June 7, 2007

Dr William S Stokes
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National Institute of Environmental Health Sciences
PO Box 12233, MD EC-17
Research Triangle Park, NC 27709

Re: 72 FR 23832; May 1, 2007; Public Comments Concerning the Draft NICEATM-ICCVAM 5-Year Plan (2008-2012)

Dear Dr Stokes:

These comments are submitted on behalf of the Doris Day Animal League, Humane Society Legislative Fund, People for the Ethical Treatment of Animals, Physicians Committee for Responsible Medicine, Alternatives Research and Development Foundation, and American Anti-Vivisection Society in response to the *Federal Register* notice cited above. This submission incorporates by reference comments filed December 31, 2006 on the same subject,[1] which do not appear to have been taken into consideration during the formulation of the draft NICEATM-ICCVAM 5-Year Plan (hereinafter, "the draft Plan").

The parties to this submission are in general agreement with the four key objectives outlined on p. 3-4 of the draft Plan, which include:

- "Identifying priorities and conducting **and facilitating** alternative test method activities"
- "Incorporating new science and technology"
- "**Fostering** regulatory **acceptance and use** of alternative methods"
- "Developing partnerships and **strengthening interactions** with ICCVAM stakeholders" (**emphasis supplied**).

We particularly support the stated aims of ICCVAM-stakeholder partnerships (i.e., "to make best use of existing resources and scientific expertise, maximize the efficiency of test method validation efforts and evaluations, minimize duplication of effort, and ensure an early exchange of information concerning test method validation"), and strongly recommend that these be adopted as guiding principles for **all** ongoing and future ICCVAM activities.

Much of the criticism leveled against ICCVAM since its establishment as a permanent standing committee in 2000 is attributable to ICCVAM's failure to abide by one or more of the above criteria. For example, despite consistently prompt reviews and endorsements of US-approved alternative methods by ICCVAM's European counterpart—the ECVAM Scientific Advisory Committee, or ESAC (Table 1)—ICCVAM has yet to even consider the great majority of alternative methods and/or testing strategies pioneered in the EU and endorsed by ESAC (Table 2). The resultant logjam created by US agencies' insistence upon ICCVAM review and endorsement as a precondition for regulatory acceptance, together

[1] http://iccvam.niehs.nih.gov/docs/5YrResponses/ICCVAM5yrplanHSLF.pdf

with ICCVAM's chronic failure to even remotely keep pace with its EU counterpart, is leading to a situation in which companies may be forced to double-test in order to satisfy increasingly divergent US and EU data/testing requirements.[2] Such a scenario is unacceptable from both animal welfare and economic perspectives, and seriously undermines the movement toward greater international harmonization to which the US has recently reaffirmed its commitment.[3]

Table 1: History of ESAC acceptance of ICCVAM-endorsed test methods

Endpoint	Name of Test	ICCVAM Final Rec.[a]	ESAC Stmt.[b]
Skin allergy	Local lymph node assay	March 1999	October 1999
Acute oral toxicity	Up-and-down procedure	March 2000	—[c]
Skin corrosion	CORROSITEX™	December 2000	December 2000

[a] http://iccvam.niehs.nih.gov/methods/methods.htm
[b] http://ecvam.jrc.it/f_home.cfm?voce=m&idvoce=3
[c] ESAC statement unnecessary given international acceptance of the UDP as OECD Test Guideline 425 since September 1998

Table 2: ESAC-endorsed alternative methods/testing strategies awaiting ICCVAM review and formal testing recommendations

