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Re: Request for Public Comments on Substances Nominated to the NTP for Toxicological Studies and Study Recommendations Made by the ICCEC

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our more than 750,000 members and supporters in response to a July 16 *Federal Register* notice from the Department of Health and Human Services. We appreciate the opportunity to comment on the testing recommendations of the National Toxicology Program (NTP) Interagency Committee for Chemical Evaluation and Coordination (ICCEC), but question both the wisdom and the value of the NTP's active solicitation of chemical nominations for toxicological evaluation.

It has been our experience that these NTP solicitations lead, almost invariably, to testing that is at best unnecessary, and at worst, extremely costly and cruel to animals. Over the past few years, PETA has commented on a number of NTP/ICCEC testing proposals, the majority of which appear to advocate testing for its own sake, and at the expense of not only animal welfare, but also basic common sense. Some of the many glaring examples of this have included the ICCEC's increasingly regular endorsement of animal testing of natural substances (e.g., grape-seed, pine bark, green tea and biliberry fruit extracts, etc.); chemicals that have already been the subject of extensive toxicological testing and hazard characterization (e.g., methanol, hexavalent chromium, etc.); and chemicals that were, at the time, also being examined by other federal agencies, such as the Environmental Protection Agency.

Although in some cases PETA submitted lengthy documentation of existing data that the ICCEC had overlooked when it proposed further animal testing, all of our comments have gone unanswered and, to the best of our knowledge, no changes have ever been made in the ICCEC's recommendations or the testing carried out by the NTP. We were therefore quite disappointed, if not surprised, to note that the ICCEC has once again called for extensive animal testing, yet provided little or no information in support of its testing recommendations. In the future, PETA strongly urges the NTP to (i) require the submission of robust summaries along with all chemical nominations, and (ii) publish these robust summaries in the *Federal Register* alongside the ICCEC's testing recommendations. The cursory summaries provided for both the nomination rationale and recommendations for toxicological studies are much too superficial to be of any utility in evaluating the ICCEC's testing recommendations. In fact, from the information provided in the *Federal Register* notice, it is not even possible to determine whether the ICCEC is recommending that specific studies be carried out *in vitro* or *in vivo*.

Our comments on specific chemical nominations and ICCEC recommendations are as follows:

ACRYLAMIDE AND GLYCIDAMIDE

The Food and Drug Administration's (FDA) recommendation is that the following areas be investigated with respect to acrylamide (CAS no. 79-06-1) and glycidamide (CAS no. 5694-00-8): toxicological characterization, toxicokinetics, toxicological mechanism, carcinogenicity, and bioavailability from food

and water. Glycidamide, an epoxide, is the principal metabolite of acrylamide, and the toxicity of the two compounds is generally considered together.

The FDA's recommendation was presumably made in response to the finding that acrylamide is present in fried foods. The highest concentrations have been found in potatoes fried at high temperatures (Fleck 2002; Mitka 2002; Nemoto 2002; Orellana 2002; Tareke 2002; Weiss 2002; Becalski 2003; Bren 2003; Ono 2003; Pelucchi 2003; Sharp 2003). However, the significance of these findings is open to debate, as one study showed the maximum realistic mean daily dose of acrylamide to be 0.3 _g/kg, which is three orders of magnitude lower than the no-observed-adverse-event level, suggesting a negligible cancer risk, if any (Shaw 2003).

It is premature to conclude that insufficient information is available regarding the toxicity of these compounds, as even a cursory search of the open literature revealed more than 800 published reports on the subject of acrylamide's toxicity, and considering the fact that acrylamide is one of only a handful of industrial substances that the Environmental Protection Agency (EPA) has attempted to ban. In particular, over the past 18 months, there has been a spate of reports regarding the danger due to acrylamide in food. Given that many of these reports were published within the past few months, it is doubtful that they were reviewed or considered by the FDA before the agency recommended this substance for further testing by the NTP. We therefore recommend that no action be taken in regard to these substances pending a full and complete review by the FDA of all available information, including unpublished in-house and corporate information.

Our specific observations regarding the four areas proposed for toxicological evaluation are as follows:

1. **Toxicological characterization and mechanism**
   The toxicity of acrylamide and glycidamide is thought to be due to their formation of adducts with DNA and various proteins (Schettgen 2002). One protein with which these compounds form adducts is hemoglobin; however, it is unclear what the FDA means by "mechanism (hemoglobin adducts)" as, rather than being a likely cause of toxicity, the presence of hemoglobin adducts in serum provides a means of measuring the concentrations of acrylamide and glycidamide (Orellana 2002; Tareke 2002; Paulsson 2003).

   The formation of DNA adducts by acrylamide may be responsible for its weak mutagenicity in murine embryonic fibroblasts (Besaratinia 2003; Tyl 2003). The formation of adducts with motor proteins and spermatid protamines gives rise to male reproductive toxicity in rats (Tyl 2003). The carcinogenicity of acrylamide is thought to be due to induction of cellular transformation (Park 2002).

   Acrylamide is also neuropathic, acting primarily on the nerve terminals in rats (Tandrup 2002a and 2002b; Lehning 2003a and 2003b; Bolt 2003; LoPachin 2003). In cattle, the neuropathic effects of acrylamide correlate well with the concentration of its hemoglobin adducts (Godin 2002).

2. **Toxicokinetics**
   The toxicokinetics of acrylamide and glycidamide in rats were investigated recently by Kirman (2003). Some work has also been carried on acrylamide pharmacokinetics in humans (Schettgen 2002).

