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These comments on the U.S. Environmental Protection Agency’s (EPA) Federal Register notice of July 24, 2008, “Testing of Certain High Production Volume Chemicals; Second Group of Chemicals” are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Alternatives Research Development Foundation, and the American Anti-Vivisection Society. PETA and PCRM have consistently commented on every test plan submitted under the HPV Challenge Program for which animal testing was proposed (see comments on test plans posted through September 2008 at http://www.epa.gov/chemrtk/pubs/summaries/viewsrch.htm). We also request the opportunity to present our comments orally in a public hearing.

While we appreciate EPA’s effort to integrate into the proposed test rule some of the basic animal welfare principles enumerated in its October 14, 1999, letter to program participants (1999 Letter; EPA-HQ-OPPT-2007-0531-0017), as well as its response to our April 25, 2001, comments (attached; EPA-HQ-OPPT-2005-0033-0090) on the first proposed test rule for HPV chemicals (EPA-HQ-OPPT-2007-0531-0020), a number of critical issues have not yet been addressed at all and need to be before the second test rule is issued.

GENERAL COMMENTS ON APPROACH TO CHEMICAL ASSESSMENT

We are very concerned that despite some recent efforts within EPA to move away from an in vivo testing paradigm, such as the National Center for Computational Toxicology’s ToxCast Program, extensive animal testing is still being proposed for HPV chemicals. As detailed below under “Chemical Specific Comments,” these proposals ignore not only existing data in many cases, but more generally the primary animal welfare principle enumerated in EPA’s 1999 Letter. This advises program participants to “conduct a thoughtful, qualitative analysis rather than use a rote checklist approach” and makes clear that “[p]articipants 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” (emphasis added). EPA agreed to incorporate the principles enumerated in this letter into any subsequent test rules. Furthermore, experience gained during the review of HPV test plans over the last eight years should be applied here in order to further reduce the number of animals killed. This experience is summarized in PCRM’s June 4, 2008, comments on the Chemicals Management and Assessment
In considering HPV chemicals, we urge OPPT to join with other offices in EPA, and
other regulatory agencies, in implementing the approach outlined in the National
Research Council’s (NRC) report, *Toxicity Testing in the Twenty-first Century: a Vision
and a Strategy*. In this report – commissioned by EPA – NRC’s Committee on Toxicity
Testing and Assessment of Environmental Agents recommended a dramatic shift away
from traditional hazard assessments focused on whole animal tests that assess apical
epipoints toward more human-relevant *in silico* and *in vitro* methods that assess the
perturbations of toxicity pathways at the molecular level. The report concluded that a
molecular and mechanistic understanding of toxic effects is absolutely essential for
accurate predictive toxicity assessment. The limitations of the current approach, not the
least of which is species extrapolation, yields unrefined assessments with little
understanding of actual impact on human health. **If the intention of the proposed test
rule is robust chemical assessment, rather than a checklist exercise to fill narrowly-
defined data gaps, then an approach consistent with NRC’s vision must be extended
to the assessment of HPV chemicals.**

**USE OF EXISTING DATA**

In response to comments on the first proposed test rule, EPA repeatedly asserted that any
request to make test plans available for public review and comment prior to initiating
testing would prolong the rulemaking process. In this regard, we note that EPA’s
response is dated May 31, 2005 – more than four years after our comments were
submitted. The final test rule for this first group of HPV chemicals was published on
March 16, 2006 – more than five years after it was proposed. As of this writing, the most
recent EPA comments on an HPV Challenge Program test plan were published on
September 24, 2008 – two years and nine months after the test plan was submitted, two
years and three months after the public comment period for this test plan had closed.
Clearly the rulemaking process is long, but a 120-day public comment period seems
insignificant compared to the lengths of time EPA requires for its own actions.

Also in response to these comments, EPA claimed that its “designation in the proposed
test rule of the tests and test methods to be required for each chemical is, in essence, the
test plan for each chemical.” Elsewhere in its response, however, EPA agreed that
publicly available databases do not provide access to all existing data on particular
chemicals and stated its hope that the test rule would “cause manufacturers to release any
in-house testing results and other existing data of which they are aware.” **If it is in part
the purpose of the proposed test rule to make this data available – during the public
comment period or after – how is the public to comment on their adequacy and
whether or not additional testing is called for?** Public advocacy organizations, such as
PETA, are left to attempt to research a long list of chemicals as thoroughly as possible
without all existing data, with limited resources, and within a single, short public
comment period.
CATEGORIES, (QUANTITATIVE) STRUCTURE ACTIVITY RELATIONSHIPS ((Q)SAR), WEIGHT OF EVIDENCE (WoE), AND INTEGRATED TESTING STRATEGIES

Given the progressive wording present in EPA’s 1999 Letter, and the commitment to incorporate those principles into any subsequent test rule, the decision to exclude the use of chemical categories or analogs, (Q)SAR tools and approaches, and Integrated or Intelligent Testing Strategies in this proposed test rule is unwarranted and unjustified and must be reversed.

