UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, DC 20207

BALLOT VOTE SHEET

Date: AUG 21 2008

TO : The Commission
Todd A. Stevenson, Secretary

THROUGH: Patricia Semple, Executive Director PS

FROM : Cheryl A. Falvey, General Counsel CAF

SUBJECT : Staff Recommendation on Response to ICCVAM on the use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing

BALLOT VOTE due: AUG 28 2008

The attached memorandum from the Health Sciences Directorate summarizes the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for the use of *in vitro* basal cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity testing. The staff recommends that the Commission accept the ICCVAM recommendations and instruct the staff to so inform ICCVAM by letter.

Please indicate your vote.

I. Accept the ICCVAM recommendations and instruct the staff to so inform ICCVAM by letter.

_____ _____
Signature Date

II. Reject the ICCVAM recommendations and instruct the staff to so inform ICCVAM by letter.

_____ _____
Signature Date

Attachment - *Staff Recommendation on Response to ICCVAM on the use of In Vitro Basal Cytotoxicity Test Methods for Estimating Starting Does for Acute Oral Systemic Toxicity Testing*, memorandum from Cassandra Prioleau, P.h.D., Directorate for Health Sciences, to the Commission, August 2008.

[illegible] recommendation has been [illegible] accepted by the Commission.
Initial TK Date 8/21/08

8/21/08

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
4330 EAST WEST HIGHWAY
BETHESDA, MD 20814

Memorandum

Date: AUG 21 2008

TO : The Commission

THROUGH: Todd A. Stevenson, Secretary
Cheryl A. Falvey, General Counsel
Patricia M. Semple, Executive Director

FROM : Robert J. Howell, Acting Assistant Executive Director
Office of Hazard Identification and Reduction

Cassandra Prioleau, Ph.D., Pharmacologist
Directorate for Health Sciences

SUBJECT : Staff Response to the ICCVAM Recommendations on the Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing

This memorandum discusses the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for the use of *in vitro*[1] basal[2] cytotoxicity[3] test methods for estimating starting doses for acute oral systemic toxicity testing. In addition, information is provided on whether these alternative methods are acceptable in the regulatory context for the purpose of classification for labeling under the Federal Hazardous Substances Act (FHSA) (15 U.S.C. 1261-1278).

I. Introduction

The National Institutes of Health Revitalization Act of 1993 directed the National Institute of Environmental Health Sciences (NIEHS) to establish a method and criteria for the validation and regulatory acceptance of alternative testing methods (Public Law No. 103-43, Section 1301). To accomplish these goals, NIEHS created an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) which was made permanent by the ICCVAM Authorization Act of 2000 (Public Law 106-545). The duties of ICCVAM are to review, optimize, and validate new, revised, or alternative test methods that encourage the reduction, refinement, or replacement of the use of animals in testing. In addition, ICCVAM is to provide test recommendations to Federal agencies and other stakeholders to facilitate appropriate interagency and international harmonization of toxicological test protocols. In 1998, the National

[1] *in vitro* – in a test tube (i.e., non-animal)
[2] Basal – pertaining to maintaining the fundamental vital activities of an organism
[3] Cytotoxicity – toxic to cells; adverse effects resulting from the interference with structures and processes essential for cell survival, proliferation, and/or function

Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established to assist ICCVAM in performing the activities necessary for the validation and regulatory acceptance of alternative test methods.

ICCVAM submits test recommendations stating the usefulness and limitations of a test method to the 15 member Federal agencies. These agencies should promote and encourage the development and use of alternatives to animal test methods for regulatory purposes, and ensure that any new or revised acute or chronic toxicity test method is valid for its proposed use under the mandate of the ICCVAM Authorization Act of 2000. Federal agencies have 180 days to identify any relevant test methods for which the ICCVAM test recommendations may be added or substituted, review such test recommendations, and notify ICCVAM if they will adopt the ICCVAM test recommendations.

ICCVAM forwarded their recommendations for the use of *in vitro* basal cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity testing to the Commission for action on February 28, 2008. The U.S. Consumer Product Safety Commission (CPSC) needs to determine if the proposed alternative methods for estimating the starting dose of acute oral systemic toxicity testing will be acceptable as an adjunct method to rodent acute oral toxicity testing. The Commission needs to respond back to ICCVAM by August 28, 2008.

