## PS 2031 ICCVAM/NICEATM/ECVAM/JACVAM SCIENTIFIC WORKSHOP ON ACUTE CHEMICAL SAFETY.

J. Strickland[1], M. Paris[1], D. Allen[1], R. Tice[2], H. Kojima[3], M. Wind[5], P. Prieto[4] and W. Stokes[2]. *[1]ILS, Inc./NICEATM, NIEHS, Research Triangle Park, NC, [2]NICEATM, NIEHS, Research Triangle Park, NC, [3]JaCVAM, Tokyo, Japan, [4]ECVAM, Ispra, Italy and [5]CPSC, Bethesda, MD.*

The evaluation and promotion of alternatives for acute systemic toxicity testing is one of ICCVAM's four highest priorities because (1) acute toxicity testing is the most commonly required product safety test worldwide and (2) it can cause significant pain and distress to test animals. We cosponsored a public workshop in February 2008 to review and consider standardized procedures to collect information pertinent to understand the mechanisms of lethality that should be included in future rat acute systemic toxicity studies to support further development of predictive mechanism-based in vitro test methods. This international workshop implemented one goal of the NICEATM–ICCVAM Five-Year Plan to identify approaches that would further reduce the potential pain and distress associated with acute toxicity testing by identifying more humane acute toxicity endpoints. The workshop reviewed public health significance and regulatory testing needs; human and animal assessments, biomarkers, and key pathways; humane endpoints; and the state of the science regarding in vitro methods that predict acute systemic toxicity. Breakout Groups identified knowledge gaps in understanding key toxicity pathways; recommended earlier humane endpoints for animal testing; suggested ways to obtain, from current in vivo testing models, mode of action and mechanistic information needed to develop and validate in vitro methods for assessing acute systemic toxicity; and explored avenues that would encourage industry to share information on in vitro and in vivo studies conducted in-house. This workshop recommended how mechanism-based in vitro test systems and earlier, more humane endpoints, could be developed to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing while ensuring the protection of human and animal health. ILS supported by NIEHS contract N01-ES-35504. This presentation may not reflect the view of the US Consumer Product Safety Commission.

## PS 2032 CONSIDERATIONS FOR CONDUCTING A CHEMICAL'S RISK ASSESSMENT UNDER REACH.

T. Petry, P. Bisht, S. Chakraborty and F. R. Tencalla. *ToxMinds BVBA, Brussels, Belgium.*

The European Union's REACH regulation for the management of risk to human health and the environment posed by chemical substances entered into force in June 2007. At its core, REACH will require producers and users of chemical substances to register these uses in a volume-triggered system. It demands the submission of chemical safety reports (CSR) containing information on the hazards, exposures and risks associated with the uses of chemical substances for review by the competent authorities and government-appointed expert committees. After a long process, the European Chemicals Agency (ECHA) published its detailed technical guidance documents supporting the REACH regulation in May 2008. These include concise guidance to support the process of risk assessment under REACH as well as in-depth guidance, which describe the required steps to risk characterization in technical detail. Due to the process established by the EU to reduce the need animal testing and foster the communication along the supply chain, hazard data will be available from a large number of sources and is expected to be of differing qualities. Conflicting information may potentially exist. The objective of the present investigation is to examine the EU's approach to conducting chemical risk assessment in this context and to detail the procedures registrants must follow to demonstrate the safety of their products. This paper will further illustrate on the basis of selected examples how special factors affecting information requirements, testing strategies and data waiving opportunities can be integrated into the risk assessment. This includes the use of grouping and read-across, as well as exposure-based and toxicokinetic arguments to waive the need for conducting animal toxicity studies. As the consideration of existing risk management measures is an integral part of the REACH safety concept, the paper will conclude by a short discussion of the iterative process of controlling risks within a chemical's life cycle under REACH.

