicity violates both the letter and spirit of the ICCVAM Authorization Act, and renders the NTP liable to legal action if this decision is not reversed.

ICCEC Testing Recommendations

PETA has reviewed and commented on a number of the testing recommendations made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC). In so doing, we have documented numerous examples of what can only be described as "thoughtless toxicology," including the following:

- Recommendations calling for the almost exclusive use of non-validated animal tests, even where validated non-animal assays are available for the same endpoint(s).

- Recommendations calling for additional — apparently endless — testing of even such thoroughly characterized substances as methanol, hexavalent chromium and turpentine, among others.

- Recommendations calling for *de novo* testing on endpoints for which data were already publicly available or could easily be inferred through bridging or batching of substances with similar molecular structure and chemical activity.

- Recommendations calling for *de novo* testing of high production volume (HPV) chemicals, the toxicities of which are already being evaluated under an Environmental Protection Agency-sponsored program.

- Recommendations calling for extensive testing of such innocuous natural substances as algae, seaweed, green tea, lemon oil, and numerous others.

As we have communicated many times in the past, PETA questions both the wisdom and the value of the NTP's solicitation of chemical nominations for toxicological evaluation. The above examples (which are expanded upon in the attached correspondence) epitomize sloppy toxicology: a "check-the-box" exercise based on non-validated animal tests, which 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.

Conclusion

In summary, PETA strongly urges the NTP to abandon the ICCEC's arbitrary recommendations and refocus its efforts "to develop and validate assays and protocols, including alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing," per the NIEHS implementation guidelines developed pursuant to the NIH Revitalization Act of 1993 § 463A(b)(3), while at the same time ensuring that the test methods it relies upon have been "determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method," per the ICCVAM Authorization Act of 2000.

Respectfully submitted,

Troy Seidle
Science Policy Advisor
757.622.PETA x 1462
TroyS@peta.org

Lori Kettler
Counsel
757.622.PETA x 1614
LoriK@peta.org

Attach.

cc: Dr. K. Olden
Dr. C. Portier

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PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

August 12, 2002

Dr. Kenneth Olden
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Mail Drop E1
Research Triangle Park, NC 27709

Dear Dr. Olden:

On behalf of our 750,000 members, People for the Ethical Treatment of Animals (PETA) is appealing to you once again to stop yet another National Toxicology Program (NTP) testing scheme that will cause suffering and death to untold numbers of animals who will be used to re-test already well-characterized chemicals.

On July 22, we wrote to you regarding the NTP's plan to subject more animals to toxicity tests for hexavalent chromium, a well-studied chemical and known human carcinogen. On July 8, we wrote regarding the NIEHS' proposal to retest methanol on animals. Last year, on January 19, May 3, and September 23, we submitted comments on the NTP's proposals to test a number of substances, including natural substances (e.g., grape seed extract), many already well-characterized substances, including metal-working fluids (in a number of cases, data from NTP-conducted studies had been overlooked), and chemicals already covered under the EPA's high production volume (HPV) chemical-testing program.

Although in some cases we submitted lengthy documentation of existing data that the NTP had ignored when it proposed further animal testing and also documented the complete lack of consideration of *in vitro* technologies, all of our comments have gone unanswered and, to the best of our knowledge, no changes were made in the NTP's final recommendations. We therefore turn to you, yet again, to request your intervention in these endless animal testing proposals that emanate from the NTP, that lack all common sense as well as scientific merit.

We are addressing these comments directly to you because your comments at the 2002 Summer Toxicology Forum indicated that you were unaware of — and concerned about — the overlap between the NIEHS' proposal to study methanol with the EPA's ongoing studies. It is these redundant testing proposals that lead to the use by the NTP of an exorbitant number of animals.

Our comments today address the NTP's June 12, 2002, *Federal Register* notice entitled, "Announcement of and Request for Public Comments on Substances Nominated to the NTP for Toxicological Studies and on Study Recommendations Made by the NTP Interagency Committee for Chemical Evaluation and Coordination." Our comments here will reiterate issues we have raised previously in written responses to similar NTP *Federal Register* notices, and then provide an example of wastefulness specific to this particular proposal.