Validation of Alternative Methods

Validation of alternative methods is required before regulatory acceptance and utilization by Federal agencies. In general, for an alternative method to be considered valid it must be relevant (i.e., the alternative test method is useful for measuring the biological effect of interest such as acute oral toxicity) and reliable (i.e., the toxicity predictions of test substances are repeatable within the same laboratory and reproducible across different laboratories).

The relevancy and reliability of an alternative test method are frequently assessed from statistical analysis of data. The relevance of an alternative test method can be determined by comparing the performance of the alternative test to the test that it is designed to replace. Performance is typically evaluated by calculating the accuracy[4], false positive rate[5], false negative rate[6], sensitivity[7], and specificity[8] of the alternative test method. The reliability of the alternative test method can be determined from the reproducibility or variability (e.g., coefficient of variation (CV), % agreement among laboratories, etc.) of test method results within and among laboratories.

Federal Hazardous Substances Act Requirements

Precautionary labeling of hazardous household substances is mandated by the Federal Hazardous Substances Act (FHSA, The Act) (15 U.S.C. § 1261-1275). Under the FHSA, to be a hazardous substance, a product must present one or more of the hazards, such as toxicity, enumerated in the statute and it must have the potential to cause substantial personal injury or substantial illness

[4] Accuracy – proportion of correct outcomes
[5] False positive rate – proportion of all negative substances that are falsely identified as positive
[6] False negative rate – proportion of all positive substances that are falsely identified as negative
[7] Sensitivity – the proportion of all positive substances that are classified as positive
[8] Specificity – the proportion of all negative substances that are classified as negative

during or as a result of any customary or reasonably foreseeable handling or use. Methodology is provided in the FHSA to aid in the classification of substances as hazardous substances.

Briefly, a "toxic substance has the capacity to produce personal injury or illness to man through ingestion, inhalation, or absorption through any body surface" (16 CFR § 1500.3 (b)(4)(iii)(5)). A substance can be determined by the Commission to be toxic on the basis of human experience or for which a positive test is obtained when tested by the method described in 16 CFR § 1500.3. To perform the acute oral toxicity test, a statistically sufficient number of animals are exposed to a test substance by oral ingestion. Test substances that produce death "when a single dose of 50 milligrams or less per kilogram of body weight is administered within 14 days in half or more than half" of the animals are considered to be "highly toxic." Substances that produce death "when a single dose of from 50 milligrams to five grams per kilogram of body weight is administered within 14 days in half or more than half" of the animals are considered to be "toxic."

Additional information should be considered when determining whether a consumer product is a hazardous substance under the FHSA. The Act states that human experience takes precedence over animal data if human results differ from the results for animals (16 CFR § 1500.4). In addition, when determining if a consumer product, which can be composed of a mixture of substances, is a hazardous substance, the mixture should be tested and not the individual components of the mixture, because synergistic or antagonistic reactions may lead to erroneous determinations concerning the toxic, irritant, corrosive, etc. properties of the substance (16 CFR § 1500.5).

Current Acute Oral Toxicity Testing

Acute oral toxicity is traditionally done by determining the lethal dose (LD_{50}) that produces death in half of the animals tested. Currently, the primary method utilized to assess the potential of the product to cause acute oral toxicity is the up-and-down procedure (UDP). A standardized test guideline (TG 425) for the UDP was developed and validated by the Organisation for Economic Co-operation and Development (OECD)[9] in 2001. Other testing guidelines for acute oral toxicity have also been developed by OECD: the Fixed Dose Procedure (FDP, TG420) and the Acute Toxic Class method (ATC, TG 423).

The original LD_{50} acute toxicity test, developed in 1927 (Botham, 2002), could use up to 100 animals. Several modifications have been implemented since the original LD_{50} acute toxicity testing was introduced that aimed to reduce the number of animals tested. In 2001, the conventional LD_{50} acute oral toxicity *in vivo*[10] test method was replaced with the acute oral toxicity UDP based on an ICCVAM technical evaluation and formal ICCVAM recommendations. Accordingly, the use of the UDP instead of the conventional LD_{50} for the purpose of classification and labeling of consumer products under the FHSA was approved by the Commission in 2003 (letter from U.S. CPSC, Office of the Secretary to Kenneth Olden, Director, National Institutes of Health [NIH], NIEHS; 2003). The UDP resulted in a major

[9] The OECD is a multilateral organization that, for one, promotes and coordinates European and international test guidelines and policies.
[10] *in vivo* – in a living body

reduction in the number of animals used compared to the conventional LD_{50} test method (Lipnick et al., 1995; Bruce et al., 1987).