## PS 2033 DETERMINATION OF THE DERIVED NO EFFECT LEVEL FOR STYRENE: AN EXERCISE IN INTERPRETING REACH GUIDANCE.

M. L. Kreider[1] and E. Williams[2]. *[1]ChemRisk, Inc., Pittsburgh, PA and [2]ChemRisk, Inc., Houston, TX.*

Under the new European chemicals regulation, REACH, a new safety value, the Derived No Effect Level (DNEL) must be established for all chemicals manufactured, imported or used in the EU in quantities greater than 10 metric tonnes per year. A DNEL, expected to be used as part of the risk characterization and for hazard communication purposes, is to be calculated for all relevant exposure pathways, exposure populations, and for different endpoints of toxicity (e.g. acute irritation, repeated dose toxicity, reproduction, etc.). The EU has published guidance on how to derive the DNEL, but with the start of the registration looming, this guidance has yet to be put into practice and is in some places not prescriptive. Using the Agency for Toxic Substances and Disease Registry (ATSDR) dataset, we have determined DNELs for styrene, based on inhalational exposure. In doing so, we considered what effect key decisions would have on the calculated DNEL (where there was flexibility in the guidance or interpretation was necessary). Key decisions included the use of animal or human data, selection of dose descriptors, estimation of human equivalent concentrations (HECs) from animal studies, and selection of appropriate modifications and assessment factors. The resulting DNELs were then compared to existing risk criteria values or occupational exposure limits. For both general population and worker based exposures, the lowest DNELs were generated based on the neurological effects of styrene. DNELs based on general population exposures were generally more conservative than analogous risk criteria (ranging from approximately 0.05 to 2.5 ppm). The DNELs based on occupational exposure scenarios are dramatically lower than existing occupational standards (ranging from approximately 0.4 to 20 ppm). To our knowledge, this work represents the first rigorous application and interpretation of the EU guidance for determination of a DNEL and will prove useful as a model for determination of other DNELs under REACH.

## PS 2034 ESTIMATION OF A TETRACHLOROETHYLENE INHALATION CANCER RISK FACTOR: COMPARISON OF MASSDEP AND U.S. EPA DOSIMETRIC DECISIONS UNDER UNCERTAINTY.

S. J. Baird, T. Zewdie, C. Smith and C. Rowan-West. *Office of Research and Standards, Massachusetts Department of Environmental Protection, Boston, MA.*

MassDEP derived a revised inhalation unit risk (IUR) for tetrachloroethylene (PCE) of $1x10^{-5}$ per ug/$m^3$ in 2007, finalized in September 2008. Following this derivation, USEPA released their draft Toxicological Review of PCE in June 2008 with IURs ranging from $2x10^{-6}$ to $2x10^{-5}$ per ug/$m^3$. Both groups selected mononuclear cell leukemia in rats as the most sensitive endpoint from the key inhalation bioassay studies, NTP 1986 and JISA 1993; concluded that mode of action was uncertain; used default linear low-dose extrapolation in calculation of the IUR; and considered multiple approaches for adjusting animal bioassay dosimetry to lower levels of human exposure. The dosimetric adjustments need to take into account potential differences in metabolism across species and dose-dependent differences in rates of metabolism in both animals and humans. Both groups selected total metabolized dose as the dose-metric because the active moiety is not known and multiple metabolites could be responsible for the carcinogenic effects. However, MassDEP used Michaelis-Menton steady-state kinetic equations to estimate animal total metabolized dose, while USEPA used a pharmacokinetic (PBPK) model. To estimate human population metabolism at relevant human environmental exposure levels MassDEP and USEPA were informed by different PBPK models and estimates of expected extent of human metabolism. MassDEP used the analysis by Chui and Bois (2006) to estimate the fraction of PCE metabolized by humans while accounting for human population variability in low dose metabolism: they estimated an upper limit of human population metabolism of PCE of 61% at 1 ppb. USEPA presented a range of IURs based on results from three human PBPK models yielding central estimates of human low dose metabolism ranging from 3–33%, as calculated by MassDEP. While different decisions were made in the dosimetric extrapolation, MassDEP's IUR falls within the range of USEPA's values.

## PS 2035 WEIGHT OF EVIDENCE PROCEDURES FOR SKIN NOTATION ASSIGNMENT IN OCCUPATIONAL HAZARD ASSESSMENT.

B. Gadagbui[1], G. Dotson[2], A. Maier[1] and G. Talaska[3]. *[1]Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH, [2]National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC), Cincinnati, OH and [3]Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

Skin contact is a significant route of exposure in occupational settings. However, many occupational health risk assessments rely on qualitative "skin" notations to provide information on the potential for dermal exposures to contribute significantly to the systemic dose, and in particular, to inform the interpretation of quantitative risk assessments based on concurrent inhalation exposures. The underlying decision criterion for making such assignments is often not documented. Moreover, the traditional skin notation approach does not provide information on other effects of dermal exposure. We have reported previously on an enhanced notation strategy developed by the National Institute for Occupational Safety and Health

(NIOSH) that provides for the assignment of multiple skin notations that address systemic toxicity, direct skin effects, and dermal sensitization. The current work presents experience in applying the NIOSH strategy to over 70 chemicals. Weight of evidence decisions to address conflicting or limited data sets is highlighted. Examples of common situations include; 1) assigning a systemic effects notation where data or model predictions indicate absorption, but no or only limited dermal toxicity data are available, 2) differentiating among irritant severity levels when relying on qualitative studies that used different material dilutions and test systems, 3) developing notations for sensitization when limited human studies and standard animal assays provide conflicting results. The lessons learned in evaluating such problematic data sets provide the basis for refining weight of evidence evaluation procedures for hazard notations.