The most glaring omission in the June 12 notice is the complete and total failure to consider the use of *in vitro* technology. The notice states: "The NTP is interested in identifying appropriate new animal models for mechanistic based research, including transgenic or knockout mice, and welcomes comments regarding the use of specific animal models to address scientific questions relevant to the nominated substances and studies under consideration."

As a result, the *Federal Register* notice calls for a large number of animal tests, including tests for the following endpoints: acute, chronic, reproductive and developmental toxicity, carcinogenicity, genotoxicity, hypersensitivity, neurotoxicity, immunotoxocity, and pulmonary toxicity. Animals proposed for use include non-human primates. A number of these endpoints could be studied *in vitro* and, since the NTP is not bound by the restrictions of regulatory agencies, it has absolutely no excuse for failing to consider and encourage the use of *in vitro* assays for these endpoints.

For example, the European Centre for the Validation of Alternative Methods recently validated an *in vitro* (rodent) embryonic stem cell test for the assessment of embryotoxicity. The ability to assess a major landmark in development toxicity *in vitro* can greatly reduce the number of animals killed in the assessment of this endpoint (i.e., by treating embryotoxicants as developmental toxicants without requiring a full developmental toxicity study). Yet this method and strategy have been completely overlooked by the NTP, as have other *in vitro* methods.

From a scientific standpoint, it is ridiculous that the NTP continues to ignore the recommendations of its own scientists by repeatedly calling for more animal carcinogenicity data. As we have pointed out repeatedly in previous comments, the lack of relevance and reliability of rodent bioassays for this endpoint has been extensively documented (see, for example, our comments to you dated July 22). Yet the NTP insists on requesting an endless number of these useless tests.

As a further note, there is absolutely no reason for the NTP to be requesting further testing at this point on substances that are part of the EPA's high production volume (HPV) chemical testing program. This represents the height of redundancy and wastefulness, and simply illustrates further the NTP's complete lack of concern with the number and suffering of animals it is condemning to death.

For the purposes of these comments, we would like to focus on just one of the chemicals the NTP is suggesting requires further animal studies. The *Federal Register* notice lists the substance turpentine as nominated by United Auto Workers' International Union for further chronic toxicity and carcinogenicity studies, with the rationale given that there is "insufficient chronic toxicity information" for this chemical. The NTP's Interagency Committee for Chemical Evaluation and Coordination concurs.

Turpentine oil is a flammable, toxic liquid that has long been known to cause serious adverse health effects in many species, including humans. Turpentine is a dermal, ocular, and respiratory irritant. Turpentine causes central nervous system depression and harmful gastrointestinal, urinary tract, and respiratory effects. It has also been implicated as a reproductive toxicant. Exposure to turpentine may cause nausea, vomiting, diarrhea, headache, dizziness, bladder irritation, chest pain, visual disturbances, choking, dyspnea, pulmonary edema, convulsions, fever, tachycardia, and death due to respiratory failure. The Occupational Safety and Health Administration, the National Institute for

Occupational Safety and Health, and the American Conference of Governmental Industrial Hygienists all concur that an acceptable time-weighted average exposure limit over a full workday is 100 ppm (560 mg/m^3).

Chronic effects in humans have been observed in employees who routinely come into contact with turpentine. Chronic skin exposure to turpentine may produce a hypersensitivity reaction, with dermatitis and/or eczema (1). A case-control study of workers in particleboard, plywood, sawmill, and formaldehyde glue factories showed a statistically significant association between chronic exposure (longer than 5 years) to terpenes (the principal component of turpentine) and the development of respiratory tract cancers (2). Occupational studies of workers with chronic exposures to turpentine have demonstrated associations between turpentine and adverse reproductive and developmental effects (3,4).

Many acute, chronic, and mechanistic animal studies have been conducted with turpentine. Turpentine has been identified as an eye, mucous membrane, and skin irritant and a central nervous system depressant in animals. Dermal application of turpentine has produced tumors in some species of animals in some experiments. Arterial, cardiac, and skeletal muscle lesions caused by turpentine-induced renal alterations were observed in rabbits (5). Adverse neurological effects have been observed in chronic animal studies (6). Liver damage has also been observed in animal studies (7,8).