Endpoint	Name of Test	ESAC Stmt.[a-b]
Antibody production	*In vitro* monoclonal antibody production	November 1997
Photoirritation	3T3 neutral red uptake (3T3 NRU) phototoxicity test	May 1998
Vaccine potency	Toxin binding inhibition (ToBI) test	December 2000
Vaccine potency	ELISA test for human tetanus vaccines	December 2000
Embryotoxicity	Embryonic stem cell test	May 2002
Embryotoxicity	Micromass assay	May 2002
Embryotoxicity	Whole rat embryo assay	May 2002
Vaccine potency	ELISA test for erysipelas vaccines	June 2002
Acute toxicity to fish	Upper threshold concentration step-down approach	March 2006
Acute neutropenia	Colony forming unit granulocyte macrophage assay	March 2006
Skin corrosion	Skinethic™ human skin model	November 2006
Chronic toxicity	Ending 1-year dog studies of pesticides	November 2006
Skin irritation	EPISKIN™-SIT	April 2007

[a] http://ecvam.jrc.it/f_home.cfm?voce=m&idvoce=3
[b] http://ecvam.jrc.it/page.cfm?voce=s&idvoce=27&idmm=4&idsm=27

[2] http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm
[3] http://www.eurunion.org/partner/summit/Summit20070430/JtReptRoadmapUSEURegCoop042007.pdf

Moreover, the handful of instances in which ICCVAM has undertaken reviews of ESAC-endorsed methods (Table 3) can hardly be claimed to "make best use of existing resources and scientific expertise," "maximize the efficiency of test method validation efforts and evaluations," and/or "minimize duplication of effort." For example:

- Whereas ESAC and the National Coordinators of the OECD Test Guidelines Program[4] endorsed a strictly non-animal testing strategy for skin corrosivity based on the ECVAM-validated human skin models EPISKIN™ and EpiDerm™, ICCVAM and its US agency members continue to require that chemicals testing negative (i.e., non-corrosive) *in vitro* be subject to "confirmatory" animal testing. Thus, while the EU and other OECD member countries have moved towards 100% replacement of animal use for skin corrosion testing, ICCVAM's position allows for only a modest reduction in animal use.
- Nearly a year after ESAC endorsed the validity of five *in vitro* human blood-based tests for pyrogenicity, ICCVAM undertook a second, full peer review of these methods, despite its stated policy that "it is inappropriate for ICCVAM to conduct such reviews for methods where there is no **substantive** disagreement with the ECVAM assessment."[5] To make matters worse, the ICCVAM-appointed reviewers failed to support even the minimal use of these methods proposed in ICCVAM's draft testing recommendations—calling instead for extensive additional testing, including further animal studies—which has left these validated and EU-endorsed *in vitro* methods in regulatory limbo in the US.
- On the basis of a retrospective ECVAM validation study, ESAC endorsed the conclusion that "the *in vitro* micronucleus test (MNT) is a scientifically valid alternative to the *in vitro* chromosome aberration (CA) assay for genotoxicity testing."[6] This endorsement led to almost immediate regulatory acceptance of the MNT under the EU REACH chemicals regulation,[7] as well as a proposal for a new OECD Test Guideline be created for the MNT. Despite this overwhelming endorsement of the MNT by EU regulators, however, ICCVAM's comments[8] regarding the draft OECD MNT Test Guideline did *not* reflect support for ESAC's position, calling instead for substantial additional work (e.g., expanded data sets on indirect-acting chemicals requiring metabolic activation, the inclusion of an optimized test protocol and performance standards, and an additional commenting round) before the MNT is accepted at OECD-level.
- Most recently, ESAC endorsement of the validity of a variant of the Local Lymph Node Assay (rLLNA), under which animal use can be reduced by as much as 50%.[9] ICCVAM's response has again been to propose a second peer review.