3. **Carcinogenicity**
   Chronic acrylamide treatment in rats and mice definitely causes cancer, probably due to the genotoxicity of glycidamide (Paulsson 2001 and 2003; Park 2002). However, it is difficult to see

the value of such research to the prevention of cancer in humans, especially as there is a marked interspecies difference even between species as closely related as rats and mice, with the latter being 10 times more sensitive to acrylamide than the former (Paulsson 2001). Mucci has commented that "species differences negate extrapolating from experimental animals to humans" (2003), and a more helpful approach therefore involves human epidemiological studies, as discussed below.

4. Bioavailability

The relevance to humans of research on bioavailability in animals is compromised by a myriad of interspecies differences. For example, serum levels of glycidamide-hemoglobin adduct per unit dose of acrylamide have been found to be 3-10 times higher in mice than in rats (Paulsson 2001).

Our principal recommendation is that, before the detailed planning of any further testing, particularly if it would involve animals, the FDA should prepare a comprehensive and up-to-date review of all research that has been carried out on these substances. The FDA should then identify the knowledge gaps, as well as their importance from the standpoint of risk mitigation and prevention of cancer in humans. It is premature to discuss possible research in detail until the above preparatory work has been carried out.

Notably, in June 2002, the United Nations' Food and Agriculture Organization (FAO) outlined the three most important areas of research needed, *none of which involved animal experiments* ("Acrylamide in food" 2002). We therefore tentatively endorse the following approaches:

1. Epidemiology

With respect to acrylamide, the FAO has stated that there is a need for "epidemiological studies of relevant cancers in humans" ("Acrylamide in food" 2002). Several epidemiological studies have been carried out on acrylamide exposure, but there is still considerable doubt as to whether either dietary or occupational acrylamide exposure results in human cancer (Granath 2003). Similarly, three studies of occupationally exposed factory workers provided little or no evidence of carcinogenicity (Sobel 1986; Collins 1989; Marsh 1999), although some uncertainty remains (Granath 2001; Marsh 2001; Schulz 2001).

Two smaller studies have also been carried out, one in Sweden (Mucci 2003), and the other in Italy and Switzerland (Pelucchi 2003). In these studies, the amount of dietary acrylamide was estimated from the fried potato consumption. However, numerous criticisms can be made of these studies, and a full-size study with actual measurement of dietary acrylamide content is recommended. For example, Reynolds (2002) has called for (i) retrospective studies, comparing the changes that occurred when boiled potatoes, until recently the staple diet in much of northern Europe, were replaced with fried potatoes, and (ii) geographic studies, comparing populations in which the popularity of fried food differs.

2. Further elucidation of the chemistry of acrylamide formation during frying

One issue that has not been addressed is whether acrylamide is formed by frying with both vegetable and animal-derived lipids. The FAO has emphasized the need to determine "how acrylamide is formed during the cooking process" ("Acrylamide in food" 2002).

3. Attention to policy issues

Even sound and comprehensive data are of little value without consideration of questions such as (i) what increase in cancer risk due to dietary acrylamide would warrant governmental intervention, and (ii) the likelihood that such intervention would be effective in terms of reducing the incidence and prevalence of acrylamide-related cancers. As Granath (2003) has pointed out, such intervention

may be superfluous, because "the high consumption of foods such as potato chips or french fries should be avoided for other and more prominent health-related reasons, such as cardiovascular disease." Similarly, the WHO already "recommends eating more fruits and vegetables and less fat-containing foods" ("WHO to hold urgent expert consultation …" 2002). In addition to the possibility that a regulatory intervention might be superfluous, it would also seem to be virtually impossible to administer, i.e., "even if acrylamide definitely was proven to cause cancer in humans, how can we get it out of food?" (McCaffree 2003).

4. Non-animal methods for investigating carcinogenicity

Currently available *in silico* models include the MULTICASE (Computer Automated Structure Evaluation) Expert System, TOPKAT (Toxicity Prediction by Computer-Assisted Technology), COMPACT (Computerized Optimized Parametric Analysis of Chemical Toxicity), DEREK (Deductive Estimation of Risk from Existing Knowledge), ONCOLOGIC, and HAZARDEXPERT.

In vitro assays for mutagenicity and genetic toxicity that have been approved for regulatory use include the bacterial reverse mutation assay, or Ames test (OECD 471), chromosomal aberration test (OECD 473), cell gene mutation test (OECD 476), and sister chromatid exchange test (OECD 479). Non-genotoxic events such aneuploidy can be investigated using metaphase analysis and chromosomal painting techniques, as well as in cell transformation assays, such as the Syrian Hamster Embryo (SHE) assay (Park 2002). While such research is not strictly non-animal, as the cells used are isolated from embryos rather than being obtained using long-term cultures (and the medium used contains fetal bovine serum), it nevertheless demonstrates the viability of *in vitro* methods.

ANTIMONY TRISULFIDE

The National Cancer Institute (NCI) has recommended that the chronic toxicity and carcinogenicity of antimony trisulfide (CAS no. 1345-04-6) be investigated. Smelted or manufactured antimony trisulfide is used industrially, mostly in the munitions, glass and pigment industries, but stibnite (CAS no. 1317-86-8), the most important antimony ore, is impure antimony trisulfide, and this should therefore be included in any assessment of toxicity (IARC 1989, pp. 291-292).

Only a limited amount of research on chronic toxicity and carcinogenicity has been carried out. The results of rodent bioassay studies have been inconclusive (IARC 1989, p. 297); however, a recent review concluded that there is a certain amount of evidence for considering antimony trisulfide to be carcinogenic (Leonard 1996).