Before the UDP is conducted using animals, all available information is considered. If the LD_{50} has already been determined for the test substance, then the toxicity of the test substance can be predicted and the test substance can be appropriately classified.

If no toxicity data for the product is available, then testing in animals may be necessary to evaluate the hazard potential of a product for the purposes of regulatory hazard classification and labeling. In these circumstances, the most humane procedures and fewest animals possible should be used while still maintaining good science. Utilizing *in vitro* basal cytotoxicity test methods to estimate the starting dose for the UDP can potentially reduce the number of animals used more than just performing the UDP alone and is consistent with the Commission's policy to reduce, refine, or replace the use of animals in testing (49 FR 22522). In fact, the Commission encouraged the regulated industry to use certain *in vitro* tests for determining the starting dose for acute systemic toxicity testing in animals (letter from CPSC to NIH, 2003).

II. Alternative Basal Cytotoxicity *In Vitro* Testing

History of the Evolution of Using *In Vitro* Cytotoxicity Test Methods to Estimate the Starting Dose for Rodent Acute Oral Toxicity Testing

1983	The Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC) program was established by the Scandinavian Society for Cell Toxicology to evaluate the ability of *in vitro* cytotoxicity assays to predict acute oral lethality in humans. The MEIC team concluded that the data from their investigation indicated that *in vitro* test methods may better predict acute oral lethality in humans than rat or mouse oral lethality data. However, optimized protocols and prediction models were needed before *in vitro* test methods could replace *in vivo* acute toxicity testing.
1992 - 1993	An international and collaborative study was organized and conducted by the Fund for the Replacement of Animals in Medical Experiments (FRAME) to identify *in vitro* test methods that could predict rodent acute oral lethality which then could be used for the classification and labeling of chemicals. Based on this study, a battery of *in vitro* tests was recommended for the purpose of classifying chemicals for their acute lethal potency in rodents.
1994	The European Centre for the Validation of Alternative Methods (ECVAM) organized a workshop to evaluate the usefulness of *in vitro* data for the purpose of classification and labeling of chemicals. At this workshop, the concept of using *in vitro* test data to estimate the starting dose for rodent acute oral toxicity testing was introduced with the purpose of reducing the numbers of animals used.
1998	The Registry of Cytotoxicity (RC) database of rodent *in vivo* acute toxicity data and *in vitro* cytotoxicity assay data was compiled from

existing data by Halle and colleagues to determine if basal cytotoxicity tests could accurately predict acute oral lethality in rodents. A model was developed from the RC data that could be used to predict rodent *in vivo* oral lethality (LD_{50}) from *in vitro* cytotoxicity data (i.e., IC_{50} or the inhibitory concentration required to kill 50% of the cells).

1999 — The German National Center for the Documentation and Evaluation of Alternative Methods (ZEBET) proposed an approach to predict a starting dose for the UDP from the IC_{50} values of the *in vitro* cytotoxicity assays.

1999 — The U.S. Environmental Protection Agency (EPA) requested ICCVAM to review the validation status of *in vitro* test methods to estimate the starting dose of rodent acute oral toxicity tests.

2000 — An international workshop on using *in vitro* methods for assessing acute systemic toxicity was organized and sponsored by NIEHS, EPA, and the National Toxicology Program (NTP) (65 FR 57203).

- The workshop participants concluded that the proposed *in vitro* methods had not been adequately assessed for reliability and relevance and therefore the *in vitro* methods could not replace the current *in vivo* acute oral toxicity test method.
- The participants recommended that *in vitro* cytotoxicity methods may be useful for estimating the starting dose for rodent acute oral toxicity testing. Consequently, a guidance document to provide sample cytotoxicity protocols and instructions for using *in vitro* data to predict starting doses for acute *in vivo* systemic toxicity tests was prepared by ICCVAM with the aid of workshop participants.
- Based on the recommendations from the workshop, ICCVAM made the following recommendation: two standard *in vitro* cytotoxicity assays, one using a human cell system and the other a rodent cell system, should be tested for their ability to estimate the starting doses for rodent acute oral toxicity testing (66 FR 49686).