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[4] http://caliban.sourceoecd.org/vl=3371732/cl=15/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s30/p1
[5] http://iccvam.niehs.nih.gov/docs/expedite.pdf
[6] http://ecvam.jrc.it/publication/ESAC25_statement_MNT_20061128.pdf
[7] http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_396/l_39620061230en00010849.pdf
[8] http://iccvam.niehs.nih.gov/methods/genetox/genetoxdoc/DraftRevMn30Jan07v4.pdf
[9] http://ecvam.jrc.it/ft_doc/ESAC26_statement_rLLNA_20070525-1.pdf

Table 3: History of ICCVAM reviews/acceptance of ESAC-endorsed test methods

Endpoint	Name of Test	ESAC Stmt.[a]	ICCVAM Rec.	Notes
Skin corrosion	EPISKIN™	April 1998	June 2002[b]	Recommended use only as "positive screens," with *in vitro* negatives subject to "confirmatory" animal testing
	EpiDerm™	May 1998		
	Rat transcutaneous electrical resistance (TER) assay	April 1998		
Pyrogenicity	Human whole blood IL-1	March 2006	—[c]	Subject to *second* peer review in Feb. 2007; final ICCVAM recommendations have yet to be transmitted to federal agencies
	Human whole blood IL-6	March 2006		
	Human cryopreserved whole blood IL-1	March 2006		
	PBMC IL-6	March 2006		
	MM6 IL-6	March 2006		
Genotoxicity	*In vitro* micronucleus test	Nov. 2006[d]	—[e]	Called for substantial additional work prior to acceptance as an OECD Test Guideline
Eye corrosion/ severe irritation	Bovine corneal opacity permeability (BCOP) test	April 2007[f]	—[g]	Final peer review report published in Nov. 2006; however, final ICCVAM recommendations have yet to be transmitted to federal agencies
	Isolated chicken eye (ICE) test	April 2007[f]		
Skin sensitization	Reduced local lymph node assay (rLLNA)	April 2007	—[h]	ICCVAM currently proposing a *second* peer review and other evaluations

[a] http://ecvam.jrc.it/page.cfm?voce=m&idvoce=3#1
[b] http://iccvam.niehs.nih.gov/methods/dermal/dermal.htm
[c] http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm
[d] Almost immediate regulatory acceptance under EU REACH chemicals regulation: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_396/l_39620061230en00010849.pdf
[e] http://iccvam.niehs.nih.gov/methods/genetox/genetox.htm
[f] Regulatory acceptance by EU Competent Authorities since November 2002: http://ecb.jrc.it/DOCUMENTS/New-Chemicals/Manual_of_decisions.pdf
[g] http://iccvam.niehs.nih.gov/methods/ocutox/ocutox.htm
[h] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

Given the extent to which international validation and regulatory acceptance criteria have now been harmonized, it is incomprehensible why ICCVAM persists in carrying out redundant peer reviews of test methods that have already been independently reviewed and endorsed according to substantially equivalent criteria. To increase the efficiency and effectiveness of ICCVAM, it is therefore imperative that ICCVAM establish formal bilateral/multilateral reciprocity agreements with ECVAM and other international validation

authorities that require immediate and abbreviated reviews of alternatives that have been validated by other authorities. Specifically, the agreements should state that once an alternative has been approved by one validation authority, there is a rebuttable presumption that the other authorities will also approve it.

Chapter 1 – Research, Development, Translation and Validation Activities for Priority Test Methods

The draft Plan states that the criteria used for setting priorities include:

- Potential impact on reducing, refining, or replacing animals for testing
- Applicability to multiple agencies
- Potential to provide improved prediction of adverse health or environmental effects

NICEATM/ICCVAM solicited public comments in November 2006, asking specifically: "Do you have comments on the priority areas for the development and validation of alternative test methods listed above?" In our December 2006 comments, we provided several suggestions for setting criteria and identifying needs, none of which have been incorporated into the draft Plan.[10] The draft Plan provides no overview or systematic analysis of priority setting for either methods under development or for planned activities. Instead, Chapter 1 contains virtually the same laundry list of methods under consideration that was presented at the November 2006 meeting of the NTP Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), with no explanation regarding the basis upon which they were chosen, or how these methods relate to the stated priorities.