With respect to chronic non-carcinogenic toxicity, administration to rats for 6 weeks gave rise to cardiac disease (IARC 1989, p. 298). Non-carcinogenic occupational toxicity reported in humans includes dermatitis, irritation of mucous membranes, cardiac disease (sometimes resulting in sudden death), gastrointestinal disorders such as nausea, vomiting, diarrhea, liver enlargement and abdominal pain, headache, muscular pain, vertigo, anorexia, altered blood chemistry, and pulmonary toxicity, including bronchitis and pneumonoconiosis (Stokinger 1981, IARC 1989, p. 300, Lobanova 1991, 1996, Health and Safety Commission 1996, p. 61). On the basis of these reports, the limit in air in the US has been set at 1 ppm (Stokinger 1981).

We suggest the following approaches:

1. Categorization

The toxicity of antimony compounds is mostly due to antimony. Therefore, with respect to toxicity, antimony trisulfide (including stibnite) should be classed together with other antimony compounds (primarily antimony trioxide and antimony pentachloride) and antimony metalloid, which exhibit similar types of toxicity. The pentachloride appears to be somewhat more toxic than the trisulfide, which should leave a safety margin with respect to the trisulfide (Health and Safety Commission 1996, pp. 59-63), and exposure to the trioxide is more common than exposure to the trisulfide, so its effects have been better studied. Industrial exposure to one antimony compound usually occurs together with exposure to other compounds and/or the metalloid, and previous medical reports, epidemiological research, legislation and industrial hygiene have therefore not usually distinguished sharply between exposure to the metalloid and the different compounds (McCallum 1989, Health and Safety Commission 1996, pp. 59-63, Leonard 1996), which makes it rather artificial to make this distinction at this stage. Studies in rats and rabbits have shown antimony compounds to have moderate chronic toxicity in the heart, liver and kidneys (Health and Safety Commission 1996, p. 61), and antimony compounds are almost certainly carcinogenic in industrial exposure (McCallum 1989).

2. Assessment of current knowledge

The NCI should have prepared a detailed review of currently available information before recommending further experimental work. Some of the previously published work might not have been taken into consideration by the Institute, because, for example, one important report on antimony trisulfide has never been translated from Russian (Melnikova 1980).

3. Epidemiological studies

Because of the likelihood of inter-species difference, and because the results of animal studies have been inconsistent, the type of research that is most likely to provide reliable information about human chronic toxicity is epidemiological, yet no full-scale epidemiological studies have either been carried out or, to the best of our knowledge, even proposed. Although a limited amount of work has been carried out on antimony miners and smelter workers, there is one exposed population in which no assessment whatsoever has been carried out: leishmaniasis patients administered antimony-containing drugs.

4. Policy issues

Most exposure to antimony compounds occurs in the process of antimony mining and smelting, and in their industrial use. Improved industrial hygiene and increased mechanization has already led to a sharp decline in industrial exposure (McCallum 1989). Thus, the need and/or value of additional research relative to continued improvement in industrial hygiene is questionable.

An additional issue that should be taken into account is the fact that stibnite and most industrial antimony compounds are contaminated with arsenic (McCallum 1989), the toxicity of which well established. As such, continued reduction of exposure would be advisable even were antimony compounds found to be safe.

CADMIUM TELLURIDE

The Department of Energy (DOE) has recommended toxicological characterization of cadmium telluride (CAS no. 1306-25-8) and investigation of chemical disposition via oral and inhalation routes.

Cadmium telluride is used in the photovoltaic and semiconductor industries (Moskowitz 1990, 1991, 1992, 1995a, 1995b). As there is now a considerable amount of human exposure to this compound—

whereas there was none until relatively recently—and this exposure can be expected to continue to increase for the foreseeable future, there is some legitimate concern about its toxicity. We have several comments to make about the suggested areas of study:

1. A considerable amount of animal research on cadmium telluride has already been carried out, mostly in rats. It is known to cause pulmonary fibrosis, inflammation and hyperplasia (Morgan 1995, 1997), and disorders of calcium metabolism and thermoregulation (Vorobeva 1981). It is associated with decreased body weight gain at doses as low as 10 mg/kg/day (Harris 1994), and is thought to cause serious chronic toxicity even in the absence of acute effects (Fadeyev 1984), thus, a threshold limit of 0.5 mg/m^3 is recommended for workplace air (Vorobeva 1981). However, it has not been shown to affect reproduction or development (Harris 1994). Before studies are planned, a full assessment should be made of all available information, including governmental reports from Moskowitz' team at the Brookhaven National Laboratory (1990, 1991, 1992, 1995a, 1995b), which have not been published in journals, and the precise areas where information is lacking should be defined.

2. Some epidemiological assessment of cadmium telluride has been carried out. It was tentatively concluded that it does not give rise to cardiovascular toxicity (Kristensen 1989).

3. Much of the most important research on cadmium telluride toxicity was carried in the former USSR during the 1980s, and published in Russian (Vorobeva 1981; Fadeyev 1984). Research published in Russian has often gone unused in the English-speaking world, and we therefore recommend a full data search in Russian, in an attempt to locate reports about chemical disposition, for example.

4. The concern expressed about cadmium telluride toxicity has mostly been in the context of occupational exposure. However, the processes used in cadmium telluride manufacture also involve exposure of employees to phosphine, hydrogen selenide and toxic gases (Moskowitz 1986), and it may therefore be the case that toxicity due to these substances is considerably greater than the likely toxicity due to cadmium telluride. If this is the case, calcium telluride toxicity becomes a purely academic concern, and attention should focus on reducing toxicity due to these other types of exposure. Therefore, considerably more information is needed about the use of and exposure to cadmium telluride before the importance of its toxicity can be discussed in detail.

VIRGINIA CEDARWOOD OIL

The NCI has recommended toxicological characterization of Virginia cedarwood oil, particularly with respect to developmental toxicity. This recommendation is presumably based on a 2001 report stating that insufficient toxicity information is available to justify the continued unregulated use of Virginia cedarwood oil in cosmetics ("Final report …" 2001).