2001 — NICEATM in partnership with ECVAM designed a multi-laboratory validation study to evaluate the use of *in vitro* basal cytotoxicity assays to estimate starting doses for rodent acute oral toxicity test methods.

In Vitro Basal Cytotoxicity Test Methods to Estimate the Starting Dose for Acute Oral Systemic Toxicity Testing

NICEATM in partnership with ECVAM designed a multi-laboratory validation study to evaluate the performance of two *in vitro* basal cytotoxicity assays: the neutral red uptake (NRU) assay in normal human epidermal keratinocytes (NHK) and mouse BALB/c fibroblast (3T3) cell lines. The protocols for the *in vitro* cytotoxicity test methods were also further optimized and standardized in this validation study. Three laboratories, the U.S. Army Edgewood Chemical Biological Center (ECBC), FRAME Alternative Laboratory (FAL), and Institute for *In Vitro* Sciences (IIVS) performed the cytotoxicity testing of chemicals (72) that represented a range of toxicities and for which human toxicity data were available. The participating laboratories independently generated IC_{50} values for the selected chemicals using the two selected *in vitro*

cytotoxicity assays. The generated IC_{50} data were used to predict LD_{50} values that could be used to estimate the starting dose for the *in vivo* acute oral toxicity testing. Finally computer modeling was done to determine if the estimated starting dose would result in a reduction in the use of animals in the *in vivo* acute oral toxicity test methods.

When the studies were completed, NICEATM with the assistance of the Acute Toxicity Working Group (ATWG) and ECVAM compiled a Background Review Document (BRD) that contains information about the validation status of the two *in vitro* alternative cytotoxicity test methods. The validation of the alternative test methods was determined by evaluating existing data that was submitted to NICEATM in response to requests for data on chemicals evaluated by *in vitro* cytotoxicity assays and data from the multi-laboratory study (69 FR 61504, 70 FR 14473). Protocols of the optimized and standardized *in vitro* cytotoxicity test methods are also included in the BRD.

The following sections describe the test method, validation status, and performance of the NRU test method in the two different cell systems. Although the *in vitro* test methods will not be used to predict the hazard classification of a consumer product as defined by the GHS or FHSA, the strategy of the validation study was to predict the LD_{50} values of the test substances from the measured *in vitro* IC_{50} values. Consequently, the relevancy (i.e., accuracy) and reliability (i.e., reproducibility) of the test methods were also evaluated. The reason for this strategy is that the use of a starting dose below or close to the reference LD_{50} of the test substance may reduce animal usage because the number of animals used depends on the starting dose and may refine animal usage because the use of a less toxic dose may decrease the pain and suffering of the animals.

In Vitro Cytotoxicity Neutral Red Uptake Test Method

There are a number of methods available to measure cytotoxicity. The NRU test method utilizing 3T3 and NHK cells was recommended by ICCVAM after the international workshop in 2000 to be used in the cytotoxicity validation studies. To perform the NRU assay, cells are treated with the test substance and cell death is measured following exposure to neutral red dye. The amount of dye retained by the cells is a measure of cell death because only viable cells take up and accumulate the neutral red dye.

1. BALB/c 3T3 Cells

The 3T3 cells are a permanent cell line made from mouse fibroblast cells. These cells were recommended to be evaluated by ICCVAM to determine if toxicity data from these cells correlate with acute toxicity data from rodent assays and to assess their usefulness and limitations to predict acute oral lethality in humans.

(i) Validation and Performance

To test the accuracy of the 3T3 *in vitro* test method, a linear regression curve was created from the *in vitro* test method data and was compared to a curve from the RC regression model[11] that was developed in 1998. An advantage of using the RC regression curve for

[11] The RC model was, in part, created from rodent (i.e., rat and mouse) LD_{50} values and thus the RC regression curve can be modeled on rat-only data or rat and mouse data and molecular weight (i.e., millimoles/liter) or weight

comparison is its reputed reliability because it is based on results from a large number (282) of chemical substances. Conversely, the *in vitro* curve was based on the data of 47 test substances. Data from the statistical analysis of the 3T3 *in vitro* curve indicated that the curve was not significantly different from the RC curve.