## PS 2036 ICCVAM PERFORMANCE STANDARDS FOR THE MURINE LOCAL LYMPH NODE ASSAY (LLNA).

J. Matheson[1], M. Wind[1], A. Jacobs[2], D. Allen[3], T. Burns[3], E. Salicru[3], J. Strickland[3], R. Tice[4] and W. Stokes[4]. *[1]U.S. CPSC, Bethesda, MD, [2]U.S. FDA, Silver Spring, MD, [3]ILS, Inc./NICEATM, NIEHS, Research Triangle Park, NC and [4]NICEATM, NIEHS, Research Triangle Park, NC.*

ICCVAM develops performance standards to facilitate the efficient validation of modified versions of adequately validated alternative test methods. ICCVAM recently developed performance standards based on the ICCVAM-recommended LLNA protocol (ICCVAM 1999). The protocol was revised recently to reduce the minimum number of mice per dose group from five to four, and to provide guidance on reducing the number of positive control animals and determining the appropriate highest test dose. The performance standards include essential test method components, a minimum list of reference substances, and standards for accuracy and reliability. Essential test method components are the structural, functional, and procedural elements of a validated test method that must be included in a modified method in order for it to be evaluated using the established performance standards. Essential components of the LLNA include topical application of the test substance to the ears of mice, measurement of lymphocyte proliferation in the lymph nodes draining the area of test substance application, and use of the maximum soluble dose that does not result in systemic toxicity or excessive local irritation. The minimum list of reference substances for these LLNA performance standards includes 13 sensitizers and 5 non-sensitizers. The accuracy and reliability standards to be achieved by a modified LLNA are based on the performance of the traditional LLNA. These LLNA performance standards will facilitate rapid and efficient validation of modified LLNA protocols, such as those using non-radioactive markers of lymphocyte proliferation. New versions of the LLNA that provide improved performance or other advantages are expected to result in broader use of the LLNA, which will further reduce and refine animal use for allergic contact dermatitis assessments while ensuring human safety. ILS staff supported this abstract by NIEHS contract N01-ES-35504.

## PS 2037 LLNA: CURRENT REGULATORY ISSUES.

D. A. Basketter[1], I. Kimber[2], R. Dearman[2], F. Gerberick[3] and C. Ryan[3]. *[1]DABMEB Consultancy Ltd, Sharnbrook, United Kingdom, [2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom and [3]Procter & Gamble, Cincinnati, OH.*

Legislation in the EU requires evaluation of the skin sensitization potential for more than 30,000 substances. For many of these the local lymph node assay (LLNA) is the required test, unless a scientific rationale can be provided for use of an alternative approach. Elements of the rationale may be that open application is unsuitable, or that the chemical is a member of a class for which there is evidence of common association with false positive or false negative results. Certain chemical classes, for example fatty alcohols, have been reported as potential false positives in the LLNA. It is important that the evidence for this is reviewed rigorously and documented, so that there is a general consensus about which substances truly represent false positives, rather than weak allergens for which hazard classification would rather be avoided. The OECD Test Guideline 429 for the LLNA provides for a minimum of 4 mice per dose group, or 5 of mice per group if statistics are required. Recent discussions at the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) have suggested a change to LLNA requirements to mandate a minimum of 5 mice per group. This has prompted a retrospective analysis of published data from our own laboratories to determine whether use of 4 or of 5 mice has had any impact on performance of the LLNA . Of the datasets for 17 chemicals in the 4 animal assay (14 positive, 1 uncertain and 2 negative), 16 results were identical in the 5 animal assay. One marginally positive result in the 4 animal assay was negative in the 5 animal assay. Where potency determinations were made, the outcomes were essentially identical in both forms of the LLNA. It is concluded there is no scientific justification for removing the option of a 4 mice/group LLNA. This will help to keep to a minimum the number of animals used for skin sensitization testing under REACH.