Virginia cedarwood oil is prepared from the wood of *Juniperus virginiana*, a conifer native to the south eastern US. It is the active ingredient in a number of insecticidal and fungicidal pesticide products, and is also a major component of many non-pesticidal cosmetics and other consumer products.

The first points to emphasize about any research plans for Virginia cedarwood oil are the considerable uncertainty as to its identity and composition:

(i) *Identity*. The NCI identifies Virginia cedarwood oil with CAS number 8000-27-9, but this CAS number is also applied to cedarwood oil in general, which may be extracted from the wood of various *Juniperus* species (e.g., *californica*, *communis*, *occidentalis*, *ashei*, *phoenicia*, *oxycedrus*,

etc.), *Cedrus* species (e.g., *atlantica*, *deodara*, etc.), or *Cupressus funebrus*, as well as *J. virginiana*. Most analytic work has not clearly distinguished Virginia cedarwood oil from other types of cedarwood oil (Lawrence 1991), and the oil is often referred to as "American cedarwood oil" (Adams 1991), which usually means Virginia cedarwood oil, but could also include oil from other species, which grow in Texas and California. Furthermore, cedarwood oil is often prepared using crude techniques, including the use of sawmill waste, which may be of unknown composition (Adams 1991), thus there can be no certainty that a given sample contains oil from a single species. These issues would matter little if the compositions of cedarwood oil from different species were similar, but the evidence as it stands suggests that there are marked interspecies differences in composition (Singh 1988; Adams 1991; Lawrence 1991). There is therefore an urgent need for clarification of nomenclature, CAS numbering, and manufacturing site and process details.

(ii) *Composition*. Even leaving aside the question of identity, only a small number of chemical analyses of oil that is probably Virginia cedarwood oil have been carried out, and the findings vary widely. In one analysis, the commercial oil was found to be composed mainly of cedrol, widdrol and a large number of oxygenated compound (Lawrence 1991), whereas in a second analysis, the principal components were alpha-cedrene and thujopsene (Adams 1991). The volatile content of the wood was also analyzed, and found to be composed mainly of alpha-cedrene, thujopsene and cedrol, and it is suggested that the cedrol might be lost due to the processing method (Adams 1991). Therefore, before any consideration of toxicity, chemical analytic data are needed on a number of samples of Virginia cedarwood oil. The above-mentioned report stating the need for more data on cedarwood oil toxicity to justify its use in cosmetics stresses that, in addition to toxicity, data are needed on uses in cosmetics, concentrations used, methods of manufacturing, impurities (especially pesticides), and ultraviolet absorption ("Final report …" 2001).

A certain amount of information is available regarding the toxicity of Virginia cedarwood. In mice, Virginia cedarwood shavings have been shown to be carcinogenic, and also to stimulate hepatic hexobarbital-metabolizing enzymes (Sabine 1975). On the other hand, in most studies the oil has exhibited little or not toxicity: it shows very low acute toxicity in animals, and it was not found to be a dermal irritant or sensitizer in either animal or human tests (Opdyke 1979; "Final report …", 2001).

There is also some information about the toxicity of oil that may have been Virginia cedarwood oil. One type of oil has been shown to cause contact dermatitis, but it is uncertain whether the oil was solely from *J. virginiana* alone, or was a mixture of oil from *J. virginiana* and *J. mexicana* (Franz 1998). Another type of oil, which was probably Virginia cedarwood oil but was described solely as "oil obtained from the red cedar tree," has been used as an abortifacient, on some occasions with a fatal outcome (Gosselin 1984).

To conclude, the first priority with respect to Virginia cedarwood oil is to define it, and to ensure that all so-described oil is extracted from a single species. It will then be necessary to analyze the oil chemically, to identify all major components, and to show how much variation in composition there is between different batches of Virginia cedarwood oil (and between this and other cedarwood oils). When these tasks have been completed, taking into account the available data on Virginia cedarwood oil, its chemical components, and other cedarwood oils, it may be found that sufficient information is already available to obviate the need for additional toxicity testing. If sufficient data are not available, human developmental and other toxicity data should be obtained primarily by way of epidemiological studies, bearing in mind the likelihood of interspecies differences, and also the facts that approximately 120,000 people are exposed to cedarwood oil each year in the US and, importantly from the point of view of epidemiological developmental toxicity studies, almost half of these are women (NIOSH).

CHONDROITIN SULFATE

The NCI recommends investigation of the chronic toxicity and carcinogenicity of chondroitin sulfate (CAS no. 9007-28-7), and the carcinogenicity of chondroitin sulfate together with glucosamine. Chondroitin sulfate, also known as chondroitin sulfates and chondroitin polysulfate, is an undefined mixture containing at least the following five distinct compounds: chondroitin sulfate A (chondroitin-4-sulfate; CAS no. 24967-93-9), chondroitin sulfate B (dermatan sulfate; CAS no. 24967-94-0), chondroitin sulfate C (chondroitin-6-sulfate; CAS no. 25322-46-7), chondroitin sulfate D (50814-15-8), and chondroitin sulfate E. Commercially available chondroitin sulfate also contains D-glucuronic acid and D-acetylgalactosamine (Chavez 1997). Therefore, available data on the toxicity of all these compounds is relevant to the NCI's recommendation.