<u>Ocular Irritaiton</u>

- Notwithstanding the fact that positive results from several *in vitro* tests have been accepted by EU Competent Authorities since 2002 for the classification of ocular irritants (Table 3), we acknowledge NICEATM/ICCVAM's role in coordinating the formal, retrospective validation of these methods, and urge ICCVAM to forward its final testing recommendations to US agencies without further delay in order that these alternative methods may enter widespread international use.
- We support the proposed NICEATM/ICCVAM efforts to evaluate the reliability and relevance of appropriate *in vitro* methods for the detection and classification of mild and non-eye irritants.
- We recognize the ongoing role of NICEATM/ICCVAM in the evaluation of a non-animal hazard assessment strategy for antimicrobial cleaning products, but are concerned with the extremely slow rate of progress of this initiative. We are also cognizant that the results of this work are of primary relevance to only one division within one program office at one agency (i.e., EPA/OPP/AD), which we do not believe necessitates the involvement of other federal agencies or ICCVAM.

[10] http://iccvam.niehs.nih.gov/docs/StrPlnPubCmts.htm

Acute Toxicity

- The parties to this submission are, on the whole, gravely dissatisfied with NICEATM/ ICCVAM's past and proposed future activities in this area. On the one hand, we were very pleased with the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity convened by ICCVAM/NICEATM in 2000 as a result of the animal protection agreement with the White House on the EPA's High Production Volume Chemical Challenge Program. In particular, the experts at the workshop concluded that, "**if the commitment** to conducting a formal validation study **was strong enough**, the scientific resources could be harnessed for this effort with facility and the *in vitro* tests studied proved good enough, **a replacement test battery might be achieved in as short a time as 2-3 years**"[11] (**emphasis supplied**). However, apart from publishing a *Guidance Document on Using* In Vitro *Data to Estimate* In Vivo *Starting Doses for Acute Toxicity*[12] in 2001, subsequent ICCVAM/NICEATM activity in this area has focused exclusively on the *possible* use of *in vitro* methods for dose-setting, rather than replacement, purposes. In contrast, the EU's ACuteTox[13] project—with a budget of €15.6 million (€9 million in government funding)—has brought together 35 regulatory, corporate, academic and other partners from 13 countries with the specific aim of developing and validating a battery of integrated, non-animal methods capable of *fully replacing* animal use for this endpoint.
- ICCVAM and its member agencies must do more than organize workshops on "humane endpoints," further validate the same two *in vitro* assays as a dose-setting tool for mixtures, and continue to trumpet the Up-and-Down Procedure (UDP) to be regarded as a constructive participant in this important area.

Biologics/Vaccines

- We strongly support the efforts of the USDA Center for Veterinary Biologics to validate *in vitro* alternatives to the hamster challenge test for *Leptospira*, the guinea pig challenge test for *Clostridium chauvoei*, and the mouse potency test for *Erysipelothrix rhusiopathaie*, among other 3Rs measures reported to SACATM in 2003.[14]
- In the interim, we strongly urge ICCVAM to circulate testing recommendations to US agencies unequivocally endorsing the validated alternative methods currently accepted by ESAC, i.e., *in vitro* production methods for monoclonal antibodies, the toxin binding inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use, and the use of ELISA for batch potency testing of human tetanus and erysipelas vaccines.[15]

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[11] http://iccvam.niehs.nih.gov/docs/acutetox_docs/finalrpt/finalall0801.pdf
[12] http://iccvam.niehs.nih.gov/docs/acutetox_docs/guidance0801/iv_guide.pdf
[13] http://www.acutetox.org
[14] http://ntp-server.niehs.nih.gov/ntpweb/index.cfm?objectid=AF6CC417-F1F6-975E-75B5F3FF7DF1CDDC #2003
[15] http://ecvam.jrc.it/page.cfm?voce=m&idvoce=3#1

Dermal Irritation

- As outlined in Table 2, ESAC recently endorsed the EPISKIN™ skin irritation test (EPISKIN™-SIT) as "a reliable and relevant stand-alone test for predicting rabbit skin irritation, when the endpoint is evaluated by MTT reduction, and for being used as a replacement … for the purposes of distinguishing between R38 skin irritating and no-label (non-skin irritating) test substances.[16] ICCVAM's review of this method should be both prompt and supportive—unlike its previous review of this method for corrosivity testing (Table 3)—and harmonized testing recommendations should be forwarded to US agencies before year-end.

