



December 22, 2006

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Re: Public Comments Regarding NICEATM/ICCVAM 5-Year Plan to Research Develop, Translate and Validate New and Revised Non-animal and Other Alternative Assays for Integration of Relevant and Reliable Methods into Federal Agency Testing Programs

Dear Dr Stokes:

These comments are submitted on behalf of the American Anti-Vivisection Society, Alternatives Research and Development Foundation, Doris Day Animal League, Humane Society Legislative Fund, The Humane Society of the United States, People for the Ethical Treatment of Animals and the Physicians Committee for Responsible Medicine in response to a *Federal Register* notice published on November 13, 2006 (71 FR 66172). The parties to this submission are national animal protection, health, and scientific advocacy organizations with a combined constituency of more than 10 million Americans who share the common goal of promoting reliable and relevant regulatory testing methods and strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals.

Key Policy Issues

Process for Public Engagement

The US Congress in report language contained in both the House and Senate versions of the Labor, Health and Human Services, Education and Other Agencies Appropriations bills for fiscal year 2007 requested that NICEATM/ICCVAM, in conjunction with the participating federal regulatory and research agencies and the NTP, craft a 5-year plan for research, development, translation and acceptance of non-animal and other alternative test methods. The Congress has repeatedly pressed the NIEHS to ensure that NICEATM/ICCVAM has

tangible appropriations with which to complete assessments and other work required by P.L. 106-545.

Congressional intent for the 5-year plan clearly embraces the need for broad stakeholder input and the general public in crafting the plan. And while animal protection organizations applaud the NICEATM/ICCVAM for beginning a process of public engagement prior to final passage of the appropriations bills, the method for creating the 5-year plan which was delineated at the recent SACATM meeting doesn't offer enough cultivated input. Therefore, we suggest NICEATM/ICCVAM consider holding three public meetings or workshops. The first workshop would be for scoping and development of models to collect and report information to address the questions and issues necessary for developing a 5-year plan¹: We also suggest that the process decided on at the first workshop consider including development of schematics for specific test methods, similar to that presented at the SACATM meeting by Dr. Stokes,² that once finalized, would provide a useful summary of near-term and long-term objectives and on-going, planned or yet to be addressed, prevalidation and validation research. The outcome of the first workshop would be the approach that each ICCVAM agency would use to gather, organize and present the information needed to develop the 5-year plan. The second workshop would be designed for presentations by the ICCVAM agencies on their progress in applying the model for collecting and reporting information. The third workshop would be for presentation and discussion of the draft final report.

The ICCVAM Authorization Act of 2000 (42 U.S.C. 2851) operates to ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for proposed use prior to an Agency requiring, recommending, or encouraging the application of such test method. In moving forward with development of the 5-year plan, we believe it is particularly important to use an approach that will clearly present information in such a way that priorities can be set. Furthermore, the trajectories of each method identified as a priority must fully integrate method validation work into the path forward. Full embracement of validation studies is necessary, because these validation studies provide the critical scientific data and information needed to understand the relevance, reliability, and appropriate use of such methods.

¹ One model that may be discussed would encompass having each ICCVAM organization describe: What are the regulatory testing programs that each ICCVAM organization currently uses which employ in vivo testing? What are the objectives of each battery with respect to the use of the data? What are the objectives of each test method within the battery with respect to the use of the data? For each regulatory testing program, the model approach would direct appropriate Agencies to develop information along the lines of: What are options for refinement? For reduction? For replacement? Near term & long term? What research efforts are already underway? What's needed? What translational efforts are already under way? What's needed? What prevalidation work is already underway? What's needed? What validation work is already underway? What's needed?

² Slide 13 <http://ntp-server.niehs.nih.gov/index.cfm?objectid=58A82A0C-F1F6-975E-72FAE774714C1F98>

However, equally as important as the validation studies required to ensure scientific rigor are two barriers that should be addressed within the framework of the plan: 1) translational research to address the manner in which a specific, proposed test method will work within the regulatory framework; and crucially important 2) significant engagement of the federal regulatory and research agencies that compose ICCVAM to suss out the reluctance to accept data from alternative methods as final results for safety studies. We strongly urge NICEATM/ICCVAM to use a section of the plan to address these two genuine concerns.