An enormous amount of research has been carried out on the toxicity, metabolism and pharmacological actions of chondroitin sulfate (the mixture) and its constituent compounds, and several thousand reports have been published. Even if attention is only given to the areas for which investigation is recommended by the NCI, several reports have been published. Firstly, chondroitin sulfates have been reported to cause dyspepsia, nausea, cephalea and euphoria in humans (Chavez 1997). Secondly, they have severe negative effects on corneal endothelium in rabbits (Ibaraki 1985). Thirdly, as anticoagulants, there was some concern about their hematologic effects, but no hematologic effects were found in humans with administration for 6 months, and a 30-day study in dogs showed only insignificant effects (Chavez 1997). With respect to carcinogenicity, chondroitin sulfates were found to have no effect on the growth of a metastatic culture of cells from murine Lewis lung carcinoma (Timar 1990), but this may have been because the different components have different effects, as chondroitin sulfate B was found to stimulate tumor growth in mice, whereas chondroitin sulfates A and C were found to inhibit it (Morita 1989), exemplifying the confusion resulting from the use of an undefined mixture of compounds. There is also some evidence for the carcinogenicity of chondroitin sulfate together with glucosamine (Chavez 1997). We suggest that carcinogenicity could best be investigated by further *in vitro* studies of these types, using cell transformation assays.

Chondroitin sulfates are widely used; however, their principal use is in the treatment of arthritis, and there is considerable doubt as to their efficacy for this condition, with one reviewer drawing the following conclusion: "Chondroitin sulfate … has not clear value and should not be prescribed" ("Chondroitin sulfate" 1995). Furthermore, this field of medicine is undergoing rapid development, so it cannot be assumed that this treatment will continue to be used indefinitely, which means that attention should be given to the projected medical use of chondroitin sulfates; there is no point in carrying out a large number of studies, which will take a considerable time to generate usable data, if the use of the compound is expected to be discontinued in the near future. This point applies also to the concomitant administration of chondroitin sulfates and glucosamine: this treatment is used for arthritis (Chavez 1997; Davis 1998), but there has recently been considerable dispute as to whether it is effective (Towheed 2000). In the context of the uncertainty about long-term continuation of chondroitin sulfate use, one other point that must be noted is that it is derived from bovine tracheal cartilage (Chavez 1997), and the use of bovine products in medicine is currently restricted or being phased out in many countries, so the relevance of this should be examined.

Finally, as numerous arthritis patients are administered chondroitin sulfates, the situation is ideal for investigation of metabolism in humans, and for carrying out epidemiology studies on carcinogenicity. Studies of these types, using the drugs as they are actually administered, in the species to which they are actually administered, in conjunction with the medical condition for which they are actually administered, would also avoid the confusion due to the different effects of the different components of the mixture, as mentioned above.

DIMETHYLETHANOLAMINE

With respect to dimethylethanolamine (CAS no. 108-01-0), the National Institute of Environmental Health Sciences (NIEHS) recommends investigation of "metabolism." The obvious interpretation is that it means the metabolism of dimethylethanolamine; however, on the same page there is the statement that "some ethanolamines can interfere with choline uptake and utilization and may also generate nitrosamines," which suggests that the recommendation is for investigation of the effect of dimethylethanolamine on choline metabolism as well as on the metabolism of dimethylethano-lamine, perhaps via nitrosamines. Attention is therefore given to both these areas here.

With respect to dimethylethanolamine metabolism, a number of reports have been published. Dimethylethanolamine undergoes endogenous methylation (La Du 1971, p. 171). In rodents, it is phosphorylated and phosphatidylated in the brain (Miyazaki 1976, Ansell 1979). It is also converted to an acetylcholine-like compound (London 1978).

It is not clear what the NIEHS means by its statement that compounds such as dimethylethanolamine "interfere with" choline uptake. The NIEHS also provides no explanation as to how this is relevant to dimethylethanolamine toxicity. Cholines originate partly from the diet, and partly by endogenous formation. In studies in rabbits and rodents, dimethylethanolamine has been found to increase (not to decrease, as "interfere with" tends to suggest) serum choline levels, resulting in cholinergic side effects. The mechanism of action of dimethylethanolamine in treating depression and tardive dyskinesia has also often been postulated to be by elevation of choline levels (Ceder 1978), but there is considerable doubt about this, as it has not been verified experimentally (Stafford 1977). The increase in choline due to dimethylethanolamine is often postulated to be due to conversion of dimethylethanolamine to cholines (Re 1974; Miyazaki 1976; Nesse 1976), but one author argues that the increase has some other, unknown cause, at least in rabbits, because there is no known route for metabolism of dimethylethanolamine to cholines, and administration of cholines themselves does not have the effects that dimethylethanolamine does (Ceder 1978). Another author argues that there is some conversion of deanol to cholines, but that report is unclear as to the quantitative importance of this (Goldberg 1977). In addition to the increase in choline level due to dimethylethanolamine, dimethylethanolamine combines with cholines in mice, probably in the brain (Miyazaki 1976).

Our recommendation is two-fold. Firstly, the NIEHS should prepare a detailed assessment of the current state of knowledge. Secondly, taking into consideration the probable inter-species difference in metabolism, metabolism should be investigated in humans. Numerous humans, including many who are hospitalized, are administered dimethylethanolamine, and there therefore seems to be little reason why the metabolism of this compound should not be investigated by collection of the patients' blood and urine.

QT INTERVAL-PROLONGING AND PROARRHYTHMIA-INDUCING DRUGS

The FDA recommends the initiation of a study program to develop *in vitro* and *in vivo* test systems for assessing drug-induced induction of torsade de pointes and/or prolongation of proarrhythmic QT interval.