Clearly, turpentine is associated with many chronic and acute hazards. Additional animal data are not needed to attempt to model how poisonous this chemical is to humans. Efforts need to be directed toward improved medical surveillance in the workplace as well as towards administrative and engineering controls of turpentine exposure. Steps should be taken to prevent worker exposure to this chemical and to reduce exposure well below permissible levels. For example, turpentine can often be replaced in the occupational setting by the petroleum product white spirit. Research efforts would be best directed toward occupational epidemiological studies of workers who are frequently exposed to turpentine such as woodworkers and painters, increased medical monitoring, and clinical observations of accidental or intentional turpentine poisoning.

Dr. Olden, surely you will agree that no more animals need to die to re-test turpentine, welding fumes, and abrasive blasting agents. We once again urge you to intervene in this important matter.

Sincerely,

Jessica Sandler, MHS
Federal Agency Liaison

cc: Dr. S. Masten

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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

July 22, 2002

Dr. Kenneth Olden, Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Mail Drop E1
Research Triangle Park, NC 27709

Dear Dr. Olden:

Last week at the 2002 Summer Toxicology Forum, you expressed concern that the National Toxicology Program (NTP) not waste the taxpayers' money by conducting redundant studies and further review of the chemical methanol. You specifically stated that the NTP could have saved taxpayer funds had you known that the EPA was conducting similar studies.

We applaud your concern that neither taxpayer dollars nor animals' lives be wasted in redundant and pointless NTP studies. We would therefore like to call your attention to another situation that represents an outrageous waste of both: the NTP's proposal to conduct additional rodent cancer studies of hexavalent chromium (see *Federal Register* notice of 5-24-02, "Availability of Data From Preliminary Studies and Proposed Study Protocols for Cancer Bioassays of Hexavalent Chromium in Rats and Mice").

Dr. Olden, how can the NTP possibly justify continued expenditure of tax dollars and the killing of more animals to test hexavalent chromium? We strenuously object to this newest NTP boondoggle, given that (1) hexavalent chromium is already well established as a known human carcinogen, and (2) rodent cancer bioasssays are recognized as being largely useless in determining carcinogenic hazards to humans.

A recent Freedom of Information Act request to the EPA regarding animal studies related to hexavalent chromium yielded a list of studies (references only — NOT the studies themselves) that was several inches thick. This substance has been, pardon the expression, studied to death. What's more, regardless of its effect on animals in laboratories and whether its reduction to trivalent chromium in drinking water ameliorates its effects, **hexavalent chromium has long been established as a known carcinogen in *humans***. Yet the NTP is insisting on subjecting ever more animals to toxicity testing of this chemical in a useless and bottomless pit of study.

Almost a decade ago, you were quoted in a *New York Times* article entitled "Many Say Lab-Animal Tests Fail to Measure Human Risk" as stating that, "many of the assumptions driving rat and mouse research do not appear to be valid" (3-23-93). In the years since, this statement has been amply corroborated and reinforced. A recent *Environmental Health Perspectives* article entitled, "Data Quality in Predictive Toxicology: Reproducibility of Rodent Carcinogenicity Experiments," concluded that, "rodent carcinogenicity assays are much less reproducible than previously expected" (May 2001).

The NTP's continued insistence on relying so heavily on the results of very crude and cruel animal poisoning tests, specifically in this case the two year carcinogenicity bioassay, which has never been formally validated to establish its reliability or relevance to human health effects, is a matter of great concern to us. You are no doubt aware of the fundamentals recognized internationally that test methods must meet in order to be consider valid. Criteria for test method validation emerged from an international conference convened in 1996 in Solna, Sweden, by the Organization for Economic Cooperation and Development (OECD). These include a determination that the test is both relevant to the endpoint of interest and reliable (reproducible). It is to these issues that the bulk of our comments are directed.