## PS 2038 NONCLINICAL RODENT STUDIES FOR A NOVEL PACLITAXEL-PEPTIDE CONJUGATE THAT TARGETS THE BRAIN.

B. Lawrence[1], R. Gabathuler[1], J. Castaigne[1], G. Goodfellow[2] and M. Demeule[1]. *[1]AngioChem Inc., Montreal, QC, Canada and [2]Toxicology, Development and Regulatory Services, Intrinsik Health Sciences Inc., Mississauga, ON, Canada.*
Sponsor: J. Daniels.

ANG1005 is a novel cytotoxic drug that combines a receptor-targeting peptide vector to 3 molecules of paclitaxel. The peptide vector can be applied to other molecular classes, including biologics, and is designed to enable transcytosis across the blood-brain barrier by targeting the low-density lipoprotein receptor-related protein. ANG1005 has been tested in Sprague-Dawley rats in safety pharmacology and toxicology studies. Following single intravenous (IV) doses of ANG1005 up to 300 mg/m$^2$, no behavioral/central nervous system effects were observed in male rats. In a single-dose IV toxicity study, ANG1005 was administered at 0 to 850 mg/m$^2$. Effects on white blood cells (and related parameters) and platelet counts were observed at dose levels ≥200 mg/m$^2$. Macroscopic findings and changes in organ weights were observed for the spleen at ≥400 mg/m$^2$ and for the testes at 850 mg/m$^2$. The maximum tolerated single IV dose was 400 mg/m$^2$. In a repeated-dose IV toxicity study, ANG1005 was administered at 0, 25, 50, or 100 mg/m$^2$ twice weekly for 4 weeks. ANG1005-related mortality was observed at 100 mg/m$^2$ after ~2 weeks (3/24 M, 2/24 F); and the dose lowered to 75 mg/m$^2$. Moderate dose-related decreases in testicular weights were observed in 50 or 100/75 mg/m$^2$ males, with partial recovery by Day 40. Corresponding macroscopic and microscopic changes were observed, with soft, small testes noted and minimal to moderate degeneration of the seminiferous tubules observed in 6/9 males at 100/75 mg/m$^2$ and in 1/10 males at 50 mg/m$^2$. These microscopic findings were not resolved during the recovery period *e.g.*, aspermia in the epidydmides; a known paclitaxel effect). The NOAEL in this study was 25 mg/m$^2$. Based on mortalities observed in this study [5/48 (10.4%) at 100/75 mg/m$^2$], the severely toxic dose in 10% of the animals ($STD_{10}$) was estimated to be 75 mg/m$^2$. The ANG1005 rodent studies supported in part the initiation of 2 Phase 1 clinical trials in brain cancer patients.

## PS 2039 BEAGLE DOG TOXICITY STUDIES FOR A NOVEL PACLITAXEL-PEPTIDE CONJUGATE THAT TARGETS THE BRAIN.

B. Lawrence[1], R. Gabathuler[1], A. Regina[1], G. Goodfellow[2] and J. Castaigne[1]. *[1]AngioChem Inc., Montreal, QC, Canada and [2]Toxicology, Development and Regulatory Services, Intrinsik Health Sciences Inc., Mississauga, ON, Canada.*
Sponsor: J. Daniels.

ANG1005 is a novel cytotoxic drug that combines a receptor-targeting peptide vector to 3 molecules of paclitaxel. The peptide vector can be applied to other molecular classes, including biologics, and is designed to enable transcytosis across the blood-brain barrier by targeting the low-density lipoprotein receptor-related protein. The toxicity profile of ANG1005 in Beagle dogs has been investigated in a series of intravenous (IV) infusion toxicology studies. ANG1005 was administered at single IV dose levels of 0, 100, 200, or 400 mg/m$^2$ with both 400 mg/m$^2$ animals not surviving past Day 4. Reversible decreases observed for platelets, reticulocytes, and WBCs (all types) at 100 or 200 mg/m$^2$. The maximum tolerated IV dose was 200 mg/m$^2$. The mean half-life for all ANG1005-treated animals following a single dose was 2.76 ± 0.53 hr. In a repeated-dose IV toxicity study, ANG1005 was administered at 0, 15, 45, or 90 mg/m$^2$ twice weekly for 4 weeks. Modest hematological changes were observed in animals administered 90 mg/m$^2$ ANG1005, with the effects fully recovered by the end of the recovery period. Microscopic changes related to ANG1005 included unilateral and bilateral degeneration of the seminiferous tubules in the testes of males (a known effect of paclitaxel) in either the 45 mg/m$^2$ (1/4) or 90 mg/m$^2$ (1/6) treatment groups, with the effect reversible for the 90 mg/m$^2$ males. Plasma concentrations decreased mono-exponentially with an overall half-life of 2.68 ± 0.49 hr. The TK profiles of ANG1005 were similar at all dose levels, with no apparent gender differences. There were no treatment-related findings for the testes at the lowest dose (15 mg/m$^2$) and the NOAEL in this study was 15 mg/m$^2$. Based on the lack of severe findings in this study, the highest non-severe toxic dose (HNSTD) is 90 mg/m$^2$. The ANG1005 dog toxicity studies supported in part the initiation of 2 Phase 1 clinical trials in brain cancer patients.