Immunotoxicity

- As outlined in Table 3, ESAC has recently concluded that "within a tiered testing strategy … a 'reduced' version of the LLNA (rLLNA), using only a negative control group and the equivalent of the high-dose group from the full LLNA, can be used as a screening test to distinguish between sensitizers and non-sensitizers," thereby reducing animal use by as much as 50%.[6] Limitations of the rLLNA include an inability to determine the potency of sensitizing chemicals, and the potential for limited false negative results when concentrations of <10% are used (although ESAC has recommended that further work be undertaken to determine whether this concentration threshold is optimal). We support this recommendation, and urge (i) ICCVAM to expeditiously review and endorse the ESAC peer review and circulate harmonized testing recommendations to US agencies before year-end, and (ii) NICEATM to collaborate with ECVAM to address the question of concentration threshold, in lieu of ICCVAM's recent proposal[17] to assemble a background review document and peer review panel "to evaluate the validation status of: (1) the [LLNA] as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification; (2) the "cut-down" or "limit dose" LLNA approach; (3) non-radiolabeled LLNA methods; (4) the use of the LLNA for testing mixtures, aqueous solutions, and metals; and (5) the current applicability domain (i.e., the types of chemicals and substances for which the LLNA has been validated"). In addition, as presented at the INVITOX Conference in Bruges, Belgium in October 2006, several laboratories are involved in efforts to develop completely non-animal sensitization testing protocols. We urge ICCVAM to spend more time fostering these methods than tinkering with the current LLNA, which, even in reduced form, uses live animals.

Endocrine Disruption

- If NICEATM/ICCVAM intend to increase their involvement in this area, we request that at least as much attention be paid to the validation and peer review of new or revised animal tests (on both the human health and ecological sides) as has

[16] http://ecvam.jrc.it/ft_doc/ESAC26_statement_SkinIrritation_20070525-1.pdf
[17] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

been paid to *in vitro* methods and (Q)SAR models. In addition to involvement at the OECD level, consideration should be given to seeking representation on EPA's FIFRA Scientific Advisory Panel (SAP), which has now replaced the Endocrine Disruptor Methods Validation Advisory Committee (EDMVAC) as the agency's primary peer review body for endocrine disruptor screens and tests.

Pyrogenicity

- The parties to this submission previously questioned the need for a separate ICCVAM peer review of the five ESAC-endorsed *in vitro* pyrogenicity tests,[18] and stand behind this position in light of panel's findings, which add nothing of value to the work done by ECVAM. This is another classic case of unnecessary duplication of effort, which must be avoided in the future. Nonetheless, we urge ICCVAM to forward final testing recommendations[19] to US agencies without further delay, in order that these alternative methods may enter widespread international use.

Chronic Toxicity/Carcinogenicity

- The draft Plan is disappointingly uninspired in regard to these endpoints, overlooking at least two opportunities for significant, near-term reduction in animal use. As we pointed out in our December 2006 comments:

  A series of science-based proposals with significant potential to reduce animal use in regulatory toxicology were published earlier this year by technical panels of the ILSI Health and Environmental Science Institute (HESI; Carmichael et al., 2006). These panels, with significant technical input and support from the U.S. Environmental Protection Agency, have recommended a number of substantial departures from conventional testing paradigms, including:

  - *Ending second-species carcinogenicity testing on mice, on the grounds that "additional information provided by [this] study is of limited value in risk assessment" (Doe et al., 2006). This would save at least 400 mice per chemical tested.*
  - *Ending second-species chronic toxicity testing on dogs, on the basis of numerous reports (i.e., Gerbracht & Spielmann, 1998; Box & Spielmann, 2005; Baetcke et al., 2005; Doe et al., 2006) documenting that data from studies of a shorter duration are sufficient for risk assessment purposes. This would save at least 32 dogs per chemical tested.*

[18] Public comments filed January 26, 2007 by PCRM on behalf of the parties to this submission regarding draft ICCVAM test recommendations for five *in vitro* pyrogenicity testing methods.
[19] ICCVAM should *not* call for further prospective validation or concurrent *in vivo*/*in vitro* pyrogenicity studies.

ESAC was quick to capitalize on one of these opportunities, issuing a statement[20] in November 2006 that:

> *Extension of a dog toxicity study beyond a 13-week duration does not provide additional essential information and reliance on the chronic rodent and 13-week dog studies would provide and adequate basis for chronic RfD derivation in pesticide risk assessment.*
>
> *There is no further need to require a one-year dog study for the evaluation of repeated dose toxicity of pesticides. The short-term oral toxicity of the active substance to non-rodents must always be reported only in a 90-day study, usually in dogs.*
>
> *Data requirements to the chronic dog studies should be harmonized between the European and North American (as well as other) regulatory agencies to avoid unnecessary testing of dogs in different time frames.*

ICCVAM should forward a comparable statement/testing recommendation to US agencies. NICEATM/ICCVAM should also collaborate with EPA/OPP and provide such additional support as may be needed to support a similar statement with respect to the mouse carcinogenicity bioassay.

Reproductive Toxicity

- Given that more animals are consumed in reproductive and developmental toxicity testing than for any other endpoint (675-2,500+ per study), it is reprehensible that these are not identified as priority areas for ICCVAM and its member agencies. Simply stating that "the development and validation of alternative test methods … will likely take longer than the five-year time frame for this strategic plan" is no excuse for US agencies to remain passive while their European counterparts—through the ECVAM-led €13.9 million ReProTect project[21]—work diligently toward developing an integrated, non-animal replacement strategy for this endpoint. To this end, we fully support the NIEHS work on *C. elegans*, and urge NIEHS to become a positive and proactive partner in ReProTect in order to promote closer US-EU collaboration and transparency in this important research area.
- Additionally, as highlighted in our December 2006 comments, an enormous reduction in animal use in this area could be realized in very short order by:

> *Moving away from reproductive toxicity studies in two generations, on the basis of several compelling studies (i.e., Ulbrich & Palmer, 1995; Cooper et al., 2006) that demonstrated for 117 pharmaceutical agents and 350 pesticides, harmful effects on reproduction could have been identified in more than 98% of cases without breeding a second generation of offspring. This would save as many as 1,200 rats per study.*

[20] http://ecvam.jrc.it/publication/ESAC25_statement_DOG_20061207.pdf
[21] http://www.reprotect.eu

A number of multi-sector, multinational initiatives are already under way to standardize and validate alternative testing protocols for modified single-generation studies and expedite their adoption into regulatory programs and test guidelines. We urge NICEATM/ICCVAM to follow the EPA/OPP's lead in these activities and be prepared to expeditiously review and endorse (i) the discontinuation of routine multigenerational breeding studies, and (ii) reduction/refinement protocols as they are developed.

Further Opportunities for Near-Term Reduction

- We note with concern that the draft Plan is silent on the subject of ecotoxicology, which is an area of ever-increasing animal use. One ESAC-endorsed strategy that could very easily be supported by an ICCVAM testing recommendation is the Upper Threshold Concentration (UTC) Step-Down Approach to acute aquatic toxicity testing in fish (Table 2), which has the potential to reduce fish use in acute lethality (LC_{50}) studies by as much as 73%.[22] In addition, we call NICEATM/ICCVAM's attention to the continued drive from certain regulatory sectors to develop and validate multigenerational reproduction studies in avian, amphibian and fish species (e.g., for endocrine testing) at a time when unprecedented efforts are being made to move *away* from such studies in mammals. If indeed F2 data contribute little or no value added to human health hazard assessments, should not the same be true in the context of ecotoxicity?
- Additionally, as stated in our December 2006 comments, we invite ICCVAM and its member agencies to give careful consideration to the following as opportunities to further minimize duplicative animal testing:

  Ending Multi-Route General Toxicity Studies: It is common for regulatory authorities in the pesticides, chemicals, and other sectors to demand multiple animal dosing studies of acute (single dose), subacute (up to 1 month repeated dose) and subchronic (3-6 months repeated dose) duration to evaluate a chemical agent's effects on body systems and general health. What is more, these toxicological "fishing expeditions" are often repeated several times using different routes of chemical exposure (e.g., oral force-feeding, forced inhalation of chemical vapors, skin exposure, etc.). The redundancy of such testing is both obvious and unnecessary: a single acute lethality study is bad enough, but the requirement that up to three such studies be carried out simply for "check-the-box" labeling purposes is unacceptable. Regulators and industry alike should make far greater use of in vitro methods and computerized biokinetic (PBBK/PBPK) modeling as a basis for extrapolating between exposure routes in lieu of duplicative animal testing.

  Ending Second-Species Developmental Toxicity Testing: Drug, pesticide, and some chemical regulators generally require that testing for toxicity to prenatal development be performed in more than one animal species—consuming up to 1,300 rats and 660 rabbits per test. The rationale behind such obviously duplicative testing is the fact that

[22] http://ecvam.jrc.it/ft_doc/ESAC%20statement%20UTC%20step%20down%20approach%2020060515.pdf

> *neither rat nor rabbit tests alone are able to detect the potential for fetal toxicity or malformations with more than 87% accuracy (Hurtt et al., 2003). Thus, regulators are concerned that limiting testing to a single species could permit a potentially large number of chemicals with birth defect-inducing properties into commerce. However, the presumption that we are surrounded by thousands of developmental toxicants is not consistent with current knowledge. For example, ECVAM has recently reviewed all substances listed in the EU's New Chemicals Database as having been tested for developmental toxicity, and determined that only 5% of these substances produced positive results leading to a regulatory classification (Bremer S, personal communication, 20 September 2006). Thus, assuming that (i) of every 1,000 chemicals, 5% (50 chemicals) are actual developmental toxicants, and (ii) developmental toxicity studies in rats are approximately 87% accurate at detecting such effects, it follows that all but six developmentally toxic chemicals could be correctly identified by testing in only one animal species.*

Chapter 2 – Advances in Science and Technology

The animal protection community supports in principle efforts by US agencies to develop and validate technologies that have the potential to reduce, refine, and ultimately replace animal use in regulatory toxicology. We regret, however, that despite the clear Congressional mandate for US agencies to advance the 3Rs,[23] agency research programs have yet to reflect this mandate in any meaningful way (i.e., although several of the programs highlighted in the draft Plan may lead to reductions or refinements in animal use, this is not their primary purpose). Furthermore, compared to such highly focused 3Rs initiatives as ACuteTox, ReProTect, Sens-it-iv,[24] BioSim,[25] PredictOmics and OSIRIS,[26] the activities of US agencies are clearly unfocused, uncoordinated, under-funded and woefully non-comprehensive from a 3Rs perspective, in that no plan is provided for identifying and prioritizing development of new technologies with regard to regulatory needs. Thus, while individual projects such as the NIEHS/FDA work on *C. elegans* may well produce a useful model for the evaluation of certain reproductive/developmental toxicity parameters, how much more could be accomplished if other agencies' intramural and extramural research divisions were also engaged in collaborative and/or complimentary projects?