Lack of relevance of the rodent cancer bioassay

Relevance is the extent to which a test correctly measures or predicts the biological effect of interest.[1] In the case of carcinogenicity testing, the biological effect of interest is cancer *in humans.* However, in continually advocating the use of rodent bioassays as a fundamental pillar of carcinogenicity testing, the NTP loses sight of this goal. This is highly inappropriate, given the exorbitant costs of such studies—in terms of time, financial resources, and animal suffering and death—coupled with the widely recognized tendency of rodent bioassays to produce results which are either equivocal or wholly irrelevant to humans.[2]

Extrapolating from one species to another is fraught with difficulties and uncertainty, as are extrapolations from high dose to low dose, from one exposure route to another, and from one exposure time frame to another.[3] [4] For example, Ennever *et al.* [5] found that rodent bioassays produce an unacceptably large number of false positive results. They report that 19 out of 20 probable human non-carcinogens tested positive in rodent bioassays, implying that the specificity of these assays may be as low as 0.05. Similarly, Gold *et al.*[6] [7] found that rodent studies identified nearly two-thirds of the 800 chemicals tested as carcinogens—an implausibly high proportion. Of even greater concern from the perspective of public health are false negative results; Salsburg[8] found that rodent assays were capable of identifying only 37 percent of known human carcinogens. In fact, the NTP's[9] own studies found that rat and mouse bioassays produced concordant results

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(both positive and negative) only 70 percent of the time. Given that rats and mice are more similar biologically than either is to humans, one must assume that rodent-human concordance is far less than 70 percent.

Rodent bioassays also provide little or no information about a chemical's mechanism of action,[10] generally lack the sensitivity to detect weak carcinogens, [11] and are unlikely to produce tumors or other effects in statistically significant numbers at realistic exposure levels. In fact, according to an NIEHS expert review committee in its report to you, "approximately two-thirds of the carcinogens would not be positive, i.e., not considered as carcinogens, if the MTD was not used." [12] There are questions about the relevance to humans of certain tumor types observed in rodents, given the high spontaneous incidence of tumors in certain animal strains. For example, because of the high background incidence of liver tumors in male $B6C3F_1$ mice, discussions continue on the relevance of this tumor to humans.[13]

Far from providing an unequivocal assessment of chemical risks to humans, the relevance of data derived from unvalidated animal tests is always in question, and therefore subject to vastly differing interpretations, and often, successful legal challenges. Even a cursory review of the Bureau of National Affairs' (BNA) *Daily Environment Report* reveals a litany of recent examples:

- The report of the Chronic Health Advisory Panel of the Consumer Product Safety Commission found that the chemical, diisononyl phthalate (DNIP), is "clearly carcinogenic to the rodent," but that DNIP appears to induce liver cancer in rodents by a mechanism not readily induced in humans under current exposure conditions involving consumer products. Therefore, the human risk has been deemed "negligible."[14]

- The NTP found conflicting results in its assessments of the chemical, *p*-nitrotoluene. For example, the NTP reported equivocal evidence of carcinogenic activity in male F344/N rats exposed to 2,500 ppm and 5,000 ppm, and some evidence of carcinogenic activity in female rats of the same strain at the same dose levels; equivocal evidence in male $B6C3F_1$ mice exposed to 5,000 ppm, but no evidence of carcinogenicity in female mice of the same strain at the same dose level.[15]

- A manufacturer of pyrethrin pesticides has sued the EPA for its classification of pyrethrins as "likely to be a human carcinogen if ingested orally," a classification that was based on default assumptions in the current revised guidelines.[16] The EPA's Cancer Assessment Review Committee reportedly "decided on the classification because of studies indicating tumors in the rat, the relevance of [which] could not be discounted in humans." However, the pyrethrin manufacturers have argued that the EPA's "assessment overestimated the significance of tumors and did not weigh the scientific

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[12] Brinkley J. Many say lab animal tests fail to measure human risk. *The New York Times*, 23-Mar-93:A-1.
[13] Schwetz *et al.*, Op cit.
[14] Werner K. BNA *Daily Environment Report*, 20-Jun-01:A-9.
[15] Phibbs P. BNA *Daily Environment Report*, 6-Apr-01:A-3.
[16] Werner K. BNA *Daily Environment Report*, 19-Apr-01:A-10.