Another method to assess accuracy is to determine the ability of the *in vitro* test method to correctly predict rodent acute oral toxicity categories. The accuracy of the 3T3 *in vitro* test method to predict the hazard classification[12] of test substances was 31% (21/67). The accuracy increased to 75% if only the test substances from the mild category (i.e., $300 < LD_{50} \leq 2000$ mg/kg) were considered. A pattern was noted for the ability of the 3T3 test method to predict classification. The toxicity of substances in the highest toxicity categories ($LD_{50} \leq 50$ mg/kg) was generally underpredicted, whereas the toxicity of substances in the lowest category ($LD_{50} > 5000$ mg/kg) was overpredicted.

If the 3T3 *in vitro* data were evaluated (not included in the BRD) for the ability to accurately predict hazard classification as defined by the FHSA (highly toxic: $LD_{50} \leq 50$ mg/kg or toxic: $50 < LD_{50} \leq 5000$ mg/kg), the overall accuracy was 75% (50/67). Test substances in the most toxic category (highly toxic: $LD50 \leq 50$ mg/kg) were by and large underpredicted. However, test substances in the least toxic category (toxic: $50 < LD_{50} \leq 5000$ mg/kg) had an accuracy of 73% and were, in general, not overpredicted.

The reliability of an *in vitro* test method can be determined from intra- and inter-laboratory reproducibility. The reproducibility of results within the same laboratory (intra-laboratory reproducibility) was determined by calculating the CV. The CV for test substances ranged from 1% to 122% and had a mean CV of 26% for the 3T3 test method. CVs less than 35% are considered to be satisfactory for biologically based test methods.

The inter-laboratory reproducibility of the 3T3 *in vitro* test method results (i.e., IC_{50} values) varied across the different laboratories. There were significant differences among laboratories in the IC_{50} values for 36% (23/64) of the test substances evaluated. The CV for the test substances ranged from 3% to 135% and had a mean CV of 47% for the 3T3 test method. For substances classified in the most toxic categories, the mean CVs were 72% ($LD_{50} \leq 5$ mg/kg) and 78% ($5 < LD_{50} \leq 50$ mg/kg). Other categorical characteristics such as solubility, volatility, chemical class, etc. did not appear to change the overall inter-laboratory mean CV of 47%.

(ii) Animal Savings

Computer modeling was done to simulate *in vivo* testing. The number of animals saved in the *in vivo* acute oral toxicity tests (i.e., UDP) when the starting dose was determined from the 3T3 *in vitro* test method was determined from statistical analyses of these models. The percent of animals saved if the 3T3 test method was used to determine the starting dose

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(i.e., mg/kg) of test substances. For the purpose of this memorandum, the *in vitro* regressions curves were compared to the rat-only, weight RC regression curve, because weight regression analysis is applicable to mixtures and substances of unknown composition and most acute oral toxicity tests are performed with rats.

[12] Based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) acute oral toxicity categories

for the UDP was 7.8%. The animal savings increased up to 17% ($2000 < LD_{50} \leq 5000$ mg/kg) or 21% ($LD_{50} > 5000$ mg/kg), depending on the selection criteria (e.g., regression curve, slope, etc.), if only test substances in the least toxic GHS categories were considered.

2. NHK Cells

NHK cells are a primary cell line of normal human epidermal keratinocytes. These cells were recommended to be evaluated by ICCVAM to assess their usefulness to predict potential toxicity in humans.

(i) Validation and Performance

To test the accuracy of the NHK *in vitro* test method, a linear regression curve was created from the *in vitro* test method data and was compared to the curve from the RC regression model that was developed in 1998. The advantage of using the RC model for comparison is its reputed reliability because it is based on results from a large number (282) of chemical substances. Conversely, the NHK *in vitro* curve was based on the data from 51 test substances. Data from the statistical analysis of the NHK *in vitro* curve indicated that the curve was not significantly different from the RC curve.

Another method used to assess accuracy is to determine the ability of the *in vitro* test method to correctly predict rodent acute oral toxicity categories. The accuracy of the NHK *in vitro* test method to predict hazard classification[13] of test substances was 31% (21/68). However, the accuracy was 81% if only the test substances from the mild category (i.e., $300 < LD_{50} \leq 2000$ mg/kg) were considered. A pattern was noted for the ability of the NHK test method to predict classification. The toxicity of substances in the highest toxicity categories ($LD_{50} \leq 50$ mg/kg) was generally underpredicted, whereas the toxicity of substances in the lowest categories ($LD_{50} > 2000$ mg/kg) was overpredicted.