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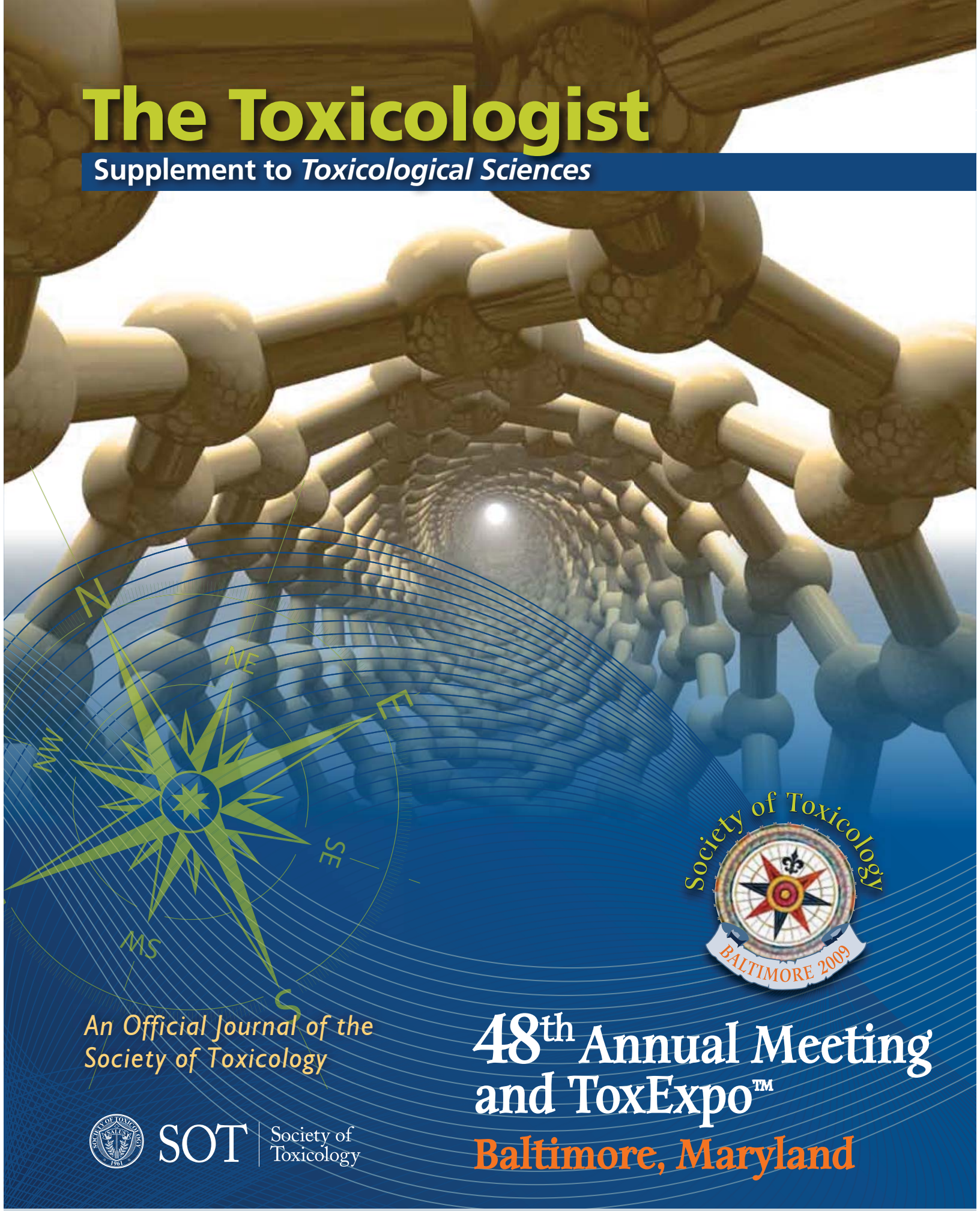


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