evidence properly."[17]

- The EPA's Science Advisory Board (SAB) Dioxin Reassessment Review Committee could not agree on the appropriate cancer classification for dioxins. "Some panel members supported EPA's proposal to classify dioxins as human carcinogens, but most did not... The majority believed that the scientific evidence is not strong enough to support classification as a human carcinogen. ... This decision was not reached because the SAB believed that the current evaluation reached fully supportable scientific conclusions, but because [panel members] believed that there would still not be adequate information available within the next several years to significantly reduce the large amount of uncertainty inherent in any current risk assessment of dioxin and related compounds."[18]

- The EPA's Federal Insecticide, Fungicide, and Rodenticide Act Science Advisory Panel concluded that the pesticide, malathion, "either is unlikely to cause cancer in humans, or the scientific evidence is insufficient to assess its carcinogenic potential... The science panel was divided on whether to place malathion in the 'suggestive evidence of carcinogenicity' category based on evidence from animal studies, or in the 'not likely to be carcinogenic to humans' classification."[19] The director of regulatory affairs for Cheminova Agro, was cited as stating: "The high exposure levels in the animal studies are not relevant to humans... One point of debate was how rare, or relevant, certain tumors in study animals were. Panel members discounted their relevance. One panel member said the pesticide should be classified as a 'likely' carcinogen, but others said there is no strong evidence of carcinogenicity nor any evidence of a mode of action relevant to humans."[20]

- Commenters on the EPA's draft guideline for evaluating the carcinogenicity and toxicity of fibers "urged the EPA to require tests on a species other than the rat, because rats are less sensitive to asbestos than people."[21] The EPA reportedly acknowledged that "while tests involving rats could identify carcinogenic fibers, they would not provide data as useful about how potent a carcinogen the fiber being tested is... Hamsters are more sensitive than rats to fiber-induced mesotheliomas, but the species does not tend to develop lung tumors and can be difficult to use in laboratory tests."[22]

- The International Life Sciences Institute (ILSI) continues to work toward the development of "general principles for determining when cancer mechanisms of action are relevant to humans."[23]

- The EPA's draft characterization of the chemical, trichloroethylene (TCE), indicates that "TCE may be more likely to cause cancer than EPA previously recognized based on new

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[17] Werner K. BNA *Daily Environment Report*, 2-Apr-01:A-8.
[18] Phibbs P. BNA *Daily Environment Report*, 20-Mar-01:A-1.
[19] Werner K. BNA *Daily Environment Report*, 20-Dec-00:A-7.
[20] Ibid.
[21] Anon. BNA *Daily Environment Report*, 1-Aug-01:A-4.
[22] Ibid.
[23] Werner K. BNA *Daily Environment Report*, 14-Aug-01:A-24.

scientific data that show humans retain TCE in their bodies longer than animals do... [Epidemiological studies] also showed an association with prostate and cervical cancers, [but] there is no good mouse or rat model that can be used to determine whether chemicals cause prostate or cervical cancer... Some scientists have argued that people would be less susceptible to TCE's carcinogenic properties due to differences between rodents and humans... For example, some scientists argue that liver tumors found in mice are not relevant to people because they are caused by peroxisome proliferation... Some scientists have also argued that people are less susceptible than mice to lung cancers that TCE has caused in laboratory studies. These scientists argue that lung tumors result from the accumulation of TCE's metabolites in Clara cells, a type of lung cell... Mice have more of these cells in their lungs than do people, which could suggest they would be more susceptible."[24]

- In its recently released risk assessment for the pesticide atrazine, the EPA concluded that the rodent studies were not relevant to humans: the agency has "determined that the mode of action for the carcinogenic potential in the Sprague-Dawley rat is not likely to be operative in humans," that "there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in SD rats is not relevant to humans," and that "there are considerable differences between the hypothalmic-pituitary-ovarian function in rats and humans."[25]

Lack of reliability of rodent bioassays

Reliability refers to the extent of reproducibility of a test's results within and among laboratories, when performed using the same protocol.[26] A recent study by Gottmann *et al.*[27] evaluated the reliability of rodent carcinogenicity bioassays by calculating the concordance of classifications from replicate studies for 121 compounds using data from the Carcinogenic Potency Database. The authors found a concordance of only 57 percent, which suggests that rodent bioassays are generally unreliable, and that the reproducibility of such assays has likely been overestimated in previous investigations.