Complementing Without Duplicating International R&D and Validation Efforts

Independent peer reviews coordinated by ICCVAM have assisted in securing U.S. regulatory acceptance of reduction, refinement or replacement (3Rs) test methods for skin corrosivity (CORROSITEX[®]), skin sensitization (Local Lymph Node Assay; LLNA), and acute systemic toxicity (Up-and-Down Procedure; UDP). ICCVAM has likewise assumed a leading role in the international review of *in vitro* methods for assessing acute systemic toxicity, ocular corrosivity/severe irritation, *Botulinum* toxins, and receptor-mediated endocrine modulation (ICCVAM, 2005).

In recent years, however, ICCVAM has invested substantial time and resources in what are regarded by many as redundant and unnecessarily duplicative evaluations of 3Rs methods that have already undergone successful validation and/or independent peer review and/or national/international acceptance in other jurisdictions. Examples include the review of ECVAM-validated tests for skin corrosivity (EPISKIN[™], EpiDerm[™] and the Rat Skin Transcutaneous Electrical Resistance Assay), and forthcoming reviews in the areas of photoirritation (3T3 Neutral Red Uptake Assay) and pyrogenicity (5 human blood-based *in vitro* pyrogen tests). According to the *ICCVAM Procedures for Test Methods That Have Been Endorsed by ECVAM*, “it is inappropriate for ICCVAM to conduct such reviews for methods where there is no substantive disagreement with the ECVAM assessment” (ICCVAM, 2001). Thus, the fact that full reviews are being proposed for the *in vitro* phototoxicity and pyrogenicity tests suggests that substantive disagreement already exists, which could obstruct U.S. acceptance of these methods as well. However, we are not aware of any such substantive disagreement.

Moreover, we are mindful of the Congressional intent behind Public Law 106-545, which clearly stipulates in § 3(b) that “the purposes of the ICCVAM shall be to—

- (1) increase the efficiency and effectiveness of Federal agency test method review;
- (2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory bodies;
- (3) optimize utilization of scientific expertise outside the Federal Government; ...”

We therefore question in principal the necessity and/or value of subjecting alternative methods to multiple peer reviews—particularly when the animal tests they are intended to refine or replace have generally not been subject to a level of scrutiny even remotely approximating that of an ECVAM or ICCVAM validation study. Similarly, such multiple peer reviews should be precluded by the otherwise extensive collaboration of ICCVAM personnel in the activities of ECVAM, which affords opportunities to raise issues early in ECVAM’s review processes.

Additionally, we remain acutely aware of the unsatisfactory results of ICCVAM’s first “expedited review” of ECVAM-validated test methods: the case of *in vitro* human skin model studies for corrosivity. As you know, EPISKIN™ and EpiDerm™ have been accepted as full replacements for rabbit skin corrosion studies for more than six years in the EU (EC, 2000), and since 2004 at the OECD level—which reflects the apparently unanimous support of all 30 OECD member countries, including the U.S. (EC, 2000; OECD, 2006). OECD Test Guideline 431 specifically “allows for the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using *non-animal* methods (*emphasis supplied*)” (OECD, 2006)—meaning that both positive and negative *in vitro* test results are regarded as definitive under the OECD guideline, with no requirement for “confirmatory” testing in animals.

In contrast, ICCVAM and several of its member agencies have indicated that they will accept these tests only as “positive screens,” whereby chemicals that appear to be non-corrosive *in vitro* will be required to undergo additional testing in rabbits (ICCVAM, 2002). Yet, as The Procter and Gamble Company noted in public comments to U.S. regulators (Nash, 2001):

The recommendation to conduct an *in vivo* corrosivity test to identify “false negatives” undermines the full validation process for this specific endpoint. Such a proposal implies that any result, positive or negative, obtained in the validated, *in vitro* tests would need to be verified using an animal test since a priori knowledge of a false positive or false negative presumably would not exist. Moreover, this approach reinforces the view that the *in vivo* animal test is the definitive assessment of skin corrosivity of a test product. Thus, the necessity of conducting any *in vitro* test is reduced to a dubious exercise of limited usefulness.