Although comments suggesting procedures for the inclusion of category and SAR approaches in TSCA section 4 rulemaking were received in response to the first proposed test rule from diverse organizations, including the Synthetic Organic Chemical Manufacturers Association, the American Chemistry Council and several animal protection organizations, EPA has again excluded these approaches from the current proposal. This is contrary to activities being pursued at other offices in the EPA, such as the Office of Pesticide Programs, and within OPPT itself.

EPA states that these approaches would require “time consuming, and intensive procedural steps, such as multi-phase rulemaking, without a corresponding benefit,” (emphasis added). However, throughout the HPV Challenge Program, category and SAR approaches have resulted in considerable benefit by reducing testing required and consequently the number of animals killed in that testing. We are aware that OPPT has available or in development a number of (Q)SAR tools and approaches that should be fully utilized where possible. In fact, EPA is a leader in the development of (Q)SAR and expert systems. For all of these reasons, we find it inexplicable that category and (Q)SAR approaches will not be allowed. These approaches are among the most important and effective ways to reduce animal testing and EPA’s stated interest in efficiency is not an acceptable reason for disallowing their use in rulemaking.

We appeal to EPA, and to chemical companies themselves, to carefully examine all publicly-available chemical databases, including OECD’s EChemPortal (http://webnet3.oecd.org/echemportal/) and the HPV Challenge Program web site. The EChemPortal draws together available data from many sources to provide master search capabilities. Both have search capabilities that make it relatively simple to find potential chemicals or categories that might contain data to “read across” to any of these test rule chemicals. As one obvious example we cite Phenol, 4,4’-methylenebis[2,6-bis91,1-dimethylethyl]- (CAS 118-82-1) as part of a populous and well-characterized category. In the course of preparing these comments, we easily identified an extensive report on phenols prepared by the Environmental Protections Division of the Government of British Columbia. We also recommend that companies first use the (Q)SAR tools EPA has made available on its web site (http://www.epa.gov/oppt/exposure/pubs/episuite.htm), or those within the OECD (Q)SAR Toolbox (http://www.oecd.org/env/existingchemicals/qsar), before planning any in vivo testing. There is no reason these tools cannot be used for well-represented chemical classes.
While consideration of WoE approaches was suggested in the 1999 Letter, it has not been taken advantage of in this proposed test rule. Ongoing work at several agencies, including OECD, EPA, International Life Sciences Institute, and the Dutch National Institute for Public Health and the Environment (RIVM) should be given consideration. The Dutch chemicals agency RIVM has recently completed a report investigating the potential for the use of Integrated (or Intelligent) Testing Strategies (ITS) under the new European REACH legislation (Vermeire, TG et al., 2007). The report gives a general discussion of alternative methods and ITS, including (Q)SAR and chemical categories, in vitro studies, toxicogenomics, and exposure-based waiving of testing; a WoE approach using Bayesian analysis of existing information from all potential sources makes this report a useful tool for anyone designing a plan for the testing of a candidate chemical.

There was broad international support for WoE approaches at a December 2007 OECD workshop titled Integrated Approaches to Testing and Assessment. We are sure EPA is aware of these principles because it was a major sponsor of the IATA Workshop. Principles that will be followed during the REACH process in an attempt to maximally avoid in vivo testing were presented, and include (http://ecb.jrc.it):

- Existing human and animal information
- Non-test based info (QSAR, categories)
- WoE
- Non-guideline toxicity tests
- In vitro methods
- Situations where testing is not feasible
- Exposure-driven regulations
- Evaluation of testing proposals

In addition, the workshop summary and recommendations for future work, once published, have the potential to be valuable tools for those seeking to use QSAR, WoE, and other “alternative” approaches to fulfilling chemical hazard information needs. A good opportunity to use such a WoE approach involves chemicals that are slated for dermal systemic testing (whether acute or chronic). For such chemicals, it is recommended that manufacturers first determine whether the chemical is absorbed through the skin. This can be accomplished with in vitro or in silico approaches, such as OECD TG 428. If no appreciable absorption is found, systemic effects cannot manifest, and further testing should not be requested. We urge manufacturers to consider this and other potential WoE strategies, or testing strategies before checking another box. Further information can be found in the attached Champ comments.