In summary, Lave *et al.*[28] have concluded that "the [rodent carcinogenicity] bioassay does not provide information commensurate with its cost, implying that regulatory policies of industrialized countries need to be changed." Similarly, your own NIEHS deputy director, Dr. Richard Griesemer, has stated that "animal research, by itself, should no longer be accepted as a reliable means of judging risks for humans," and your NIEHS expert review committee concluded that "the government should no longer rely on animal studies."[29] We could not agree more.

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[24] Phibbs P. BNA *Daily Environment Report,* 11-Sep-01:A-5.
[25] Atrazine: Response to Public Comments on the EPA's January 19, 2001, Revised Preliminary Human Health Risk Assessment and Associated Documents for the Reregistration Eligibility Decision, 4-16-02.
[26] ICCVAM, Op cit.
[27] Gotmann E, Kramer S, Pfahringer B & Helma C. Data quality in predictive toxicology: Reproducibility of rodent carcinogenicity experiments. *Environ. Hlth. Perpsect.* 2001;109(5):509-14.
[28] Lester *et al.*, Op cit.
[29] Brinkley, Op cit.

Dr. Olden, we submitted comments on the same methanol testing issue that you addressed last week at the Toxicology Forum. We will shortly be submitting yet another set of comments on the NTP's proposal to re-test such substances as turpentine, blasting agents, ephedrin and thimerosol on more animals. We urge you to put an end to such redundant, excessive and wasteful animal testing, of which the NTP's proposal to continue testing hexavalent chromium is yet another blatant example. We respectfully request your serious consideration of this issue and look forward to your timely response.

Sincerely,

Jessica Sandler, MHS
Federal Agency Liaison

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

July 8, 2002

Dr. Michael Shelby
NTP Center for the Evaluation of Risks to Human Reproduction
P.O. Box 12233
MD EC-32
Research Triangle Park, N.C. 27709

Dear Dr. Shelby:

On behalf of the 750,000 members and supporters of People for the Ethical Treatment of Animals (PETA), I am again submitting comments in opposition to the National Toxicology Program's (NTP) highly inappropriate proposal to conduct still more animal-based toxicity studies on methanol. PETA is the largest animal rights organization in the world, and is committed to using the best available science to protect animals from suffering and to promote the acceptance of alternatives to activities that harm animals.

Although it is acknowledged in Section 5.9 of the NTP report that further animal testing of methanol is not "critical," the report concludes that more "studies are needed to elucidate the basis for the developmental toxicity of methanol," and calls for "data from developmental toxicity studies using concurrent exposures to methanol and ethanol." For the reasons cited below, this recommendation is seriously flawed on both scientific and policy grounds.

Methanol is among the most over-studied chemicals in existence — having been subject to a veritable laundry-list of cruel and non-validated animal-poisoning tests, both in rodents as well as dogs and non-human primates. The NTP has truly refined the process of "paralysis by analysis" to an art form— subjecting chemicals to a useless and bottomless pit of study with little or no regulatory action in the end. This situation is appalling on a policy level, given the extremely high costs of NTP-mandated testing — both financially and in terms of animal suffering and death. In addition, the NTP's current paradigm represents the least public health protective application of the precautionary principle, which often results in protracted delays in risk management decisions, which can in turn have a serious adverse impact on human health.