Beyond the questionable logic of relying on a non-validated animal test to “confirm” the results of a validated non-animal test, the U.S. position (i) undercuts the potential for any meaningful reduction in animal testing for this endpoint (since more than 90 percent of new chemicals are non-corrosive and would therefore be subject to “confirmatory” animal testing in the US; Hartung, 2003), and (ii) appears to contravene both the letter and spirit of the OECD Council Decision Regarding the Mutual Acceptance of Data (OECD, 1981). Moreover, despite repeated attempts by several of the parties to this submission to persuade ICCVAM and its member agencies to reconsider their position on this subject, such efforts have yet to bear fruit.

The experience chronicled above strongly reinforces our existing doubts regarding the need for an ICCVAM review of test methods that have undergone successful validation and peer review under the auspices of ECVAM or another international validation authority. We are also mindful of the fact that at least 23 alternative methods and/or testing strategies have been successfully validated and peer reviewed to date, of which only a tiny handful have been accepted formally or otherwise by U.S. agencies, as documented in the table below.

Endpoint	Name of Test	1st Endorsement	US Acceptance
Skin corrosion	EPISKIN™	April 1998 ¹	June 2002 ^{4,5}
Skin corrosion	EpiDerm™	May 1998 ¹	June 2002 ^{4,5}
Skin corrosion	Rat TER assay	April 1998 ¹	June 2002 ^{4,5}
Antibody production	<i>In vitro</i> production of monoclonal antibodies	November 1997 ^{1,6}	November 1997 ⁶
Photoirritation	3T3 NRU PT test	May 1998 ¹	May 2003 ⁷
Skin absorption	<i>In vitro</i> skin absorption test	— ³	— ^{4,8}
Skin allergy	Local lymph node assay	March 1999 ²	October 1999 ⁴
Vaccine potency	Toxin binding inhibition test	December 2000 ¹	--
Vaccine potency	ELISA test for human tetanus vaccines	December 2000 ¹	--
Skin corrosion	CORROSITEX™	December 2000 ²	—
Embryotoxicity	Embryonic stem cell test	May 2002 ¹	—
Embryotoxicity	Micromass assay	May 2002 ¹	—
Embryotoxicity	Whole rat embryo assay	May 2002 ¹	—
Vaccine potency	ELISA test for erysipelas vaccines	June 2002 ¹	—
Paralytic shellfish poison	Lawrence method of high performance liquid chromatography	June 2005 ¹⁰	—
Pyrogenicity	Human whole blood IL-1	March 2006 ¹	—
Pyrogenicity	Human whole blood IL-6	March 2006 ¹	—
Pyrogenicity	Human cryopreserved whole blood IL-1	March 2006 ¹	—
Pyrogenicity	PBMC IL-6	March 2006 ¹	—
Pyrogenicity	MM6 IL-6	March 2006 ¹	—
Acute toxicity to fish	Upper threshold concentration approach	March 2006 ¹	—
Acute neutropenia	CFU-GM assay	March 2006 ¹	—
Eye corrosion/ severe	Bovine corneal opacity-	March 2006 ²	— ⁹

irritation	permeability test		
Eye corrosion/ severe irritation	Isolated chicken eye test	March 2006 ²	—
Genotoxicity	<i>In vitro</i> micronucleus test	—	— ¹¹

¹ ECVAM Scientific Advisory Committee (ECVAM, 2006).
² ICCVAM Expert Panel (ICCVAM, 2006).
³ OECD Expert Consultation.
⁴ OECD Test Guideline subject to OECD Mutual Acceptance of Data requirements (OECD, 1981; 2006).
⁵ Partial acceptance as a “positive screen” according to which negative results *in vitro* (ICCVAM, 2002).
⁶ OLAW (1997).
⁷ FDA (2003).
⁸ *In vitro* methods required under EPA/OPPT test rule (69 FR 22402, 26 April 2004), but not generally accepted in EPA/OPP for pesticide registrations (70 FR 12276, 11 March 2005).
⁹ Case-by-case acceptance by EPA for pesticide labeling (Harbell J, personal communication, Sept 2004).
¹⁰ AOAC (2005).
¹¹ Requirement under EU REACH regulation (Annex VIII, Information Requirement 8.4.2.) for chemicals manufactured in volumes of ≥10 tons per year.