Fundamentally, these scientifically-sound principles must first be utilized before any company sponsors any new in vivo testing, and we request that EPA amend the proposed test rule to reflect the latest best practices in chemical hazard identification.
**TEST METHODS**

As a general comment, chemical properties and environmental fate data are lacking for many of the substances included in the proposed test rule. **With such poorly characterized substances, it is senseless to conduct any toxicity tests until this fundamental information – on boiling point, water solubility, hydrolysis, vapor pressure, octanol-water partition coefficient, for example – is known.** In many cases, simply knowing these properties of a compound can preclude the need to conduct further tests.

**Acute toxicity**

Acute toxicity tests – by design – inflict suffering so extreme that they result in the deaths of half of the animals to whom the test substance is administered during the tests’ short duration. The proposed test rule requires mammalian acute toxicity testing for seven of the nineteen chemical substances listed. These include: sodium glucoheptonate (CAS 31138-65-5), a substance listed by the U.S. Food and Drug Administration (FDA) as a secondary direct food additive permitted in food for human consumption (FDA, 2008); C.I. Leuco Sulphur Black 1 (CAS 66241-11-0), a sulphur dye for which there is existing acute toxicity data in rats (European Chemicals Bureau, 2000a); and sulfated and oxidized forms of castor oil (CAS 68187-76-8 and 68187-84-8), a naturally-occurring vegetable oil listed by FDA as generally recognized as safe (GRAS; FDA, 2008) that is familiar through its long history of use in pharmaceuticals, food and other industries.

Recent changes in the requirements of some regulatory entities, as well as activities at the European Center for the Validation of Alternative Methods, call into question the acute toxicity testing requirement altogether. A coalition of European pharmaceutical companies determined that regulatory decisions were almost never predicated on the results of acute oral toxicity tests, prompting the International Council on Harmonization and its regional pharmaceutical regulators to propose removing the requirement for an acute oral toxicity testing from the testing guidelines (Robinson S et al., 2008; European Medicines Agency, 2008), and obtain any necessary information on acute toxicity from available repeated dose studies. One of the chemicals for which acute toxicity data is requested, C.I. Leuco Sulphur Black 1, has repeated dose data available, and we suggest that the chemical manufacturers investigate strategies to use this data. ECVAM reports that it was able to correctly classify a majority of chemicals using this approach (Kinsner, A et al., 2008). Combined with data already available showing this chemical is likely non-toxic (see below), a WoE evaluation leads to the conclusion that no further testing needs to be considered.

Evidence shows that more than 85% of industrial chemicals are non-toxic (Kinsner, A et al., 2008). **Efforts to validate the in vitro 3T3 NRU cytotoxicity assay (ICCVAM, 2006) also indicate that this assay correctly discriminates non-toxic chemicals (those with an LD$_{50}$ $\geq$ 2000 mg/kg) from more toxic ones, and shows very good correlation with mammalian LD$_{50}$ data at both extremes of the toxicity spectrum (i.e. very toxic and non-toxic).** ECVAM is currently conducting a definitive validation of this
hypothesis with thousands of chemicals (Kinsner, A et al., 2008); data are expected in early 2009. Consideration of the current knowledge of these chemicals leads one to conclude that they are likely not acutely toxic, and could be assessed adequately within the HPV program by using the 3T3 NRU cytotoxicity assay. **If further supportive evidence is needed, waiting a few months for the ECVAM results in order to avoid in vivo oral acute toxicity tests is completely appropriate.**

For industrial chemicals that might be tested by the inhalation route, the narcosis hypothesis could be helpful (Veith, G et al., 2008; Mackay, D et al., 2008). Starting with the understanding that the water and air solubility (vapor pressure) of a chemical represent the effective dose that an animal would “inhale” (for fish or mammals respectively), this concept predicts the inhalation toxicity of non-reactive chemicals using available acute fish test data, and vice versa. The International QSAR Foundation has preliminary data showing very good correlations, and is using this concept to build a QSAR model. This emerging method is exactly the kind of thoughtful toxicology that the EPA and chemical manufacturers should be using within the HPV program. Much more information can be found here: http://mckim.qsari.org/Presentations2007.html.

For all of these reasons, it is clear that animals should not be subjected to acute toxicity tests on such substances as those mentioned above.