From a scientific perspective, the NTP's report is replete with references to the fact that "the rodent data are *assumed* to be relevant for humans." However, such an arbitrary and chronically unconfirmed leap of faith does not befit a supposedly science-based institution. Because none of the NTP's laundry list of animal-poisoning tests used has ever been validated for its relevance to humans, calling for more such tests will only confuse matters further and prolong testing *ad infinitum*. We call your attention to a publication by the National Academy of Sciences in 2000, entitled Scientific Frontier in Developmental Toxicology and Risk Assessment, and highly recommend that your expert panel review the section on limitations in developmental toxicity risk assessments. The report discusses at great length the dubious relevance of animal tests to the

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understanding of human development, the major limitations of the default assumption that "outcomes for rodent tests are relevant for human risk prediction," and the failure of animal tests to generate useful mechanistic data — a fact which could not be more clear based on the NTP's current recommendations regarding methanol.

Other widely recognized limitations to animal-based studies of reproductive and developmental toxicity include, but are not limited to the following:

- the fact that human and test species' reproductive systems and cycles are very different;
- the influence of immune, physiological and dietary status on the interpretation of results of testing is fraught with problems;
- genetic constitution profoundly affects the reproductive toxicity of chemicals and this varies in humans and animals;
- organs such as the testes and ovaries respond to the test substances differently in human and animal species;
- the time course of the metabolism and elimination of any test substance influences the ways that repeat doses elicit a response, for example, in some animals but not others, the chemical accumulates in the body over time causing a more toxic effect which will complicate any extrapolation to humans; and
- the binding of the test substance to various organs and cells within the body means that there will be different distribution and concentration of the toxic substance in the internal organs of different species which will affect the interpretation of both single and multiple doses and the necessary extrapolation to humans.

Given all the admitted problems in interpreting the results of animal tests for developmental and reproductive toxicity, it is appalling that the NTP fails to consider non-animal test methods. For example, the European Centre for the Validation of Alternative Methods (ECVAM) recently validated an *in vitro* embryonic stem cell test as a sensitive and reliable method for detecting chemicals with embryotoxic potential — making it a valuable screen for potential developmental toxicants. The test uses rodent-derived stem cells, which survive in culture indefinitely and can develop into specialized cells such as heart cells. Embryotoxicity is determined by the ability of a test substance to prevent or limit the development of embryonic stem cells into specialized heart cells in culture (Genschow *et al.* 2002). Several *in vitro* methods are also available to study the mechanisms by which toxicity to reproduction occurs. The NTP should be championing their further development, validation and regulatory acceptance, per the NIEHS implementation guidelines developed pursuant to the NIH Revitalization Act.

We strongly urge the NTP to retract its call for further, unnecessary testing of methanol on animals.

Sincerely,

Jessica Sandler
Federal Agency Liaison

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

September 23, 2001

Dr. Scott Masten
Office of Chemical Nomination & Selection
NIEHS/NTP
P.O. Box 12233
Research Triangle Park, N.C.27709

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.[30] 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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[30] 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.[31]

- **Metalworking fluids**

  A literature review of four randomly selected metalworking fluids—1,1,2-Trichloro-1,2,2-trifluoroethane [76-13-1], Polypropylene glycol [25322-69-4], Tetraethylene glycol [112-60-7]

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[31] 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.

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 not 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."[32] 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.[33] 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."[34] 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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32 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.

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

34 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
Science Policy Advisor

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*)

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

	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

PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

September 6, 2001

Dr. Michael Shelby, Director
Center for the Evaluation of Risks to Human Reproduction
National Institute of Environmental Health Sciences
79 T.W. Alexander Drive, Building 4401, Room 103
P.O. Box 12233, MD EC-32
Research Triangle Park, NC 27709

Dear Dr. Shelby:

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our over 750,000 members in response to a *Federal Register* notice of July 16, 2001, soliciting public comment on the draft expert report on methanol released by the National Toxicology Program's (NTP) Center for the Evaluation of Risks to Human Reproduction's (CERHR).

It is impossible to tell from the draft that is available from the NTP web site, http://cerhr.niehs.nih.gov/news/methanol_report.PDF, whether or not the CERHR's conclusion is that insufficient animal data exist on the potential reproductive and developmental risks of methanol to humans. The report is not presented in a "user-friendly" manner and omits the preface and conclusions of the draft expert report, including such critical information as the data needs section. As a result, the public cannot fully review the conclusions of the expert panel in order to gain valuable insight into the panel's priorities when formulating its conclusions.