In addition to the test methods listed above, ECVAM has recently reported that as many as 171 alternative methods are currently in the “validation pipeline” (Hartung, 2006), which suggests that the current bottleneck will only grow with time. This is unacceptable—both in principle and in view of the statutory language of P.L. 106-545, which calls for greater efficiency, less duplication, and increased reliance on scientific expertise outside the U.S. government.

- *The parties to this submission therefore strongly urge ICCVAM and its member agencies to establish formal bilateral and/or multilateral reciprocity agreements with ECVAM and other international validation authorities whereby an endorsement by one authority is recognized and automatically accepted in all participating jurisdictions.*

Responses to Specific Questions Posed by NICEATM/ICCVAM

1. Comments on the priority areas for the development and validation of alternative test methods

We appreciate ICCVAM’s efforts to establish U.S. government-wide priorities for the development and validation of new or revised regulatory testing methods that reduce, refine or replace animal use. However, we question whether the current rank-ordered list adequately reflects the regulatory endpoints with multi-agency applicability in the U.S., which according to P.L. 106-545 should generally be the primary focus of ICCVAM’s activities.³ More specifically, we recommend that ICCVAM assign the highest priority to endpoints/ activities that satisfy one or both of the following criteria:

³ This criterion should in no way be perceived to discourage individual ICCVAM member agencies from pursuing the full range of 3Rs opportunities in their respective regulatory sector(s).

- Endpoints for which partial or full replacement of animal use is achievable in the near-term (e.g., skin and eye irritation, mechanistic endocrine screening, endpoints that traditionally involve the use of multiple species and/or exposure routes, etc.).
- Endpoints for which conventional test methods consume the greatest number of animals (e.g., reproductive and developmental toxicity, carcinogenicity, etc.; Appendix 1).

2. Development, translation and validation activities most likely to have the greatest impacts within the next five years on refining, reducing or replacing animal use

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.
- 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.

In addition to the recommendations from the HESI technical panels, 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 vapours, 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 computerised 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 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.

We would also encourage ICCVAM to expand its efforts to reduce animal use in acute toxicity testing to enforcement and full replacement.

- Since beginning the task of reducing and replacing animal use in lethal dose testing (NIH Pub. No. 01-4499), ICCVAM has issued a single Guidance Document: “Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity” (NIH Pub. No. 01-4500). To the best of our knowledge, no additional prevalidation studies of any pharmacodynamic models were initiated while the larger cytotoxicity study was in progress nor were any of the intermediate-term activities, recommended in the 2000 workshop report, implemented: “Continued development and optimization of such systems (as gut absorption, BBB passage, key kinetic parameters, and metabolism) for this application should be encouraged and should receive regulatory support” (NIH Pub. No. 01-4499). This neglect to date on ICCVAM's part of the cytotoxicity methods as full replacement methods for the continued use of animals in lethal dose testing is inexcusable. We encourage ICCVAM to further pursue cytotoxicity methods that would allow full replacement of animals in lethal dose testing.
- In addition, a quick review of the tests proposed and conducted under the HPV program shows that, of the approximately 20 acute systemic toxicity tests conducted and currently pending, only one cytotoxicity test was apparently conducted to set the starting dose (this equates to a use rate of only 5%). We urge ICCVAM to fulfill its obligation of coordinating the acceptance of the use of cytotoxicity data for estimating starting doses for acute toxicity.