**Aquatic toxicity testing**

Based on the OECD’s TG 203, each fish acute toxicity test will kill approximately 120 animals. The proposed test rule requires fish acute toxicity testing for as many as twelve of the nineteen chemical substances listed depending on the octanol-water partition coefficient. In addition to the substances mentioned above, these include: 1,3-Propanediol, 2,2-bis[(nitrooxy)methyl]- dinitrate (ester) (CAS 78-11-5), an explosive for which there is existing data in fathead minnow and bluegill (Bentley, RE et al., 1975); ethanedioic acid (CAS 144-62-7), a naturally-occurring metabolic product for which there is existing toxicity data in goldfish and bluegill (Ellis, MM, 1937; Buzzell JC et al., 1968); phosphorochloridothioic acid, O,O-diethyl ester (CAS 2524-04-1), a chemical intermediate that is insoluble in water; and benzenediamine, ar,ar-diethyl-ar-methyl (CAS 68479-98-1), a polymer curing agent for which there is existing toxicity data in goldfish (Bayer MaterialScience LLC, 2007).

*In vivo* aquatic toxicity testing is unnecessary considering the availability of suitable *in silico* and *in vitro* test methods such as ECOSAR and the DarT test. In fact no endpoint is better suited to the use of (Q)SAR tools than aquatic toxicity. As EPA is aware, many industrial chemicals fit into a narcosis model of toxicity, and those that don’t may fit into other predictive “mode of action” categories. HPV chemicals are the perfect chemical space for (Q)SAR tools built by EPA, and EPA and producers must take advantage of them.

The EPA guidance document “The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program” notes that if a QSAR model is
available, it may be used with the appropriate rationale for its applicability to the HPV candidate chemical and identifies ECOSAR as an available model to estimate aquatic ecotoxicity (EPA-HQ-OPPT-2007-0531-0049).

Moreover, fish acute toxicity testing is intended to predict the potential for economic loss and ecological damage resulting from fish death on a large scale. If exposure to test substances is toxic to the food on which fish subsist, fish populations could be effected even without direct fish toxicity. It is therefore reasonable to characterize the toxicity of test substances to aquatic plants and invertebrates prior to consideration of acute toxicity testing on fish.

The validated DarT test uses fertilized zebrafish eggs as a surrogate for living fish (Nagel, R, 2002). Since the eggs do not hatch during the test period, the DarT is classified as a non-animal test. The exposure period is 48 hours, and assessed endpoints comparable to lethality in vivo include incomplete gastrulation and somite development, absence of a heartbeat and coagulation of eggs. Additional endpoints provide a more detailed assessment of the effects of test substances. The reliability and relevance of the DarT test have been confirmed through an international, multi-laboratory validation study coordinated by the German Environmental Protection Agency. Predictions of acute toxicity from the DarT test were highly concordant with in vivo reference data (Schulte, C et al., 1996). This in vitro test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius, T et al., 1995) and has since been nominated for development into an OECD test guideline. It is clearly suitable for immediate use as a replacement for the use of fish in SIDS screening studies.

If a fish acute toxicity test is still perceived to be required, the European Center for the Validation of Alternative Methods’ Ecotoxicology Task Force evaluated a fish acute threshold (step-down) test concept with the potential to reduce the number of fish used in ecotoxicity testing by approximately 50%-70% (Jerama, S et al., 2005). Since fish are less sensitive than algae or Daphnia in acute aquatic toxicity tests roughly 85% of the time, an upper threshold concentration (UTC) is set at the lowest EC50 value observed in the algae and daphnia tests. An acute test is carried out at the UTC using five test and five control fish. If no toxicity is observed, no further tests are carried out and the acute fish toxicity result (LC50) is reported as greater than the UTC value. If toxicity is observed, a second test is performed at a step-down concentration using a dilution factor of 3.2, based on a semi-logarithmic concentration series. The testing continues to lower concentrations until no toxicity is observed. The LC50 96-hour value can be obtained from all step-down threshold test data by applying the binominal method of interpolation. An additional refinement could be obtained by terminating the test after 24 hours of exposure, when lethality and/or serious morbidity are observed in two out of five fish (Hutchinson, TH et al., 2003). We strongly urge the use of this new testing strategy, which is consistent with the goal of obtaining screening-level toxicity data, when no replacement for the acute fish toxicity test is perceived to be applicable.
Finally, we would like to call your attention to what we assume is a typographical error. In column C (Special Conditions) of Table 3 on page 43339, the following contradictory instructions are given:

If log Kow < 4.2: Test Group 1 is required.
If log Kow ≤ 4.2: Test Group 2 is required.