More than 50 non-cardiac drugs, including antibiotics, histamine blockers, and antipsychotic medications, are known to induce torsade de pointes or prolong the QT interval, largely by blocking potassium channels in myocardial cells (Haverkamp 2000). Arrhythmias are serious side effects, and QT prolongation is often used as a surrogate marker for cardiotoxicity. Canine telemetry models for assessment of QT prolongation are currently used to assess preclinical safety, as mentioned by the FDA. These presumably involve the implantation of electrodes into dogs, but the FDA refers to the "canine telemetry model," in the singular, without providing details as fundamental as whether the dogs used are

anesthetized (as in Killingworth 2000) or conscious (as in Takahara 2001), or whether or not they are killed. The FDA also does not mention that most canine telemetric methods have only recently been developed, and are not universally accepted. In all, the FDA's rationale and recommendations are so vague that it is difficult to make any worthwhile comments on them. However, we do support the initiation of "a study program to develop *in vitro* … test systems for assessing QT interval prolongation." Several *in vitro* models already exist, as a basis for this program. Some of these used *Xenopus* oocytes, and others recombinant mammalian cells (e.g., Chinese hamster ovary cells), and they are all based on testing the blocking of heterologous expression of the potassium channel (Haverkamp 2000).

One comment we can make at this preliminary stage is that, according to the most up-to-date research, involving a review of almost all published work (Viskin 2003), most patients who develop proarrhythmia have risk factors that can readily be identified from their medical history. These include female sex, organic heart disease, hypokalemia, and a history of long QT interval and/or arrhythmias. Patients with no risk factors (or without at least one in addition to female sex) are at very low risk, and the most important preventive measure for these patients is to avoid concurrent administration of two or more QT-prolonging drugs or administration of a drug that impairs the metabolism of a QT-prolonging drug. Even for patients with risk factors, the best improvement in reduction of harm is achieved by electrocardiography before treatment.

A great deal of recent research has also focused on the specific drugs that give rise to QT interval prolongation (Crouch 2003), and this should make it possible to estimate the risks to patients on this basis. Placebo-controlled clinical studies are the most effective means for estimating QT-prolonging effects in humans, and these are "now an integral part of the evaluation of new medications" (Viskin 2003). Information is also obtained from careful post-marketing surveillance. A recent review gave far more attention to *in vitro* and clinical investigation of QT-prolonging and arrhythmia-inducing drugs than to animal studies, and stated that the latter are "not appropriate for screening" (Haverkamp 2000).

To summarize, it is the responsibility of the FDA to show how any information it intends to obtain from further animal studies can be expected to save more human lives than the above simple, practical advice.

GLUCOSAMINE

The NCI recommends investigation of the chronic toxicity and carcinogenicity of glucosamine (CAS no. 3416-24-8), and the carcinogenicity of chondroitin sulfate and glucosamine combined. The latter is discussed above, under chondroitin sulfate, so only the former is discussed here.

A certain amount of research has been carried out on glucosamine toxicity (Bonadonna 1996; Giaccari 1996). With respect to cancer, in rats and mice, glucosamine has been found to be antineoplastic rather than carcinogenic: it inhibits the growth of transplanted tumors (Bekesi 1970), and even leads to the necrosis of tumor cells in lungs (Molnar 1972). In addition, some bacterial glucosamine-derived lipids stimulate macrophages to produce tissue necrosis factor (Lasfargues 1988). The NCI should prepare a comprehensive summary of all available information, and detail precisely what additional information is necessary.

Glucosamine is widely used as a medication, most commonly for the treatment of osteoarthritis (Barclay 1998). It therefore offers an ideal situation for epidemiological studies, as samples can be collected and measurements taken readily from patients. We therefore strongly maintain that research into glucosamine toxicity should concentrate on epidemiology.

NANOSCALE MATERIALS

Rice University recommends that the following areas should be investigated with respect to nanoscale materials: (i) size- and composition-dependent biological disposition of nanocrystalline fluorescent semiconductor materials; (ii) toxicological characterization of high-aspect-ratio carbon nanomaterials; (iii) role of particle core and surface composition in immunotoxicity; and (iv) phototoxicity of representative metal oxide nanoparticles. This recommendation is presumably a response to the concerns raised by three groups of researchers at the March 2003 meeting of the American Chemical Society (Service 2003).

The use of nanoscale materials is a new area of research, so work on the toxicity of these materials has only recently started. Preliminary findings include granulomas caused by graphite nanotubes in the lungs of mice, and the lethality of polytetrafluoroethylene nanoparticles in rats (Service 2003). Some of the most important work in this area, on adriamycin-carboxymethyldextran magnetic nanoparticles, has as yet only been published in Chinese (Shi 2003), and we therefore advise Rice University to access Chinese-language databases to make a full search of available information.

Our central point is that Rice University has made a number of vague, confusing and extremely broad recommendations. This recommendation in the *Federal Register* does not even distinguish between toxicity due to the compounds of which nanoparticles are composed or with which they are coated, and toxicity due to the physical fact of their being nanoparticles. This is a crucial point, as it is the latter about which very little is known, and polytetrafluoroethylene nanoparticles 20 nm in diameter were lethal in rats, probably due to the inability of macrophages to eliminate them from tissues, whereas polytetrafluoroethylene nanoparticles 150 nm in diameter had no negative effects (Service 2003). To summarize, Rice University should prepare a document detailing the projected use of the different types of nanoparticles, and the exact information needed about their toxicity.

TRANS-RESVERATROL

The NIEHS recommends toxicological characterization of trans-resveratrol (resveratrol; CAS no. 501-36-0), and investigation of its carcinogenicity and reproductive toxicity. Resveratrol is a naturally occurring compound found in various soft fruits, red wine and peanuts. Our central position is that research on the toxicity of compounds that occur in such a wide range of foods, which have been eaten for thousands of years, is only necessary if there is a very strong reason to suppose them to be dangerous. This position is clearly common sense; its rejection would necessitate the exhaustive toxicity testing of all combinations of all of the enormous number of compounds that occur in foods.