ICCVAM should also be aware of the activities of the European Partnership for Alternative Approaches to Animal Testing (EPAA; <http://www.epaa.eu.com>), which recently surveyed its more than two dozen corporate partners to identify 3Rs methods used in-house that could potentially be brought into the mainstream. The EPAA reports that as of November 12, 2006, 114 candidate 3Rs methods have been identified, of which:

- 56 percent were geared at replacement; 31 percent reduction; 13 percent refinement.
- 57 percent were applicable to the chemicals sector; 35 percent pharmaceuticals; 17 percent animal health; and 5 percent agrochemicals (Webb, 2006).
 - *Given the tremendous potential of these recommendations to reducing animal use, ICCVAM member agencies representing sectors that require or recommend testing for any of the above endpoints should begin to take immediate steps to implement these recommendations, as applicable, in their regulatory programs.*

3. Research and development activities holding the greatest promise in the long-term for refining, reducing or replacing animal use

European government institutions and corporate partners are currently providing more than €80 million in funding under for 13 targeted, multi-year 3Rs research projects (Hartung, 2006), including the following:

- *ReProTect* (<http://www.reprotect.eu>): An EU integrated project budgeted at €13.9 million (EC contribution €9.1 million) aimed at developing the concepts required to develop 3Rs testing strategies in the areas of reproductive and developmental toxicity.
- *ACuteTox* (<http://www.acutetox.org>): An integrated project budgeted at €15.7 million (EC contribution €9 million) aimed at optimizing and prevalidating an *in vitro* testing strategy for predicting acute toxicity in humans.
- *Sens-it-iv* (<http://www.sens-it-iv.eu>): A multi-stakeholder integrated project to develop novel testing strategies for *in vitro* assessment of allergens.
- *PredictOmics*: An integrated project budgeted at €3.4 million (EC contribution €2.3 million) aimed at developing short-term *in vitro* assays to evaluate long-term toxicity. A parallel EPAA initiative is also slated to begin in the coming year.
- *BioSim* (<http://www.biosim-network.net>): An EU Network of Excellence comprised of 26 academic, 10 industrial and 4 regulatory partners mandated to develop *in silico* simulation models of cellular, physiological and pharmacological processes to provide a deeper understanding of biological processes.
- *OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through the Integration of Non-test and Test Information)*: Integrated project with an EC contribution of €10 million and estimated 4.5 year duration, which addresses the reduction of animal tests in the

implementation of the REACH regulation through the application of “Intelligent Testing Strategies.”

Ongoing developments in the field of computational toxicology also hold great promise as means to reduce (by means of chemical grouping and bridging/batching techniques), and ultimately replace, animal testing. For example, parties to this submission have contributed more than \$500,000 to the scientific charity, the International QSAR Foundation to Reduce Animal Testing (IQF), which is chaired by retired EPA senior scientist Gilman Veith, who also coordinates (Q)SAR activities for the OECD. The IQF organizes workshops to develop strategies and modeling solutions focused on 1) filling crucial gaps in modeling needs and 2) finding cost-effective ways to integrate QSAR modeling to decrease regulatory testing needs. IQF research programs currently focus on predicting:

- Chemical reactivity with model nucleophiles.
 - Skin irritation/sensitization for chemical reactivity profiles.
 - Inhalation and aquatic toxicity from chemical reactivity profiles.
 - Environmental risk assessment.
- *Financial support and/or other constructive involvement in these initiatives by U.S. regulatory agencies would no doubt be welcomed.*

4. Appropriate measures for evaluating progress in enhancing the development and use of alternative test methods

As articulated on numerous occasions at meetings of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), we strongly support the implementation of measures by corporations to permit the quantitative tracking and reporting of all vertebrate animal use for regulatory testing purposes. Key details that should be collected include the following:

- Number and species of animal used
- USDA category of invasiveness (i.e., B, C, D or E)
- Primary sector for which testing was conducted (e.g., pharmaceutical, chemical, etc.)
- Type of testing (i.e., human/environmental safety, efficacy)
- Toxicological endpoint
- Test guideline
- Whether testing was explicitly requested/required by a regulatory agency
- Whether testing was required in addition to U.S. requirements for export to a foreign country

U.S. regulatory agencies that require or recommend animal testing should (i) require registrants to include details such as these in all data submissions, and (ii) establish internal databases for collection and annual reporting of these data. The first such report from each agency could be used as a benchmark against which future trends in animal use can be judged.