If the NHK *in vitro* data were evaluated (not included in the BRD) for the ability to accurately predict the hazard classification as defined by the FHSA (highly toxic: $LD_{50} \leq 50$ mg/kg or toxic: $50 < LD_{50} \leq 5000$ mg/kg), the overall accuracy was 75% (51/68). Test substances in the most toxic category (highly toxic: $LD_{50} \leq 50$ mg/kg) were by and large underpredicted. However, test substances in the least toxic category (toxic: $50 < LD_{50} \leq 5000$mg/kg) had an accuracy of 72% and were, in general, not overpredicted.

An approach used to measure intra-laboratory reproducibility is to assess the variability of the *in vitro* test method endpoint (i.e., IC_{50} value). The reproducibility of results within the same laboratory was determined by calculating the CV. The CV for test substances ranged from 1% to 129% and had a mean CV of 26% for the NHK test method. CVs less than 35% are considered to be satisfactory for biologically based test methods.

The inter-laboratory reproducibility of the NHK *in vitro* test method results (i.e., IC_{50} values) varied across the different laboratories. There were significant differences among

[13] Based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) acute oral toxicity categories

laboratories in the IC_{50} values for 9% (6/68) of the test substances evaluated. The CV for the test substances ranged from 1% to 91% and had a mean CV of 28% for the NHK test method. The overall inter-laboratory mean CV (28%) did not appear to change if the *in vivo* classification of the test substance was considered separately. Other categorical characteristics such as solubility, volatility, chemical class, etc. also did not appear to change the overall inter-laboratory mean CV.

(ii) Animal Savings

Computer modeling was done to simulate *in vivo* testing. The percent of animals saved if the NHK test method was used to determine the starting dose for the UDP was 6.8%. The animal savings increased up to 14% ($2000 < LD_{50} \leq 5000$ mg/kg) or 20% ($LD_{50} > 5000$ mg/kg), depending on the selection criteria (e.g., regression curve, slope, etc.), if only test substances in the least toxic GHS categories were considered.

III. Conclusions and Recommendations on the Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing

Peer Review Panel Conclusions and Recommendations

In May 2006, a peer review panel (Panel) at an ICCVAM sponsored meeting independently assessed the validation status, usefulness and limitations of the *in vitro* NRU basal cytotoxicity test methods to predict starting doses for acute oral systemic toxicity test methods. "The Panel agreed that the applicable validation criteria have been adequately addressed for using these *in vitro* test methods in a weight-of-evidence approach to determine the starting dose for acute oral *in vivo* toxicity protocols." Thus, the Panel concluded that the test method may be useful to determine the starting dose for acute oral *in vivo* toxicity protocols. However, the Panel recommended that future work be done to develop a tiered testing strategy that included using basal cytotoxicity test methods. The Panel also recommended that other *in vitro* test methods be investigated for their ability (usefulness) to accurately predict acute oral hazard classification.

ICCVAM Conclusions and Recommendations

In November 2006, ICCVAM finalized its conclusions and recommendations. After reviewing the BRD, the report from the peer review panel, and public comments, final test method recommendations for the use of *in vitro* basal cytotoxicity test methods to estimate starting doses for *in vivo* acute systemic toxicity testing were issued. ICCVAM recommendations as they relate to the use of *in vitro* basal cytotoxicity test methods for CPSC regulatory purposes are as follows:

- "The 3T3 and NHK NRU test methods are not sufficiently accurate to predict acute oral toxicity for the purpose of regulatory hazard classification."... "For the purpose of acute oral toxicity testing, the 3T3 and NHK NRU test methods may be used in a weight-of-evidence approach to determine the starting dose for the current acute oral toxicity protocols."
- "...*in vitro* basal cytotoxicity test methods as part of a weight-of-evidence approach to estimate the starting dose for acute oral *in vivo* toxicity test methods should be considered

and used where appropriate before testing is conducted using animals. For some types of substances, this approach will reduce the number of animals needed."