At a minimum, US agencies each need to better coordinate their 3Rs research efforts—both domestically and internationally—in order to avoid duplication of effort (e.g., how many federal bioinformatics, computox and nanotox programs do we really need?), while ensuring that important research is not overlooked or neglected (e.g., how long have metabolism and biokinetic factors been recognized as barriers to the acceptance of *in vitro* methods, and what have US agencies done to overcome this challenge?). *To this end, we request that ICCVAM member agencies commit to convening an annual meeting of senior science and program office staff from each*

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[23] P.L. 106-545
[24] http://www.sens-it-iv.eu
[25] http://www.biosim-network.net
[26] Optimized Strategies for Risk Assessment of Industrial Chemicals through the Integration of Non-test and Test Information

agency, together with representatives from ECVAM and key US stakeholders, to establish a comprehensive, coordinated, multi-year 3Rs research agenda.

Chapter 3 – Fostering Acceptance and Appropriate Use of Alternative Test Methods

We recognize NICEATM/ICCVAM's aggressive promotion of reduction/refinement approaches such as the LLNA and UDP, and look forward to witnessing an equal or greater level of enthusiasm from US agencies with respect to the acceptance of *in vitro* and other non-animal methods as full replacements. This Chapter provides a golden opportunity for NICEATM/ICCVAM to outline a specific plan for improving US regulatory acceptance of validated alternative methods. Such a plan would involve agency input that prioritizes regulatory endpoints currently subject to animal testing; contain specific descriptions of replacement methods; and delineate an integrated process for test method validation and regulatory acceptance.

As the draft Plan currently stands, NICEATM/ICCVAM have not crafted an approach to foster interest and acceptance of alternative methods by US federal agencies. The disconnect between the reports of ICCVAM peer review panels and the final decisions regarding applicability or robustness made by ICCVAM member agencies (or the converse) is a stifling reality. Therefore, under the statutory authority granted NICEATM/ICCVAM, a significant piece of the final Plan should be devoted to this necessary facilitation. Simply "continuing to do" what ICCVAM has been doing to date—a reference repeatedly found throughout this draft Plan—will not move the ball forward.

Chapter 4 – Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

This Chapter represents yet another missed opportunity. The draft Plan contains only descriptions of past approaches to developing partnerships and fostering interactions, with several promises to continue these same approaches, all of which have achieved limited success over the past decade. The point of requesting a 5-year plan is to *re-strategize*, to develop *new* approaches to *improve* and *strengthen* interactions. Again, several suggestions were provided in the animal protection community's December 2006 comments, none of which have been incorporated into the draft Plan.

Conclusion

The draft Plan is disappointing in its lack of direction, lack of specificity, and apparent lack of commitment to a coherent process to achieve its stated goals. The implicit purpose of the Appropriations Committees' request for a 5-year plan was to allow NICEATM/ICCVAM to develop and articulate a *new* approach for the future. However, the draft Plan is largely a review of past activities and a collection of statements that suggest that future activities will proceed much as before. Over the past decade, this approach has been proven to be woefully insufficient and unsatisfactory to animal protection stakeholders, both in the US and internationally. Failure by ICCVAM and its member agencies to make the most of this opportunity to develop new approaches—together with the fact that previous comments

have largely been ignored—again leads us to question US agencies' commitment to ICCVAM and its Congressionally-sanctioned 3Rs mandate.

As we have stated previously, "the parties to this submission have always endeavored to regard ICCVAM and its member agencies as federal partners who share our commitment to reducing, refining, and ultimately replacing the use of animals in regulatory toxicology. However, the abbreviated number of methods reviewed by ICCVAM and accepted by federal agencies in recent years raises concern over the genuine commitment to progress in the 3Rs by some federal agencies and/or their representatives on ICCVAM."[18] The extent to which this view is maintained or modified in the coming years will depend greatly on the extent of NICEATM/ICCVAM's responsiveness to the comments and recommendations above.

Please direct any questions to Sara Amundson at 202.676.2341 or samundson@hslf.org.

Sincerely,

(signature redacted)

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