In vitro genetic toxicity and combined repeated dose toxicity with reproduction/developmental toxicity screening

The proposed test rule states that “[p]ersons who would be required to conduct testing for chromosomal damage are encouraged to use in vitro genetic toxicity testing.” Clearly, a rule must stipulate the agency’s requirements, not merely “encourage” actions by regulated entities. Further, as described in the proposal, companies may use in vivo tests and then submit their rationale along with the results: “A subject person who uses one of the in vivo methods instead of the in vitro method… would be required to submit to EPA a rationale for conducting that alternative test in the final study report,” (emphasis added). If the company's rationale is flawed, there will have been no opportunity to spare animals from these tests. We call upon EPA to amend the proposed rule to mandate the use of internationally accepted in vitro genetic toxicity screening tests.

Companies proposing to use in vivo genetic toxicity testing should be required to submit a compelling justification for an exemption from a requirement to use in vitro tests prior to the testing being conducted, and opportunity for public comment should be made available prior to EPA’s decision. EPA should only grant exemptions when the physical properties of the chemical make the use of in vitro tests impossible.

Similarly, EPA proposes that “a subject person who uses the combination of the Reproduction/Developmental Toxicity Screening Test and the Repeated Dose 28-Day Oral Toxicity Study in place of the Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screen would be required to submit to EPA a rationale for conducting these alternate tests in the final study reports.” Again, if this rationale is flawed, there will have been no opportunity to reduce the number of animals killed – by hundreds – by using the combined protocol. EPA should amend the proposed rule to require companies to use the combined protocol unless a compelling justification to test a chemical using both protocols is submitted prior to the testing being conducted, and opportunity for public comment should be made available prior to EPA’s decision.

In response to similar comments on the first proposed test rule, EPA noted that it “anticipates that test sponsors will generally choose to conduct the less costly and less resource intensive in vitro and combined tests unless there are sound scientific reasons not to do so.” In our retrospective analysis of all test plans submitted to the HPV Challenge Program, we find that 21% of in vivo chromosomal aberration tests originally proposed were subsequently replaced by the in vitro test in revised test plans saving the lives of more than 500 animals. Similarly, 35% of individual repeated dose, reproductive and developmental toxicity tests were subsequently replaced by combined repeated dose...
with reproduction/developmental toxicity tests saving the lives of more than 16,000 animals. In addition, EPA once again suggests that publication of study plans in order to provide an opportunity for public comment on the inclusion of any alternative tests could cause “a significant delay in the initiation of testing.” We refer to our comments above under the use of existing data and reiterate that EPA’s dubious concern for efficiency is not an acceptable reason for allowing test sponsors to justify the use of *in vivo* methods after the fact.

**Developmental toxicity testing**

Further, with regard to developmental toxicity testing, we note that an embryonic stem cell test (EST) has been validated by the European Centre for the Validation of Alternative Methods (ECVAM) as a test for embryotoxicity – a critical parameter and manifestation of developmental toxicity (Genschow, E et al., 2002). The test uses rodent-derived stem cells, which survive in culture indefinitely and are capable of differentiation. Embryotoxicity is determined by the concentration of a test chemical required to inhibit differentiation by 50% together with growth inhibition by 50% relative to controls. This validated test method is ideally suited for immediate use as a reduction measure in a basic, screening-level program like EPA’s HPV Challenge – whereby chemicals that test positive for embryotoxicity could be classified as probable developmental toxicants without further testing. **We strongly urge the use of this new testing strategy, which is consistent with the goal of obtaining screening-level toxicity data.**

**CHEMICAL-SPECIFIC COMMENTS**

The following list is not comprehensive. It represents a cursory review of the chemicals in the proposed test rule. The information presented demonstrates EPA's failure to thoroughly review existing data or to consider the most basic principles of thoughtful toxicology applied to reducing the number of animals killed in the proposed tests. This existing data must be carefully evaluated, and these principles must be considered for each chemical in the proposal.

**Acetaldehyde (CAS 75-07-0)**

EPA has requested reproductive and developmental testing of acetaldehyde, again illustrating a rote checklist approach to chemical assessment. This test would kill approximately 675 animals. There are plentiful data available on the reproductive and developmental effects of acetaldehyde including various routes of exposure. A search for acetaldehyde in the DART database of developmental toxicology literature revealed 332 unique citations (http://toxnet.nlm.nih.gov/cgi-bin/sis/search 10/16/08). Some examples of these data include the results of intraperitoneal studies indicating that acetaldehyde causes adverse developmental effects including delayed ossification, skeletal malformations, growth retardation, delayed skeletogenesis, and malformation of the face and limbs (ACGIH, 1991); a chronic inhalation study in rats indicated growth retardation (US EPA, 1994 IRIS). There is also a wealth of data on the reproductive and
developmental effects of ethanol, the metabolic precursor to acetaldehyde, which could be applied to the assessment of acetaldehyde. Since acetaldehyde is the major metabolite of ethanol, alcohol consumption represents the main source of acetaldehyde exposure (DHHS Report on Carcinogens, 11th ed.). Many of the adverse effects of alcohol are attributed to acetaldehyde (US EPA 749-F-94-003a, 1994), therefore data on the reproductive and developmental effects of ethanol are an excellent measure of the human health effects of acetaldehyde.