- "The starting doses for substances with certain toxic mechanisms that are not expected to be active in 3T3 or NHK cells (e.g., those that are neurotoxic or cardiotoxic) will likely be underpredicted by these *in vitro* basal cytotoxicity test methods. Therefore, the results from basal cytotoxicity testing with such substances may not be appropriate for estimating starting doses."
- "Compared to the NHK NRU test method, the 3T3 NRU test method appears to be less labor intensive and less expensive to conduct; therefore, the 3T3 NRU test method is recommended for general use."

IV. **Discussion by CPSC Staff**

Precautionary labeling of hazardous household substances is mandated by the FHSA (15 U.S.C. § 1261-1275). Hazardous substances that are ingested orally are either highly toxic ($LD_{50} \leq 50$ mg/kg) or toxic ($50 < LD_{50} \leq 5000$ mg/kg) and must be labeled appropriately. Currently, the acute oral hazard is determined from the lethal dose that produces death in half of the animals tested, but prior human experiences, literature results, and expert opinion are considered first before testing in animals is done. However, if testing is necessary to evaluate the hazard potential of a product for the purpose of labeling, the most humane procedures and fewest animals possible should be used.

Utilizing *in vitro* basal cytotoxicity test methods to estimate the starting dose for the UDP can potentially reduce and refine animal use. The estimated LD_{50} values (calculated from the IC_{50} values) of a number of the test substances in the validation study were generally underpredicted or close to the true or reference LD_{50}. For both the 3T3 and NHK cells, the estimated LD_{50} values of test substances classified as "highly toxic," based on FHSA criteria, were largely underpredicted when compared to the reference LD_{50} values obtained from *in vivo* testing. Furthermore, the LD_{50} values of test substances classified as "toxic" were close to the reference *in vivo* LD_{50} values (accuracy of 73% for the 3T3 cells and 72% for the NHK cells). Use of the estimated LD_{50} value as the starting dose for the acute oral toxicity test may save a considerable number of animals because the number of animals used in the rodent acute oral toxicity tests depends on the starting dose. In addition, using a starting dose below the reference LD_{50} will refine animal usage by using a less toxic dose thereby decreasing pain and distress.

Commission policy supports limiting animal tests and using procedures that eliminate or reduce the pain or discomfort associated with animal testing. Therefore the Commission encourages the utilization of validated alternative testing methods whenever possible. The *in vitro* basal cytotoxicity tests evaluated in this validation study are based on sound science and are scientifically valid to be used as part of a weight-of-evidence approach to estimate the starting does for acute oral *in vivo* toxicity test methods but not to predict acute oral toxicity for the purpose of regulatory hazard classification. Although these test methods are not adequate to replace testing in animals, they can reduce the number or animals required for acute oral toxicity tests. Thus, CPSC staff believes they should be considered for use as part of a weight-of-evidence approach before using animals.

Options

The Commission can vote to:

1. Accept the ICCVAM recommendations and instruct the staff to draft a letter to ICCVAM indicating acceptance of its recommendations.

2. Reject the ICCVAM recommendations and instruct the staff to draft a letter to ICCVAM indicating rejection of its recommendations.

Recommendations by CPSC Staff

The staff recommends that the Commission accept the ICCVAM recommendations that the *in vitro* basal cytotoxicity test methods should be considered and used where appropriate as part of a weight-of-evidence approach to estimate the starting does for acute oral *in vivo* toxicity test methods but not to predict acute oral toxicity for the purpose of regulatory hazard classification. These alternative *in vitro* test methods encourage the reduction, refinement, or replacement of animals in testing and the data indicate that the methods are scientifically valid methods. The staff also recommends that the Commission instruct the staff to draft a letter to ICCVAM indicating acceptance of its recommendations.

V. References

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66 FR 49686. NIEHS; NTP Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. 2001.

69 FR 61504. NIEHS; NTP; NICEATM Availability of Updated Standardized *In Vitro* Cytotoxicity Test Method Protocols for Estimating Acute Oral Systemic Toxicity; Request for Existing *In Vivo* and *In Vitro* Acute Toxicity Data. 2004.

70 FR 14473. NTP; NICEATM Request for Nominations for an Independent Peer Review Panel to Evaluate *In Vitro* Testing Methods for Estimating Acute Oral Systemic Toxicity and Request for *In Vivo* and *In Vitro* Data. 2005.

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