Other Issues

Gathering and use of human data to improve evaluations of test method relevance

According to the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2004):

It is recommended that human data should be more often used for risk assessment, since they form the most direct evidence for human health risk. It is also recommended that if sufficient quality human data as well as animal data are available, the human data should be given priority regardless of their effect on the risk assessment.

The same could be said of the use of human data for validation purposes. Indeed, it was a consensus recommendation of the 2002 OECD Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment that an international expert workshop be convened to:

- Identify sources of existing, high quality human data (e.g., occupational biomonitoring, clinical trials, accidental exposure/poison control, epidemiology, etc.)
- Discuss the existence/creation of centralized databases, toxicity endpoints covered, data quality issues, etc.
- Develop consensus positions and recommendations for moving forward.

Regrettably, in the nearly five years since the OECD validation conference, no perceptible effort has been made on the part of regulatory authorities or industry to implement this recommendation.

- *ICCVAM and its member agencies are invited to take a leading role in the organization of an international workshop on this topic.*

Summary

Thank you for your attention and responsiveness to these comments. Please direct any questions to the undersigned at samundson@hslf.org.

Sincerely,



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Page 12

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APPENDIX 1 – ANIMAL USE CALCULATIONS

Endpoint	OECD guideline ¹	Avg. # of animals/test ¹
<i>Human Health Effects</i>		
Acute systemic toxicity – oral	420/423/425	7-20 rats
Acute systemic toxicity – inhalation	403 (draft 433/436)	20 rats
Acute systemic toxicity – dermal	402 (draft 434)	20 rats, rabbits <i>or</i> guinea pigs
Acute eye irritation	405	3 rabbits
Acute skin irritation	404	3 rabbits
Skin sensitisation	429/406	16 mice <i>or</i> 32 guinea pigs
Subacute (21/28 d) toxicity – dermal	410	40 rats
Subchronic (90 d) toxicity – rat	408	120 rats
Subchronic (90 d) toxicity – non-rodent	409	32 dogs
Subchronic (90 d) toxicity rodent – dermal	411	120 rats
Subchronic (90 d) toxicity rodent – inhalation	413	120 rats
Acute neurotoxicity	424	80 rats
Acute delayed OP neurotoxicity	418	24 hens
Delayed (28 d) OP neurotoxicity	419	40 hens
Subchronic (90 d) neurotoxicity	424	80 rats
Chronic toxicity/carcinogenicity – rat	453	400 rats
Chronic (1-year) toxicity – non-rodent	452	32 dogs
Carcinogenicity – mouse	451	400 mice
Mutagenicity – <i>in vivo</i> chromosomal aberration	475	80 rodents
Mutagenicity – mouse micronucleus	474	80 rodents
Mutagenicity – rodent dominant lethal	478	80 rodents
Reproductive toxicity in 2 generations	416	2,600 rats
Developmental toxicity – rodent	414	1,300 rats
Developmental toxicity – non-rodent	414	660 rabbits
Developmental neurotoxicity	(draft 426)	1,300 rats
General metabolism	417	4-32 rats
Dermal penetration	427/428	96 rats

Ecotoxicological Effects

Avian oral LC ₅₀	OPPTS 850.2100	60 birds
Avian dietary LC ₅₀	205	80 birds
Avian reproduction	206	1,450 birds
Freshwater fish LC ₅₀	203	30-120 fish
Estuarine fish LC ₅₀	203	30-120 fish
Fish early life stage – freshwater	210	360 fish
Fish early life stage – saltwater	210	360 fish
Fish life-cycle	212/215	360 fish
Fish bioconcentration	305	12 fish

¹ Calculations based on OECD (2006).