There is clearly adequate information on this chemical to meet the screening level requirements of this proposed test rule without additional animal testing. If additional testing is perceived as necessary, we ask EPA to strongly recommend screening via an EST. If the EST indicates acetaldehyde is a reproductive and/or developmental toxicant, then it should be regulated as such without animal testing.

**1,3-Propanediol, 2,2-bis[(nitrooxy)methyl]- dinitrate (ester) (Pentaerythritol tetranitrate; CAS 78-11-5)**

Pentaerythritol tetranitrate is listed in the proposed test rule as requiring a fish acute toxicity test. This test would kill approximately 120 animals. A U.S. Army study reported data on fathead minnow and bluegill, including a mean LC50 of 27,000 mg/L for fathead minnow, and classifies the substance as not acutely toxic (Bentley, RE et al., 1975). In addition, we note that Dyno Nobel Inc., has submitted the results of their testing under the first test rule and notes that since degradation rates exceed water solubility rates, there is no logical justification for completing any additional aquatic toxicity tests (EPA-HQ-OPPT-2007-0531-0062.1). We call upon EPA to review these studies and to reconsider its testing requirement for this substance in the proposed test rule.

**1H,3H-Benz[1,2-c:4,5-c’] difuran-1,3,5,7-tetrone (CAS 89-32-7)**

Acute toxicity testing is requested for this chemical. EPA indicates that there are four studies conducted with this chemical in mice, rats, and guinea pigs. These studies are in a different language but not necessarily inadequate, and it is incumbent upon the EPA to determine the usefulness of this data before requesting additional data. We urge the manufacturers of the chemical to participate in this effort as well. We also found, on Pubmed Toxicology, many more records with potential usefulness, which should be vetted prior to in vivo testing.

**2,4-Hexadienoic acid,(E,E)- (sorbic acid; CAS 110-44-1)**

Sorbic acid is listed in the proposed test rule as requiring a reproduction/developmental toxicity screening test. This test would kill approximately 675 animals. Several multi-generation studies in mice and rats reported no adverse effects of sorbic acid on reproductive function or post-natal development (Demaree, GE et al., 1955; Shtenberg, AI and Ignat’ev, AD, 1970; Gaunt, IF et al., 1975). EPA must review these studies and reconsider its testing requirement for this substance in the proposed test rule.
Further, we note that sorbic acid is a naturally-occurring fatty acid similar in structure to linoleic and oleic acids. It is metabolized in mammals in a similar manner to other fatty acids and can thus serve as an energy source (Walker, 1990). The metabolic pathway for fatty acids is the same for all chain lengths and does not distinguish between saturated and unsaturated fatty acids. Sorbic acid falls within the molecular range of fatty acids normally metabolized by animals and humans and has no structural characteristics that would indicate that it requires toxicity testing. In comments on Crompton Corporation’s HPV Challenge Program test plan for eicosenoic acid, EPA also encouraged the use of analog data to address human health endpoints which should be done here as well (http://www.epa.gov/chemrtk/pubs/summaries/eicoamez/c14960ct.pdf).

Sorbic acid has been safely used as a preservative and antimicrobial agent in foods, cosmetics and personal care products since the 1960s. In 2006, the Personal Care Products Council’s Cosmetic Ingredient Review (CIR) Expert Panel considered available new data on sorbic acid and potassium sorbate and concluded that sorbic acid was practically nontoxic in acute oral toxicity studies noting that no significant adverse effects were observed at dietary concentrations as high as 10%. CIR also noted that WoE indicates that sorbic acid is not mutagenic or carcinogenic, and no developmental effects have been observed with potassium sorbate (CIR, 2006). The potassium salt is the form more widely-used as a preservative, since it is more soluble in water than sorbic acid.

FDA lists sorbic acid as GRAS as a preservative for direct addition to food (FDA, 2008). In addition, the European Commission Cosmetic Directive has approved the use of sorbic acid without restrictions or warning labels at levels up to 0.6% (Eastman Chemical Company, 1998). GRAS substances generally have extensive databases demonstrating their lack of toxicity. In its 1999 Letter, EPA recommends that participants should specifically consider whether the information available makes it unnecessary to proceed with further testing involving animals and whether any additional information obtained would be useful or relevant. Considering existing data, WoE and GRAS status, it is imperative that EPA reconsider its testing requirements for sorbic acid in the proposed test rule. Testing sorbic acid in animals as required in the proposed test rule is particularly inappropriate.