We sincerely hope that the U.S government does not believe that additional animal tests should be conducted on methanol. The CERHR draft report itself details the abundant existing data on the reproductive and developmental toxicity of methanol to rats and non-human primates. The test plan for methanol, submitted by the American Methanol Institute Testing Group, also provides extensive data on this substance—including reproductive and developmental data—and calls for no further testing under the Environmental Protection Agency's high production volume chemical testing program.

A wealth of existing epidemiological and toxicological data provides more than adequate information to understand the potential health implications of methanol exposure. Information on the extensive natural, food-additive-related (e.g., aspartamine), and industrial human exposure to methanol provide a rich set of existing cohorts by which to evaluate the potential reproductive effects of methanol on human populations. Further, the fundamental biochemical basis of methanol metabolism and toxicity—in which methanol toxicity is related to the rate of formate metabolism—is well understood and
can provide additional data on the toxicity of this ubiquitous compound. Any further testing of methanol on animals would be pointless and cruel.

Finally, we would like to notify the CERHR of our intention to submit more extensive public comments jointly with the Physicians Committee for Responsible Medicine at the Methanol Expert Panel Meeting scheduled for October 15-17, 2001, in Alexandria, Virginia.

Thank you for your attention to these comments.

Sincerely,

Jessica T. Sandler, MHS
Federal Agency Liaison

PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

May 3, 2001

Docket Control Office (7407)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Docket Control Number OPPTS-41055
47th Report of the TSCA Interagency Testing Committee

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our over 700,000 members who are concerned about the use of animals in laboratories.

We appreciate that the Interagency Testing Committee (ITC) is not currently requesting that toxicological testing be undertaken for chemicals identified in the 47th Report, but rather, is calling for reports of existing data via TSCA section 8(a) and 8(e) rules. However, in the event that future testing of these chemicals is considered by the ITC, we submit the following recommendations to guide the conduct of such testing:

- We strongly encourage the development of categories for these compounds and their assessment by means of structure-activity relationships (SARs).

- The indium compounds should be evaluated as a group, and their toxicity should be considered based on trends in existing indium compound behavior, as well as the chemical trends and behavior observed in metals nearby in the periodic table.

- If there is a concern of the chloroalkanes being persistent and bioaccumulative, the ITC should consider existing field data at places where these compounds have been released to evaluate any bioaccumulative affects.

We look forward to closely following how the ITC incorporates animal protection concerns into any proposed testing of these substances.

Sincerely,

Troy Seidle
Research Associate

January 19, 2001

Dr. Scott Masten
NIEHS/NTP
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Masten:

The following is in response to your *Federal Register* notice of 4 December 2000 requesting comments on substances nominated to the National Toxicology Program (NTP) for toxicological studies and on testing recommendations made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEE). These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our over 700,000 members.

Given that the NTP's stated objective in soliciting nominations for toxicological studies is "to identify and select for study chemicals and agents with the highest potential for adversely impacting public health...based on human exposure and suspicion of toxicity," we find it highly objectionable that natural dietary supplements have been included in the proposed testing scheme. As you know, many of the identified substances have been in routine use in various cultures for centuries or more, without adverse health effects having been noted.

A case in point is the proposed testing of the extract from biliberry fruit (a relative to the common blueberry), for both *in vitro* and *in vivo* genotoxicity. The only rationale for this testing is a stated "lack of toxicity information." As we trust you are aware, the proposed *in vitro* genotoxicity assay 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."

We likewise submit that the proposed use of animals in subchronic and two-generation reproductive and developmental toxicity studies of black cohosh, subchronic toxicity and neurotoxicity studies of blue-green algae, photogenotoxicity testing of lemon oil, and possible subchronic toxicity testing of S-Adenosylmethionine, is cruel and unnecessary. We regard the proposed ICCEC testing of herbal dietary supplements as little more than the curiosity-driven poisoning of animals. We urge the NTP to consider the impact of this proposal on animal suffering, and withdraw this proposed testing requirement.

Sincerely,

Troy Seidle
Research Associate

PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457