There have been numerous reports about the health benefits due to resveratrol:

(i) It inhibits cardiovascular damage, due to biological properties such as antioxidant activity, modulation of hepatic apolipoprotein and lipid synthesis, inhibition of platelet aggregation, and stimulation of pro-atherogenic eicosanoid production by human platelets and neutrophils (Goldberg 1995; Soleas 1997; Sun 1997; Das 1999; Bradamante 2003; Cal 2003; Floreani 2003; Haider 2003; Miura 2003).

(ii) It counters the developmental toxicity of ethanol (Drdova 2001; Glogarova 2001).

(iii) It inhibits the growth of *Helicobacter pylori* (Mahady 2003).

(iv) It has a wide range of anti-cancer actions, as reported in hundreds of publications (e.g., Jang 1997, 1998a, 1998b, 1999; Steele 1998; Cardenas 1999; Surh 1999; Adhami 2003; Bernhard 2003; Cal 2003; Dong 2003; Liang 2003; Miura 2003; Schwarz 2003; She 2003; Zhou 2003). Its inhibition of breast cancer development is discussed below.

The health benefits of resveratrol, especially its cardioprotective properties (the putative cause of the so-called "French paradox"), have led to its widespread use as a dietary supplement. We do acknowledge the possibility of risk due to the use of even naturally occurring compounds in the form of high-concentration dietary supplements. However, as discussed below, the evidence points against resveratrol having serious toxicity, and *in vitro* methods are the most appropriate for investigating those areas about which there are legitimate grounds for concern. Two other points should also be noted at this stage. Firstly, the use of a highly concentrated dietary supplement offers a suitable population for conducting epidemiological studies. Secondly, the most appropriate response to concerns about the use of resveratrol as a dietary supplement is to encourage consumption of resveratrol-containing foods rather than the supplement. Almost all dieticians consider that a marked increase in the US consumption of fresh fruit would result in health advantages, and it seems strange that, in response to a hypothetical risk due to resveratrol, the NIEHS does not advocate a redoubling of efforts to encourage fruit consumption, but instead recommends initiating a program of animal testing. This looks very much like taking an overly technological approach to a problem in preference to a practical, common-sense approach.

To turn to the specific areas of concern, the NIEHS states vaguely that there is "suspicion of toxicity based on estrogenic and genotoxic activity." We therefore discuss these two areas as follows:

1. Estrogenicity

Resveratrol is usually considered to be a phytoestrogen, although there is still some uncertainty about the validity of this classification (Stahl 1998). That a compound is a phytoestrogen means that it acts estrogenically in at least some tissues (Calabrese 1999), and the human tissues in which resveratrol acts estrogenically, in some species, are the cardiovascular system (Ashby 1999) and the uterus. Taking into account the fact that the NIEHS' recommendations are to investigate carcinogenicity and reproductive toxicity, two areas with respect to the estrogenicity of resveratrol may warrant concern:

(i) *Breast cancer*. Estrogens such as estradiol and diethylstilbestrol inhibit cancer in the uterus yet stimulate it in the breast. However, there are several reasons why the estrogenicity of resveratrol does not mean that it is likely to have cancer-stimulating estrogenic effects in the breast. Firstly, resveratrol is only very weakly estrogenic, with an affinity for uterine estrogen-receptors five orders of magnitude lower than estradiol and diethylstilbestrol, and with apparently no activity in the rat uterotrophic assay (Ashby 1999). Secondly, it is now known that the vast majority of estrogen-system-interacting compounds are not strictly estrogenic, but are selective estrogen receptor modulators (SERMs), which means that they are capable of acting estrogenically in certain tissues (e.g., inhibiting cancer in the uterus), yet anti-estrogenically in others (e.g., inhibiting cancer in the breast). Several recently developed drugs, such as raloxifene, are SERMs, enabling treatment of menopausal symptoms without increasing the probability of breast cancer, and the available evidence with respect to resveratrol suggests that it also is a SERM, as both *in vitro* and *in vivo* studies suggest that it inhibits breast cancer development despite its usual classification as an estrogen (Clement 1998; Mgbonyebi 1998; Gaubatz 1999; Subbaramaiah 1998, 1999; Otake 2000; Sgambato 2001; Dobuisson 2002; Cal 2003; Wietzke 2003). Its anti-cancer action in the breast is not necessarily due to anti-estrogenic activity, but may be due instead, or additionally, to estrogen-receptor-independent activity (El-Mowafy 2003; Levenson 2003). The SERM

nature of resveratrol, if in the last analysis considered to be important, should therefore be investigated by *in vitro* investigation of an array of cell lines from various tissues. A battery of *in vitro* tests based on this principle is available (Safe 1998).

(ii) *Male reproductive toxicity*. Currently, there is intense concern about male sterility and genital malformation due to environmental xenoestrogens. It is therefore premature and illogical to investigate the effects of resveratrol in isolation, and they should be seen in the context of the overall global plan for investigating and resolving this issue. However, the above points about the role of resveratrol with respect to breast cancer (its weakness, and its probable SERM nature), apply equally to male reproductive toxicity.

2. Genotoxicity

There is a limited amount of evidence that resveratrol causes DNA cleavage (Fukuhara 1998). However, there is more evidence for its suppression of mutagenicity (Uenobe 1997), and this is also supported by its anti-cancer action, as discussed above.

TETRABROMOBISPHENOL A

The NIEHS recommends toxicological characterization of tetrabromobisphenol A (CAS no. 79-94-7), and investigation of its neurodevelopmental toxicity and carcinogenicity.