**Ethanedioic Acid (Oxalic acid; CAS 144-62-7)**

Data on genotoxicity and reproductive and developmental effects are requested for ethanedioic acid despite the availability of numerous studies which address these endpoints including data from the National Toxicology Program (NTP). Several studies indicate reproductive and developmental effects including decreased growth rate, decreased body weight, decreased weight of reproductive organs, lesions in male and female reproductive tissues, increased number of abnormal sperm, increased estrus cycle, decreased pup weights, litter size, and number of live pups (Goldman M, et al, 1977; Gulati DK, et al, 1985; Lamb JC IV, 1997; Sheik-Omar AR and Scheifer HB, 1980)

For genotoxicity, several *in vitro* methods were negative for mutagenicity and clastogenicity. A 2004 assessment from the Health-Based Reassessment of...
Administrative Occupational Exposure Limits in the Netherlands determined the primary target organs for toxicity appear to be the kidneys and nervous system, whereas genotoxicity was not a major concern (Health Council of the Netherlands, 2004).

Several physicochemical and environmental fate data are also requested. As a general rule, these data should be generated prior to making decisions on additional tests because the chemical may possess properties that would preclude additional testing. In the case of ethanedioic acid, water solubility and the octanol-water partition coefficient should be determined prior to conducting acute fish toxicity testing.

**D-gluc-Hep-tonic Acid (Sodium Glucoheptonate; CAS 31138-65-5)**

Data on multiple endpoints are requested for sodium glucoheptonate including physicochemical properties, as well as data from acute fish and mammalian, genetic, and combined repeated dose with reproduction/developmental toxicity studies. These tests would kill approximately 800 animals. This substance is listed by FDA as a secondary direct food additive permitted in food for human consumption (FDA, 2008). As noted above, with regard to GRAS substances, EPA has recommended that participants specifically consider whether the information available makes it unnecessary to proceed with further testing involving animals and whether any additional information obtained would be useful or relevant. EPA must reconsider its testing requirements for sodium glucoheptonate in the proposed test rule. Also, we recommend that the physicochemical data be generated prior to any animal testing since certain properties may preclude further animal testing.

**C.I. Leuco Sulphur Black 1 (CAS 66241-11-0)**

C.I. Leuco Sulphur Black 1 is listed in the proposed test rule as requiring a mammalian acute toxicity test and possibly a fish acute toxicity test. A European Chemicals Bureau IUCLID Dataset summarizes data from industry studies of acute toxicity in mammals and fish that report an LD50 of greater than 2000 mg/kg bw in rats and an LC50 of 401 mg/L in salmon trout, respectively (European Chemicals Bureau, 2000a). EPA must review these studies and reconsider its requirements for this substance in the proposed test rule.

**Castor oil, sulfated, sodium salt (CAS 68187–76–8) and oxidized (CAS 68187–84–8)**

Sulfated and oxidized derivatives of castor oil are listed in the proposed test rule as each requiring a mammalian acute toxicity test, a combined repeated dose with reproduction/developmental toxicity test and possibly a fish acute toxicity test. These tests would kill approximately 1,350 – 1,610 animals. A 1982 study reports fish acute toxicity data including a mean LC50 of 2.1 mg/L for medaka (Tonogai, YS. et al., 1982). EPA must reconsider this requirement for this substance in the proposed test rule.

These substances share a long history of safe use and consequently widespread human exposure. Castor oil is a natural vegetable oil obtained from the castor bean that is used in pharmaceuticals, food and other industries. FDA lists castor oil as GRAS for over-the-
counter use as a laxative. In folk medicine, it has also been traditionally used to treat skin problems, as a rub for various ailments and to induce childbirth. Sulfated castor oil, also known as Turkey red oil, completely disperses in water and, as a result, is widely used in cosmetics and personal care products as well as in lubricants, softeners, and as a dye aid. It is listed by Health Canada as a substance not requiring further work for human health. Oxidized castor oil, also known as blown castor oil, is produced by bubbling air through castor oil at elevated temperatures to increase its viscosity and specific gravity. It is used as a plasticizer for inks, lacquers and adhesives.

Again, the first principle enumerated in EPA’s 1999 Letter makes clear that “[p]articipants 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.” This principle is directly applicable to the castor oil derivatives given the wealth of human experience with them, and it is inconceivable that animal tests would be required. Considering existing data and WoE, EPA must reconsider its testing requirements for these substances in the proposed test rule.

**Benzenediamine, ar,ar-diethyl-ar-methyl- (Diethyltoluenediamine, DETA; CAS 68479-98-1)**

DETA is listed in the proposed test rule as requiring a fish acute toxicity test and a combined repeated dose with reproduction/developmental toxicity test. These tests would kill approximately 800 animals. DETA is a polymer curing agent. We find reference to an LC50 value of approximately 194 mg/l in Golden orfe (Bayer MaterialScience LLC, 2007). In addition, the Toxic Substance Control Act Test Submission database retrieves records of communications to EPA from Ethyl Corporation reporting results from subchronic and chronic toxicity tests for DETA. If available, data from the histopathology of reproductive organs in these studies may be used to fulfill the reproductive toxicity endpoint. This approach is accepted by OECD and has been recommended by EPA in HPV Challenge Program test plans. EPA must review these studies and reconsider its requirements for this substance in the proposed test rule.

**Alkenes, C12-24, chloro (CAS 68527-02-6)**

EPA requests aquatic, acute mammalian, and mutagenicity testing; however, information on all of these endpoints is available within IUCLUD data sheets for this CAS number (European Chemicals Bureau, 2000b). Also, this chemical mixture is another good candidate for SAR or category data fulfillment. We urge the EPA and the manufacturers to investigate these potential avenues.

**Hydrocarbons, C > 4 (CAS 68647-60-9)**

Hydrocarbons, C > 4 is listed in the proposed test rule as requiring a mammalian acute toxicity test, a combined repeated dose with reproduction/developmental toxicity test and possibly a fish acute toxicity test. These tests would kill approximately 800 animals.
The fish acute toxicity test is unnecessary, since it is well known that hydrocarbons kill fish via non-polar necrosis, an effect that has little dependence on the specific structure of a compound. EPA must reconsider this requirement for this substance in the proposed test rule. In addition, it is not clear whether EPA has carefully analyzed the composition of these class 2 chemical substances in order to identify the known bioactive agents.

**Phenol, 4,4’-methylenebis[2,6-bis91,1-dimethylethyl)- (118-82-1)**

Acute toxicity to fish is requested for this phenol. EPA should examine the data submitted by Shell Oil Company to EPA on 06/09/1992, which includes acute toxicity to steelhead trout and chronic toxicity to fathead minnows (EPA TSCATS Database, 2008) There may also be data available from ExxonMobil as their MSDS for 10W-30 motor oil, of which Phenol, 4,4’-methylenebis[2,6-bis91,1-dimethylethyl)- is one of two ingredients, states that it’s not expected to be harmful to aquatic organisms based on available data (Exxon Mobil, MSDS, 2007).

The use of categories and QSAR analysis would also be a logical approach for this chemical in order to provide screening level data requested by the proposed test rule. Given the size and extensive knowledge of this chemical class, this rationale was used in British Columbia by the Ministry of the Environment to develop water quality guidelines for phenols as a related group (Government of British Columbia, 2002).

**SUMMARY**

While EPA incorporated some of the animal welfare principles enumerated in its 1999 Letter, these efforts again fall far short of the agency’s commitment to incorporate them into related test rules and much more must be done to reduce the number of animals who would be killed in the proposed tests. Once again, EPA has failed to consider the totality of what is known about each chemical – including human experience – before concluding that all endpoints need to be tested. The inclusion of such familiar substances as acetaldehyde, sorbic acid, oxalic acid and castor oil derivatives is evidence that this primary principle has been grossly ignored. Also, EPA must allow for public comment after any in-house testing results or other existing data are made available in a test plan as a result of this proposal. This would also provide test sponsors with the opportunity to consider the use of categories and SAR analyses and to submit any justifications for exemptions from requirements to use in vitro genetic toxicity and combined repeated dose toxicity with reproduction/developmental toxicity screening tests prior to the testing being conducted. We also urge EPA to take this opportunity to reconsider the alternatives to mammalian acute toxicity, fish acute toxicity and developmental toxicity tests detailed above. Finally – and most urgently – EPA must thoroughly evaluate the existing data we have cited for many of the substances included in this proposed test rule as it has failed in this proposal to do even minimum due diligence in searching for existing data.
Sincerely,

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**Attachments**

1. 2001 comments on first HPV test rule.pdf  
3. ChAMP letter--PCRM to EPA.pdf  
4. Request for presentation of oral comment.pdf
REFERENCES


CIR (Cosmetic Ingredient Review). 2006. CIR Compendium, containing abstracts, discussions, and conclusions of CIR cosmetic ingredient safety assessments. Washington DC.


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