A great deal of work has been carried out on the toxicity of tetrabromobisphenol A, a fire retardant that is widely used in the building industry and in electronic equipment. A certain amount of this work has been published; for example, tetrabromobisphenol A is embryotoxic in rats (Akita 2002; Yokoyama 2002) and domestic fowl (Berg 2001), and is slightly hepatotoxic in rats (Szymanska 2000). *In vitro* studies have also shown that tetrabromobisphenol A induces the hemolysis of erythrocytes (Inouye 1979). However, the vast majority of the work has been carried out within corporations, and remains unpublished, although reports have been submitted to the US Environmental Protection Agency (EPA). Before planning any additional studies, we suggest that the NIEHS examine the reports of all previous work that has been carried out, and detail the areas in which information is required.

With respect to thyroid toxicity (mentioned by the NIEHS), *in vitro* research suggests that tetrabromobisphenol A acts as a thyroid hormone agonist (Kitamura 2002), analogously to its (slight) estrogenicity (Korner 1998; Olsen 2003). It also stimulates the proliferation of cultured breast cancer cells at a certain range of doses (Samuelsen 2001). It is premature to discuss the work that is needed until all previous findings have been analyzed carefully, but we suggest that much information on thyroid and neurodevelopmental toxicity and carcinogenicity can be obtained by continuing *in vitro* research of this type.

TETRABROMOBISPHENOL A BIS (2,3 DIBROMOPROPYL ETHER)

The NIEHS recommends toxicological characterization of tetrabromobisphenol A bis (2,3-dibromopropyl ether), referred to below as TBBPA-bis (CAS no. 21850-44-2), and investigation of its *in vivo* genotoxicity, metabolism and carcinogenicity.

Little work has been carried out on the toxicity of TBBPA-bis. It has been found to be a direct mutagen in *Salmonella typhimurium*, but it was shown not to have certain other types of *in vitro* mutagenicity ("Tetrabromobisphenol A and derivatives" 1995). These *in vitro* studies were carried out by companies, and the reports have not been published, although they have been submitted to the EPA.

We consider that it is highly premature to discuss animal experiments on TBBPA-bis, when not even the most fundamental information is available about its physicochemical properties, and the amount of human exposure. The World Health Organization agrees with us on this point, and makes the following statement: "This substance cannot be evaluated until adequate data become available on physical and chemical properties, production and use, environmental transport, distribution and transformation, environmental levels and human exposure" ("Tetrabromobisphenol A and derivatives" 1995, p. 78).

TUNGSTEN

The National Center for Environmental Health (NCEH) recommends toxicological characterization of tungsten (CAS no. 7440-33-7), and investigation of its carcinogenicity, with these studies focusing on a representative soluble tungsten compound.

A considerable amount of *in vitro*, animal and human work has already been carried out on the toxicology of tungsten and its compounds, with more than 1,000 reports having been published. The first task for the NCEH should therefore be to assess the available data. Most human exposure to tungsten is in the form of tungsten metal and its alloys (e.g., tungsten steel), rather than compounds, and there is little evidence that tungsten gives rise to toxicity that is quantitatively or qualitatively similar to that of its compounds. It is therefore difficult to understand why the NCEH recommends that "studies should focus on a representative soluble tungsten compound," and the below discussion is concerned only with the toxicity of tungsten. However, far more research has been carried out on tungsten compounds, such as sodium tungstate, tungsten dioxide, tungsten trioxide, tungsten carbides, tungsten silicide and ammonium paratungstate.

With respect to animal studies, inhalation studies in rats have shown tungsten to have pulmonary toxicity (Delahant 1955; Mezentseva 1964; Song 1990), and tungsten also increases mammary cancer frequency in rats (Wei 1985). On the other hand, several chronic oral administration studies in mallards, carried out to investigate the environmental impact of tungsten steel shot, have found no toxicity due to tungsten (Kelly 1998; Mitchell 1999).

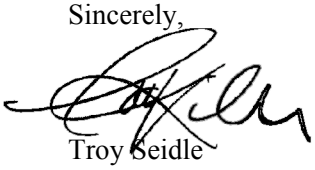
With respect to human data, there is probably a slight increase in lung cancer risk in welders who use thoriated tungsten electrodes. However, this is thought to be due to the thorium, which is radioactive, and it is suggested that the risk could be eliminated by using electrodes containing cerium or lanthanum instead of thorium (Vinzents 1994). In addition, workers in a tungsten-alloy-manufacturing plant had elevated urinary tungsten levels, and showed various complaints, including dyspnea, coughing, tachycardia, headaches, dizziness, nausea, loss of appetite, and olfactory disorders (Vengerskaya 1962). Several reported human cases of pulmonary fibrosis may also have been due to inhaled tungsten (Heuer 1962; Rochemaure 1972; Balmes 1987).

As stated above, we recommend that the highest priority for determination of tungsten toxicity is to assess all previous reports in detail. The second priority should be to carry out a full exposure and epidemiology study of industrially exposed workers. Reports on the medical importance of tungsten exposure have mostly been small-scale or anecdotal, and there is therefore an urgent need for statistical data. Svartengren (1994) has stated that: "The effects of tungsten exposure on humans have not been determined as no cases of exposure to tungsten or tungsten compounds alone without concomitant exposure to other agents have been reported;" however, it is difficult to imagine that this difficulty, exposure to multiple compounds, cannot be resolved by the use of appropriate statistical techniques.

SUMMARY

A thorough review of the most recent chemical nominations and ICCEC testing recommendations only serves to reinforce the concerns expressed in our opening remarks: that the 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. Although we trust that our comments have amply demonstrated the inappropriateness of the proposed animal testing in each 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
Dr. C. Portier

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